BARRIER DISRUPTION IN STAT6VT TRANSGENIC MICE AS A POTENTIAL MODEL FOR ATOPIC DERMATITIS SKIN INFLAMMATION

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Submitted to the faculty of the University Graduate School
in partial fulfillment of the requirements
for the degree
Master of Science
in the Department of Biochemistry and Molecular Biology,
Indiana University

November 2010

Accepted by the Faculty of Indiana University, in partial fulfillment of the requirements for the degree of Master of Science.

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This is dedicated to my parents, and my best friends Jorge & Tim…

"There are only two mistakes one can make along the road to truth;

not going all the way, and not starting"

– Siddhartha Gautama, Buddha

Acknowledgments

"We don't accomplish anything in this world alone..."

-Sandra Day O'Connor, U.S. Supreme Court

I would like to thank Dr. Jeffrey Travers for his constant mentorship, understanding, and confidence in me. Thank you for the opportunity to shadow you in your AD clinic. I would also like to thank Dr. Dan Spandau and Dr. Raymond Konger for the constant encouragement and their utter patience. I owe my gratitude to Dr. Sonal Sanghani for educating me in all the biotechnological techniques that I used in pursuit of my degree. I am very fortunate to have wonderful mentors guiding me and willing to let me interrupt their work with my questions.

I would also like to thank Dr. Mark Kaplan for generously providing me with the STAT6VT transgenic mice, and Dr. Michael Southall for hosting me at Johnson & Johnson, NJ.

I thank all the members of the labs where I completed each segment of my project and were instrumental to the technical aspects of this project. For Dr. Ravi Sahu, Dr. Mohammed Al-Hassani, Dr. Sarita Sehra, Dr. Simarna Kaur, Qiaofang Yi, Davina A. Lewis, Badri M. Rashid, Evelyn T. Nguyen, and Pamela Durant.

And finally, to Tim, I owe my sincerest gratitude for encouraging me every time that I thought I had lost it. You gave me comfort and hope whenever I needed it.

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Abbreviations

AD	atopic dermatitis
BCA	bicinchoninic acid
cDNA	complementary deoxyribonucleic acid
CE	cornified envelope
EDC	epidermal differentiation complex
FLG	filaggrin
GM-CSF	granulocyte-macrophage colony-stimulating factor
IFNγ	interferon gamma
ICD	irritant contact dermatitis
IL-1ß	interleukin-1 beta
IL-4	interleukin-4
IL-12	interleukin-12
IL-13	interleukin-13
IVL	involucrin
JAK	Janus kinase
KLK7	kallikrein 7
KRT14	keratin 14
KO	knockout
LOR	loricrin
PBS	phosphate buffered saline
PCR	polymerase chain reaction
qRT-PCR	quantitative real-time polymerase chain reaction
SPINK5	serine peptidase inhibitor Kazal type 5
SH2	Src homology domain 2
STAT6	signal transducer and activator of transcription 6
STAT6VT	STAT6 V547A/T548A mutation
SC	stratum corneum
TBS	tris buffered saline
TEWL	transepidermal water loss
T_H1	T-helper Type 1 cells
T_H2	T-helper Type 2 cells
TNFα	tumor necrosis factor alpha
WT	wild-type

Introduction

Atopic dermatitis (AD) is a pruritic, chronic inflammatory skin disease with a lifetime prevalence of 10-20% in children and 1-3% in adults, worldwide. It is the most common cause of occupational skin disease in adults. In the past three decades, prevalence of the disease has increased by two to three-fold in industrialized countries, with higher incidences in urban regions compared to rural regions (Leung & Bieber, 2003). A recent review has described how AD impacts both the child and immediate family (Sehra, et al., 2008). The pruritic feature of AD can cause mental exhaustion, mood changes, lack of concentration in school or work, and an increase in parental and child morbidity. The clinical features of AD include mild cases of erythema in localized areas of the body to acute lesions that appear as erythematous macules. Lesions tend to occur on the cheeks, scalp and extensor areas of the arms and legs in infants, and in the flexural regions of the extremities in older children (Sehra, et al., 2008). The pathogenesis of AD includes complex interactions between susceptibility genes, environment, and immunological factors (Leung, 2000). Although recent studies have resulted in an enhanced understanding of the pathogenesis of AD, there is still a need for an improved elucidative animal model.

The systemic immune response of AD involves an increase in blood serum IgE antibodies to nonpathogenic allergens and a decrease in interferon γ levels (IFNγ). Approximately 80% of children with AD will develop either allergic rhinitis or asthma (Leung & Bieber, 2003). Biopsy samples from unaffected skin of patients with AD, compared to healthy, normal skin, show an increase in the number of T_H2 cells that regulate interleukin-4 and interleukin-13 (IL-4, IL-13, respectively) (Hamid, et al., 1996).

Histologically, there are differences between acute and chronic atopic dermatitis. Acute AD lesions demonstrate focal parakeratosis, spongiosis of the epidermis, and inflammatory cell filtrates in the dermis made up of T-cells expressing IL-4 and IL-13, inflammatory dendritic epidermal cells, macrophages, eosinophils, mast cells, and antigen-presenting cells like Langerhans cells. On the other hand, chronic AD shows a less pronounced dermal infiltrate and a significant reduction in IL-4 and IL-13 expression. Interleukin-5 (IL-5), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-12 (IL-12), and IFNγ are also increased in comparison to the acute phase of AD and there is acanthosis (Dubrac, Schmuth, & Ebner). The increase in IL-5 is generally seen during the transition from acute to chronic AD and may be responsible for prolonged eosinophil survival and function (Hamid, Boguniewicz, & Leung, 1994).

Those with a history of AD are more prone to developing irritant contact dermatitis (ICD) of the hands. The clinical result of ICD is inflammation caused by the release of proinflammatory cytokines from keratinocytes in response to chemical stimuli (Hogan, 1990). Naive T-cells will differentiate into having either a Type 1 or Type 2 helper T cell phenotype, (T_H1 or T_H2 respectively), depending on the source of antigen-presenting cells. Generally, the T_H1 pathway is stimulated in response to intravesicular pathogens in response to infected macrophages. On the other hand, T_H2 cells are generated when basophils present antigen to the T cell receptor triggering antibody production in B-cells (Kaiko, Horvat, Beagley, & Hansbro, 2008).

The skin is responsible for preventing loss of water from the body as well deterring the entry of environmental particulates. The function of the epidermal

permeability barrier is characterized by the stratum corneum (SC); a complex made up of intracellular lipids, corneocytes, and a structural arrangement (Nielsen, 2000). The SC has been likened to a brick wall, with the corneocytes acting as the brick and the lipid intracellular matrix as the cement. During epidermal differentiation, there is a reduction in phospholipids but an increase in fatty acids and ceramides. The final stages of epidermal differentiation are marked by the discharge of lipid-containing granules, or lamellar bodies, from the keratinocytes into the extracellular spaces in the upper granular layer, forming intercellular membrane bilayers (Loden, 1995).

Skin transepidermal water loss (TEWL) is the rate at which water travels from the viable dermal and epidermal tissues through the SC to the external environment. It is a widely accepted technique to evaluate the integrity of the SC and used to study skin barrier function (Loden, 1995). TEWL (g/hm^2) can be evaluated with a Tewameter® TM 300 (Courage + Khazaka electronic GMB, Cologne, Germany). It is important that certain considerations be taken into account, as published by the Standardization Group of the European Society of contact dermatitis, specifically room temperature and ambient humidity. Air convection may interfere with the readings, so they should be taken in a draught protected area such as a hood (Pinnagoda, Tupker, Agner, & Serup, 1990). TEWL measurements can be used to monitor barrier repair as a function of time. An increase in TEWL is an indicator of skin barrier dysfunction (Loden, 1995).

Skin erythema is caused by dilation of the blood vessels with an infiltration of blood cells close to the skin surface and can be elicited by detergents, allergens or UV light. Erythema is a major assessment in determining the irritation potential of a compound, and is typically determined by a visual score. Certain factors can skew this

score including skin color, scorer subjectivity, and reproducibility between experiments (Ahmad Fadzil, Ihtatho, Mohd Affandi, & Hussein, 2009). A noninvasive bioengineered tool that measures erythema is the Mexameter® MX18 (Courage + Khazaka electronic GMB, Cologne, Germany).

Intact skin barrier is dependent on undisturbed epidermal differentiation which is coded for by a cluster of genes termed the epidermal differentiation complex (EDC). Variation within the EDC plays a major role in the pathogenesis of AD. Microarray analysis of AD lesions have indicated altered gene expression of genes in the EDC, specifically downregulation of filament aggregating protein or filaggrin (*FLG*) and loricrin (*LOR*) (Hoffjan & Stemmler, 2007).

The EDC spans 1.62 megabases on Chromosome 1q21.3 and contains about 50 genes that are directly involved in the barrier function. These genes encode proteins including (pro)filaggrin, involucrin, and loricrin. (Toulza, et al., 2007).

FLG is the gene for the protein profilaggrin which is cleaved in the suprabasal keratinocytes in a complex biochemical cascade involving phosphatases and proteases into the active form filaggrin (FLG) (Koch, et al., 2000). FLG has a high affinity for keratins and bundles the keratin intermediate filaments into packs (Koch, et al., 2000; Sehra, et al., 2008). In the SC, FLG is eventually completely hydrolyzed freeing amino acids that are thought to contribute to water retention in the SC (Koch, et al., 2000). Loss-of-function mutations in FLG have been correlated to atopic dermatitis (Torma, Lindberg, & Berne, 2008). In addition, the abnormal skin barrier seen in patients with a *FLG* null mutation and AD and T_H2 polarization may be caused partly by an elevated allergen penetration through the skin (Leung, 2009). On the other hand, overexpression

of murine filaggrin (*Flg)* in a transgenic model has been shown to accelerate the rate of barrier repair following chemical disruption (Presland, et al., 2004).

IVL and *LOR* encode for involucrin (IVL*)* and loricrin (LOR), respectively, which have been shown to facilitate terminal differentiation of the epidermis and skin barrier formation (Kim, Leung, Boguniewicz, & Howell, 2008). LOR is a major protein (constituting up to 70% of mass in mice) of the epidermal cornified cell envelope (CE) found in terminally differentiated epidermal cells. The CE acts as a lamellar structure that is essential for the barrier function of the skin, preventing the loss of water and ions and providing protection from environmental factors (Koch, et al., 2000). IVL is also found in the CE and acts as a scaffold to which other proteins become cross-linked (Kim, et al., 2008).

KRT14 is responsible for encoding keratin, type 1 cytoskeletal 14, or keratin-14 (KRT14), an intermediate filament protein that forms the structural framework of certain cells including those that make up skin, hair and nails ("Keratin, type 1 cytoskeletal 14," 2010). KRT14 is expressed in the basal layer of stratified squamous epithelia, specifically in the epidermis (Rosenberg, RayChaudhury, Shows, Le Beau, & Fuchs, 1988).

SPINK5 encodes a multidomain serine peptidase inhibitor Kazal type 5 that contains 15 potential inhibitory domains. A recent study of Caucasian AD families showed that maternally derived alleles of the *SPINK5* gene are associated with development of AD and asthma.

The role of the innate immune system has been studied in the pathogenesis of AD (Niebuhr, Lutat, Sigel, & Werfel, 2009). It is known that white blood cells, specifically

macrophages accumulate in the inflamed skin of both acute and chronic AD lesions (Kiekens, et al., 2001). Macrophages differentiate from blood-derived monocytes *in situ* and are responsible for the phagocytosis of invading pathogens and dead cells, as well as the stimulation of cytokines. The cytokines constitute a diverse group of soluble proteins and peptides that act as regulators of individual cells and tissues under both normal and pathological conditions. Furthermore, they mediate intracellular interactions as well as processes occurring in the extracellular environment. Cytokines behave similarly to hormones by acting at a systemic level by affecting inflammation. However, they differ from classic hormones in that they act on a wider range of target cells, and are not produced by specialized cells that are organized in specialized glands. They are classified as lymphokines, interferons, colony stimulating factors, and chemokines (Balkwill & Balkwill, 2000). Macrophages are an important source of proinflammatory mediators including IL-1ß, TNFα, IL-6, CXCL8 (Bloemen, et al., 2007).

Early events in atopic skin inflammation involve mechanical trauma and skin barrier disruption resulting in a rapid upregulation of the proinflammatory cytokines IL-1α, IL-1ß, TNFα and GM-CSF (Homey, Steinhoff, Ruzicka, & Leung, 2006). The relative contribution of an individual cytokine is dependent on the duration of the skin lesion, thus implicating both T_H1 and T_H2 cytokines in the pathogenesis of skin inflammation exhibited in AD (Werfel, 2009). Studies have shown that IFNγ is upregulated in spontaneous or older lesions in AD (Grewe, et al., 1995). AD patients have activated T-cells that express high levels of IFNγ that undergo apoptosis in circulation, skewing the immune response towards a T_H2 phenotype. This provides a

mechanism for the T_H2 predominance seen in the circulation and in acute AD lesions (Akkoc, et al., 2008).

The cytokine IL-4 plays a critical role in the pathosphysiology of asthma and allergic diseases. The IL-4 pathway cascade is mediated through the activation of the latent transcription factor, signal transducer and activator of transcription 6 (STAT6). The STAT proteins consist of 750 to 800 amino acid residues and contain a Src homology 2 (SH2) domain and a carboxyl-terminal tyrosine phosphorylation site. Seven STAT proteins have been identified. STAT proteins require both tyrosine phosphorylation for dimerization and translocation to the nucleus and serine phosphorylation for transcriptional activation (Akira, 1999).

STAT6 is a monomeric protein that is activated by the Jak/STAT pathway. When IL-4 binds to its receptor, the Jaks phosphorylate tyrosine residues in the IL-4Rα and γc receptor chains, recruiting STAT6 to the IL-4Rα chain (Bruns, Schindler, & Kaplan, 2003). The STAT6 SH2 domain is integral to its interaction with the two phosphotyrosine-containing regions in IL-4. As a result, STAT6 becomes phosphorylated on tyrosine 641 by Jak1 and/or Jak3 (Jak family of tyrosine kinases). The phosphorylated monomers then dimerize and migrate to the nucleus, where STAT6 binds *cis*-acting elements to activate the transcription of IL-4 target genes (Daniel, Salvekar, & Schindler, 2000). Previous experiments using STAT6 -deficient mice have shown that most IL-4 mediated functions are lost with the absence of STAT6 (Bruns, et al., 2003). These losses of function include the inability to regulate Ig isotype expression by promoting a class switch to IgE and IgG1 in antigen-activated B cells. In addition, there is no induction of IL-4-induced cell surface markers, and lymphocytes have

impaired proliferative responses to IL-4. Finally, the STAT6-deficient mice cannot promote the differentiation of naïve $CD4^+$ T-cells into the T_H2 phenotype (Bruns, et al., 2003).

Mice with an activating mutation in STAT6, known as STAT6VT, constitutively express STAT6 in T-cells. The difference between STAT6VT and wild-type (WT) mice is that the mutant contains two residue substitutions at positions V625A and T626A in the SH2 domain. The STAT6VT is constitutively phosphorylated on the critical tyrosine residue, Y641, and transcriptionally active in the absence of IL-4. The conformational change in the protein caused by the mutation hinders the association of STAT6VT with the unphosphorylated IL-4R chains (Bruns, et al., 2003).

The ability of the constitutively active STAT6 to mimic the IL-4 activated functions *in vivo* was evaluated by generating a transgenic mouse that expressed STAT6VT only in lymphoid tissues; it was engineered using a C-terminal FLAG tag. The vector containing the STAT6VT cDNA was digested with *Eco*RI, and the fragment was cloned into VACD2, a plasmid containing the CD2 locus control region (LCR). Next, the CD2: STAT6VT vector was digested with *Kpn*I and *Xba*I, resulting in a 15.7 kb fragment that was purified and used to generate the transgenic mice. The resulting mice contain a conformational change in the STAT6 protein that allows for it to be phosphorylated, bind DNA and activate transcription independent of IL-4 stimulation. The STAT6VT transgenic mice have altered lymphocytic behavior. The expression of STAT6VT in the B lymphocytes causes increased expression of IL-4 related inducibility and increased production of IgG1 and IgE, but no activation of the B lymphocytes. The same STAT6VT expression in the T lymphocytes results in T cell differentiation towards

a T_H2 phenotype (Daniel, et al., 2000). The STAT6VT transgenic mice develop AD symptoms under specific pathogen-free conditions and do not require overexpression of effectors in the skin.

Sodium lauryl sulfate (SLS), an anionic detergent, is a common ingredient found in soaps, shampoos, and various other skin care products. It has been shown to trigger a prolonged barrier disruption of the skin that lasts up to a week following a single 24-hour application. SLS is commonly used in studies involving skin barrier damage (Patil, Singh, Sarasour, & Maibach, 1995; Torma, et al., 2008). The underlying molecular mechanisms to explain barrier repair after chemical disruption of the skin are currently limited, although it is known that altered keratinocyte differentiation may occur (Ponec & Kempenaar, 1995). SLS penetrates the skin to a depth of 5-6mm below the site of application in a hairless rat model (Patil, Singh, Sarasour, et al., 1995). The radial spread of SLS can elicit increased TEWL and decreased skin capacitance in areas adjacent to the application sites, as far as approximately 0.75cm away (Patil, Singh, & Maibach, 1995). It appears that SLS acts in a time-and-dose dependent manner to disrupt the barrier function (Nielsen, 2000). The goal of the present studies was to assess the effects of topical SLS on skin in normal and STAT6VT mice. Our hypothesis is that treatment of STAT6VT mice, at a young age where they have no clinical abnormalities, with SLS will result in an increased barrier disruption in comparison to WT mice. Our preliminary data suggests significant differences between the STAT6VT transgenic mice from WT littermate controls treated with SLS. These findings correlate with evidence that there are abnormalities in the barrier function between these mice (Sehra, et al., 2010).

Polymerase chain reaction (PCR) is a standard laboratory technique that amplifies a specific DNA sequence in vitro (Mullis, 1990). Quantitative real-time PCR (qRT-PCR) is used to determine the copy number of target DNA in a particular sample. Taqman PCR is a probe-based system that uses a fluorogenic probe containing a reporter fluorescent dye and a quencher dye to provide a fast and reliable method for semi-quantitative analysis of gene expression (Medhurst, et al., 2000). Cytokine analysis of several analytes can be done simultaneously using an array system based on Luminex® xMAP technology. This method is based on flowmetric analysis of beads acting as a solid support for individual reactions using a common fluorophore reporter. The measurement of an array of cytokines is important for studying inflammatory diseases and diseases of unknown etiology (Skogstrand, et al., 2005). The bicinchoninic acid (BCA) assay is a colorimetric assay used to quantify the amount of protein in a sample (Sapan, Lundblad, & Price, 1999).

Materials and Methods

Wild-type (WT) and STAT6VT transgenic mice

The STAT6VT transgenic mice have been generously provided by Dr. Mark Kaplan at Indiana University School of Medicine, Indianapolis, IN for our breeding purposes. These heterozygous mice were bred with C57BL/6 mice (Harlan Laboratories, Indianapolis, IN) to generate additional transgenic mice and WT controls. All mice were maintained in standard microisolator cages under a simulated 12-hour day/night cycle and were fed and watered *ad libitum.* All animal experiments were in compliance with and approved by the Indiana University School of Medicine Animal Care and Use Committee.

Mouse Genotyping using a Taq Polymerase

PCR-quality mouse genomic DNA was prepared by using a hot sodium hydroxide and tris (HotSHOT) method. Mouse tail snips (0.2-0.5 cm long) were collected into 0.5 ml PCR tubes and placed on ice. If lysis could not be done immediately, the tails were placed at -20^{o}C for storage. The stock reagents include an alkaline lysis reagent prepared with 25 mM NaOH, 0.2 mM disodium EDTA (pH=12) dissolved in water without adjusting the pH; and a neutralizing reagent of 40 mM Tris-HCl (pH=5) also dissolved in water without adjusting the pH. Next, 0.75 µl of the alkaline lysis was added to the tail snip, heated to 95^{o}C for 30 minutes, and cooled to 4^{o}C. Then 0.75 µl of neutralizing reagent was added, spun down and either used immediately or the supernatant was stored at -20^{o}C (Truett, et al., 2000). The PCR conditions to screen for the STAT6VT transgene (5′ sequence: gcctaccatggtgccttcttatg, 3′ sequence: tatgcttgtcatcgtcgtccttgtagtcac)

consisted of a 1 minute denaturation step at 94^{o}C and 34 cycles of 94^{o}C for 20 s, 58^{o}C for 30 s, and 72^{o}C for 45 s.

Induction of chronic irritation and biophysical measurements

The development of chronic atopic symptoms in mice was induced by treating the intact dorsal skin of both STAT6VT and WT sibling controls with 0.1 ml of a 5% aqueous solution of sodium lauryl sulfate (BioRad Laboratories Inc., Hercules, CA) and distilled water (vehicle). The animals were kept in clean rooms with controlled temperature (22±2^{o}C) and humidity (35-40%), and lit with a 12-hour on/off cycle. Female mice were used in all experiments unless otherwise indicated. Approximately two to three mice were assigned to each of the following four groups: STAT6VT Vehicle, WT Vehicle, STAT6VT SLS and WT SLS during each of the three experiment trials. The SLS was applied in a regime of three sets of daily exposure for six consecutive exposures followed by a 14-day period with no application. On Day 0, the mice were briefly anesthetized with ketamine/xylazine (80/10 µg/kg body weight) intraperitoneally and the dorsal hair was shaved. Twenty-four hours later, baseline transepidermal water loss (TEWL, g/hm^{2}) measurements were taken with a Multi Probe Adapter (MPA) fitted with a TM300 Tewameter (Courage + Khazaka, Cologne, Germany), and 0.1 ml 5% SLS was applied topically to irritate the skin. Subsequent TEWL readings were taken daily during the application time course, and every other day during non-application days. The same sample size of WT sibling control mice were treated similarly. A final six day treatment period of 10% SLS was done. The accepted practice of TEWL measurement using an open chamber system is based on assessing the

vapor pressure that builds up inside the probe tunnel when it is placed on the skin surface. To minimize the possible errors of this method caused by fluctuations in the relative humidity and skin surface temperature, standard procedures were followed. All measurements were performed at standard laboratory conditions (temperature of 20-25^{o}C and humidity of 30-40%). The probe was applied so that it was in full contact on the dorsal median and the probe temperature was given time to acclimate to the skin temperature (approximately 20 s). The change in TEWL for each mouse was compared to each individual mouse's baseline value and the fold increase was plotted.

Tissue Processing

The dorsal skin of age-matched mice (STAT6VT and WT) that was treated with SLS or water was harvested within 72 hours following the final treatment. The skin tissue was processed for each of the following: tissue was harvested and preserved in RNA*later* RNA Stabilization Reagent (Qiagen, Valencia, CA) for qRT-PCR analysis, 7 mm skin biopsies were harvested to assess cytokine levels, and 0.5 cm^2 skin sections were harvested and fixed in 10% neutral buffered formalin for 1.5 hours for paraffin-embedding and histologic assessment and immunohistochemical analysis. The remaining tissue was snap-frozen in liquid nitrogen and stored at -80^{o}C.

Immunohistochemistry for Ki-67 and quantification of positive staining

Mouse dorsal skin sections were formalin-fixed for 1.5 hours and changed to 70% EtOH, trimmed and placed in cassettes for further processing and embedding. For Ki-67 immunolabeling, standard deparaffinization and hydration were performed followed by

heat-induced antigen retrieval using 10 mM citrate buffer, pH 6.0 for 20 min. All reagents were purchased from DAKO, Carpinteria, CA unless otherwise noted. Sections were incubated with 3% hydrogen peroxide for 10 min at room temperature. Sections were washed with TBS followed by incubation with Ki-67 (SP6) (Thermo Scientific). The sections were then washed and incubated with Envision+ System-HRP (DAB) for use with rabbit primary antibodies for 30 min at room temperature. DAB+ was used for substrate detection. Sections were then counterstained and mounted. All images taken with a Nikon Eclipse E400 microscope (Nikon Corp., Melville, NY) at 200X magnification were saved as .tiff files and subjected to a background subtraction in NIH Image J. For the background subtraction, the light background, separate colors, and sliding parabaloid functions were checked. The images were opened in Metamorph Meta Imaging Series 7.5 (Molecular Devices, Downington, PA). In Metamorph, the epidermis was selected using the region drawing tool and the images were color separated into blue, red, and green images. For thresholding on Ki-67 positive cells as a percentage of the total epidermal area, the blue image was utilized. Total epidermal nuclei were estimated by thresholding on a grayscale image in Metamorph. All images were analyzed using the same threshold settings for K-i67 and total nuclei. Results are shown as the ratio of Ki-67 positive thresholding relative to total epidermal nuclei.

Quantification of epidermal thickness by IHC

Changes in overall morphology were visualized following hematoxylin and eosin (H&E) staining of paraffin-embedded tissue samples. Epidermal thickness was measured by an optical micrometer in a blinded manner using a Nikon Eclipse E400 microscope at

200X total magnification. Measurements (µm) were taken from the base of the stratum corneum to the basement membrane of the inter-rete ridges along the entire length of each section, in 3 random fields in an observer-blinded manner for each condition.

Total RNA extraction and cDNA synthesis

To quantify the relative gene expression for each mouse, the skin tissue preserved in RNA*later* was first weighed (<30mg) and total RNA was isolated using the RNeasy Fibrous Mini Kit (QIAGEN Inc, Valencia, CA). The skin tissue was disrupted and homogenized in Buffer RLT with ß-Mercaptoethanol and a stainless steel bead (5 mm mean diameter) with a TissueLyser (QIAGEN Inc, Valencia, CA) for three repeated bursts of 2 min at 20 Hz. After a proteinase K digestion step, DNase was used to remove any traces of DNA that may copurify with the total RNA. The eluate was collected and the concentration of total RNA(1 µl) was determined by using a NanoDrop 2000 UV-Vis spectrophotometer (Thermo Scientific, Wilmington, DE) at 280 nm, and all samples were blanked with 1 µl of RNase-free water. RNA (0.85 µg) was reverse-transcribed in a 20 µl reaction containing Random Primers (50 ng/µl: Invitrogen, Carlsbad, CA), dNTP (10 mmol/L; Invitrogen) with a 5 min denaturation step at 65^{o}C; then 10X First Strand RT buffer (Invitrogen), DTT (0.1 mol/L; Invitrogen), Superscript III RT enzyme (200 U/µl; Invitrogen) for 10 min at 25^{o}C, 50 min at 50^{o}C and 5 min at 85^{o}C; and RNase H (Invitrogen) for 20 min at 37^{o}C. The cDNA was either stored at -20^{o}C or used for qRT-PCR immediately.

Quantitative real-time PCR

Relative gene expression was studied by qRT-PCR in a 10 µl volume reaction in MicroAmp® 96-well reaction optical tubes in the 7900HT Fast Sequence Detection System (Applied Biosystems, Stockholm, Sweden). cDNA was used as template (0.85 µg total RNA) along with a dual-labeled fluorogenic probe method using Taqman primers. All primers and probes for murine genes were purchased from Applied Biosystems. Relative gene expression levels were calculated using the $\Delta\Delta C_T$ method. Quantities of all targets in each sample were normalized to the corresponding *Krt14* in the skin biopsies.

Cytokine analysis

Punch biopsies of 7 mm diameter were taken from mouse dorsal skin that had been challenged with SLS or vehicle and stored at -80^{o}C. The skin was minced on a glass plate using a razor blade and homogenized on ice in 800 µl cold PBS containing protease inhibitor cocktail and 0.1% Triton X-100 (Sigma Aldrich, St Louis, MO). The samples were centrifuged at 1000 RPM for 10 min at 4^{o}C and the supernatant was used for cytokine analysis. Cytokine and chemokine analysis for murine GM-CSF, IFNγ, IL-10, IL-12 (p70), IL-13, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, MCP-1, and TNFα was done using the MILLIPLEX™ MAP cytokine and chemokine panel, based on the Luminex xMAP platform (Millipore, Billerica, MA) at Johnson & Johnson, Skillman, NJ.

Normalization of protein levels

Cytokine levels were normalized to total protein levels determined by a Pierce BCA protein assay kit (Thermo Scientific, Rockford, IL.). 10 µl of all standards and samples were diluted in 200 µl of the BCA working reagent (50:1, Reagent A: B) to yield a sample to working ratio of 1:20) in a microplate. The standards used were known concentrations of bovine serum albumin (BSA) diluted in the same homogenization solution given above. Plate was covered and incubated at 37^{o}C for 30 min. The absorbance for both the standards and the samples were read at 562 nm in a Beckman spectrophotometer blanked with 800 µl cold PBS containing protease inhibitor cocktail and 0.1% Triton X-100 and 200 µl of BCA working reagent. The absorbance values for the standards were plotted using Microsoft Excel for a linear regression fit to generate a standard curve from which the protein concentration of experimental standards could be determined.

Statistics

Statistical analysis was conducted using Graph Pad Prism, version 5.0 (San Diego, CA). Statistical differences between groups were determined using the student *t*-test with significant differences conferred when $p<0.05$. In cases where multiple groups were compared, data were analyzed by a one-way analysis of variance (ANOVA). Where the direction of change was known, a one-tailed student *t*-test was employed.

Results

Chronic irritant treatment of STAT6VT mice augmented barrier disruption

Initially, we assessed whether the STAT6VT transgenic mice exhibited baseline differences in barrier function by measuring TEWL using the Tewameter® TM 300. The baseline TEWL levels were similar in C57BL/6 controls versus STAT6VT transgenic mice (data not shown). Next, we assessed the effect of chronic irritant SLS by two groups of six daily applications separated by a 14-day interval. As shown in Fig. 1, the fold increase in TEWL (plotted as $TEWL_{given\ day}$ / $TEWL_{baseline}$) was increased in STAT6VT mice repeatedly treated with SLS compared to WT mice similarly treated, with significant (* $p<0.05$) differences between the two groups were seen on days 10, 12, 24, 26, 52, 54, and 57. Furthermore, TEWL was increased in STAT6VT mice during each of the application periods, whereas the reactions seen in the WT were not as pronounced. Next, we assessed whether a higher concentration of SLS would elicit a greater increase in TEWL, so we applied a 10% SLS solution to the mouse dorsum. It should be noted that the fold increase in TEWL does not appear to be further increased with the higher SLS dose. The chronic SLS treatment resulted in irritation of the mouse dorsal skin as shown in Fig. 2A. Visually, the STAT6VT transgenic mice exhibit increased erythema and excoriations compared to WT littermate controls which did not develop dermatitis. As shown in Fig. 2B, the change in TEWL ($TEWL_{endpoint}$ - $TEWL_{baseline}$) was significantly increased in both STAT6VT and WT mice following repeated SLS applications compared to controls.

Concurrently with the tewameter studies, we assessed the irritation potential of SLS on the STAT6VT transgenic and WT mice by measuring erythema with the

Mexameter® MX18. The change in erythema ($erythema_{endpoint}$ - $erythema_{baseline}$) in both the WT and STAT6VT transgenic mice treated with SLS was significantly augmented as shown in Fig. 2C compared to the vehicle control groups (data not shown). However, there were no differences in the mexameter readings in SLS treated STAT6VT compared to WT mice. The present studies indicate that the STAT6VT transgenic murine model exhibits increased barrier disruption following chronic treatment with SLS.

Effects of SLS on inflammation

Previous studies have shown that the STAT6VT transgenic mice have increased scratching behavior that result in dermatitic plaques with alopecia on the face, dorsum and other areas (Sehra, et al., 2010). We therefore examined the effect of SLS on the inflammatory response in murine skin. As shown in Fig. 3, histological evaluation revealed a marked influx of inflammatory cells in SLS-treated STAT6VT transgenic mice compared to similarly treated WT littermate controls. Importantly, the vehicle-treated STAT6VT mice exhibited low-grade inflammatory cell infiltration of the dermal region; whereas, the SLS treatment resulted in more severe inflammation.

Repeated SLS treatment augments hyperproliferation as determined by epidermal thickness (hyperplasia) and Ki-67 immunolabeling

Previous studies have provided evidence that topical acetone treatment or tape stripping induces epidermal hyperplasia in mice (Denda, et al., 1996). The degree of the epidermal hyperplasia correlated with the duration of the treatment period. In addition, the hyperplasia was attributed to increased cell proliferation. In our studies, we were

interested in examining the effect of SLS on the STAT6VT transgenic mice on these responses. First, we evaluated the ability of SLS to induce hyperplasia by assessing epidermal thickening (Fig. 4A). Quantitative epidermal measurements indicate a marked increase in STAT6VT transgenic mice treated chronically with SLS (* $p<0.05$). No differences are noted between the STAT6VT transgenic mice treated with vehicle or the WT mice treated with SLS or vehicle.

Next, we assessed whether the increased epidermal thickness in SLS-treated STAT6VT mice was due to increased cellular proliferation. Ki-67 is a nuclear protein that provides a reliable method to evaluate the growth fraction of cells during late S, M and G2 phases (Gerdes, et al., 1984). As shown in Fig. 4B, quantification of Ki-67 following chronic SLS treatment indicated that there is a greater proliferative effect in the SLS-treated STAT6VT. This trend is consistent with that of the epidermal thickening suggesting that the increased epidermal thickening correlates with proliferation.

EDC gene expression is altered due to skin barrier disruption by SLS

Our next studies were designed to assess the role of EDC genes in the enhanced SLS-mediated barrier disruption found in STAT6VT mice. Thus, the expression of specific EDC genes in skin of vehicle and SLS-treated mice was analyzed through qRT-PCR. As shown in Fig. 5A, although the expression of *Flg* appears greater in STAT6VT transgenic mice as well as in the WT mice compared to control mice, the differences did not achieve statistical significance. There are also no obvious changes in expression of *Ivl* and *Klk7* (Fig. 5B and 5D, respectively) compared to controls. On the other hand, a major reduction is seen in the *Lor* expression in both the STAT6VT vehicle and WT

SLS-treated mice compared to WT vehicle-treated littermate controls in Fig. 5C (* $p<0.05$). Similarly, the *Spink5* mRNA expression (Fig. 5E) is downregulated in STAT6VT vehicle and SLS-treated mice compared to WT vehicle-treated littermate controls (** $p<0.01$). These studies indicate that the STAT6VT transgene may contribute to a downregulation of *Lor* and *Spink5* gene expression following SLS treatment.

Gene expression and protein levels of inflammatory murine cytokines are altered following exposure to SLS

Previous studies have implicated T_H2 immune response in acute, and more mixed T_H1-T_H2 immune response in chronic AD (Akdis, et al., 2003; Akkoc, et al., 2008). Our next studies were designed to characterize the inflammatory cytokines in our murine model. As shown in Fig. 6A, *Il-13* gene expression is upregulated in SLS-treated WT mice compared to vehicle-treated WT controls. However, as seen in Fig. 6B, *Il-1ß* gene expression is significantly downregulated in SLS-induced WT and STAT6VT mice compared to vehicle-treated WT mice (* $p<0.05$). Similarly, *Tnfα* gene expression (Fig. 6C) is significantly (* $p<0.05$) reduced in the SLS-treated STAT6VT mice compared to both vehicle and SLS-treated WT mice. There are no obvious changes in mRNA expression of *Ifnγ* and *Mcp-1* (Fig. 6D and 6E, respectively) compared to controls.

Next, we measured protein expression level to attempt to correlate with cytokine gene expression. Table 1 provides an overview of the protein expression of various cytokines normalized to total protein (represented as pg/mL cytokine per µg protein). Previous studies have reported that AD patients have an increased activation and apoptosis of high IFNγ producing T_H1 cells which may result in a predominant T_H2

profile (Akkoc, et al., 2008). Our studies show that IFNγ was significantly upregulated in SLS-treated STAT6VT and WT mice compared to littermate controls, but the gene expression results were not statistically significant.

Discussion

Development of an irritant contact dermatitis by repeated applications of SLS in STAT6VT mice

In this study, we were interested in whether the STAT6VT transgenic mouse would be an appropriate model to study irritant contact skin inflammation, and compare a normal mouse versus a mouse with immunological abnormalities associated with AD. Since approximately 40% of STAT6VT transgenic mice develop spontaneous skin inflammation characteristic of AD, we wanted to assess the effects of barrier disruption in this model. As a means to this end, we used previously described methods to induce irritant-induced dermatitis (De Jongh, et al., 2006; Torma, et al., 2008). In regards to permeability barrier homeostasis, we used biophysical parameters to measure barrier function. TEWL measurements were elevated in the transgenic model compared to controls with significance observed on treatment days and at the conclusion of the chronic phase. The STAT6VT transgenic mouse has impairment in barrier recovery following skin irritation as evidenced by a marked increase in the difference for both TEWL and erythema at the study endpoint compared to baseline. Interestingly, the SLS-treated WT mice seemed to have decreased responses, or hardening after multiple treatments. However, this effect was not seen in the STAT6VT transgenic mice. Of particular note, the TEWL of the WT mice returned to baseline levels during the interval period between treatments, unlike the STAT6VT transgenic mice.

Our study found that there were significant differences in the TEWL and erythema responses amongst the SLS-treated STAT6VT transgenic mice. A possible explanation is the induction of hair growth in the experimental (SLS) groups, a finding

not observed in the control groups. Of note, a study by another group showed visible acceleration of hair growth on SLS-treated skin at three weeks from the start of treatment (Li, Fiedler, & Kumar, 1999). SLS permeability is impacted by accelerated hair growth during a long-term study. Another difficulty of using a murine model in the C57BL/6 background was that a completely accurate erythema visual score was limited due to the melanin content of the murine skin. For future studies, we have bred the STAT6VT transgenic mouse into a SKH1-*Hr* ^{hr} (hairless, albino) background which is currently used for research studies in skin inflammation, wound healing, and safety and efficacy testing. This model should allow for better penetration of a topical irritant and easier visualization of skin plaques and lesions that form.

Epidermal thickness and active proliferation data supports the inflammatory infiltration in the STAT6VT transgenic mouse treated with a topical irritant resulting in a hyperproliferative dermatitis. Another group has similarly shown that irritant skin reactions caused from repeated applications of SLS result in increased epidermal thickness (Anderson, Sundberg, & Groth, 1986). Previous and current studies by our lab have shown that STAT6VT murine ear tissue, even in mice without obvious lesions, exhibits a marked thickening of the dermis and epidermis with cellular infiltration of eosinophils and lymphocytes (Sehra, et al., 2008). A poor correlation was found between hyperplasia and increased TEWL in our murine model (data not shown) indicating the complex nature of barrier disruption.

It has been previously reported that *Flg* mRNA expression is significantly reduced in older (>4 months of age) STAT6VT transgenic mice compared to WT controls (Sehra, et al., 2008). Contrarily, our present studies found that the *Flg* levels

were increased, but these differences did not achieve statistical significance. This may be in part due to the fact that SLS treatment was started in 10- to 14-week old STAT6VT and WT mice. The SLS-induced irritation introduced at this age may have affected *Flg* gene expression. Others have shown an induction in profilaggrin mRNA expression in humans four days after exposure to 1% SLS; although there was an initial reduction six hours postexposure (Torma, et al., 2008). Further studies in keratinocytes overexpressing filaggrin have suggested an addition role for filaggrin. This group reported that filaggrin disrupted actin microfilament distribution, altered the cell shape, and disrupted the distribution of desmosome proteins that occurs at the granular to cornified cell transition that happens during terminal keratinocyte differentiation (Presland, Kuechle, Lewis, Fleckman, & Dale, 2001). Previous studies by another group show that filaggrin expression is increased in lesional skin from patients with AD without the 2282del4 *FLG* mutation (Howell, et al., 2009), which may account for the results seen in this study.

Our findings indicate that *Lor* gene expression is diminished in the STAT6VT transgenic mice (Kim, et al., 2008; Sehra, et al., 2008). Interestingly, the WT mice treated with SLS also have a marked decrease in *Lor* mRNA expression. However, these mice did not develop visual or biophysical (augemented TEWL and erythema) signs of dermatitis which further suggests that the downregulation of loricrin is not the only contributing factor of barrier function. Of interest, it has been reported that loricrin-deficient mice are prone to desquamation but not to increases in TEWL (Koch, et al., 2000).

The cytokine multiplex studies indicate a potential, although not significant, increase in T_H2 cytokines including IL-1ß and IL-5 in the STAT6VT vehicle and SLS

groups. As an established model of AD arising from T-cell specific expression of an active STAT6, our transgenic mice are predisposed to a T_H2 response. As a coordinator of eosinophilic-based inflammatory responses, an increase in IL-5 would provide a link between barrier disruption and allergic inflammation. Of interest, the T-regulatory (T-reg) cytokine IL-10 appears to be reduced in STAT6VT transgenic mice compared to similarly SLS-treated WT mice. Previous work has shown that the augmentation in IL-10, produced primarily by monocytes and partly by lymphocytes and having pleiotropic effects in immunoregulation and inflammation, may provide one explanation for the downregulation of T_H1 cytokine expression (Moore, de Waal Malefyt, Coffman, & O'Garra, 2001). Our studies also show that IFNγ was significantly upregulated in SLS-treated STAT6VT and WT mice compared to littermate controls. However, the mRNA expression results were not statistically significant possibly explained by previous studies showing that AD patients have higher levels of activation and apoptosis of IFNγ expressing T_H1 cells in circulation, skewing the immune response towards a T_H2 phenotype (Akkoc, et al., 2008). The lack of further significant findings in cytokine protein expression may partly be due to incompletely homogenizing the murine skin tissue.

Our findings further corroborate the complexity of the molecular mechanisms towards a T_H2 bias at play in atopic diseases. The STAT6VT transgenic mice spontaneously develop skin inflammation and have abnormalities in EDC genes as well as abnormal barrier function. As a result, these mice provide evidence that there is a link between allergic inflammation and permeability barrier homeostasis. The STAT6VT transgenic murine model is clinically relevant because it provides a platform involving

the complex interactions between susceptibility genes, pharmacological abnormalities and immunological abnormalities. Consequently, our murine model can be used in future studies such as the determination the point at which barrier function can no longer be repaired.

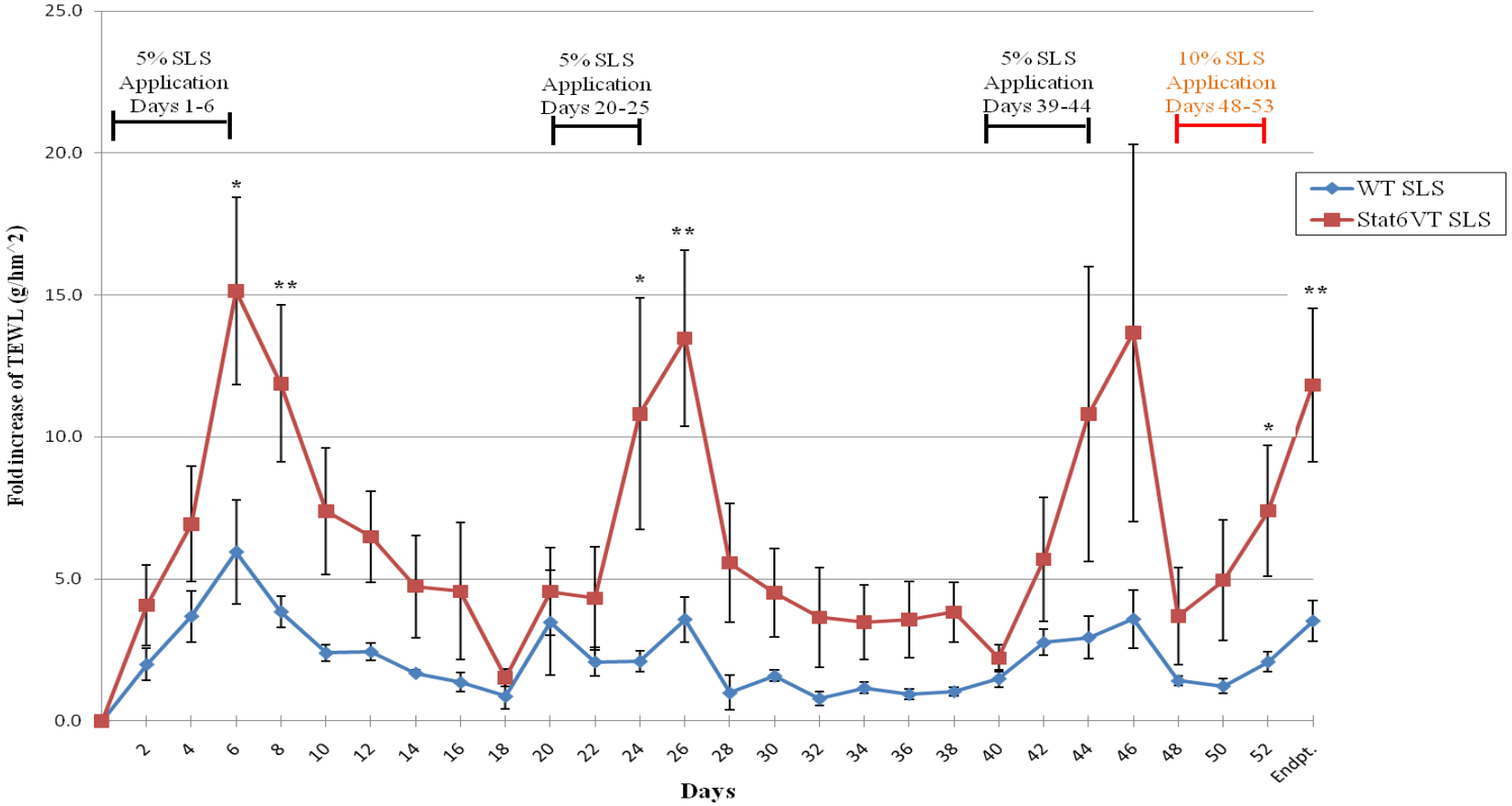
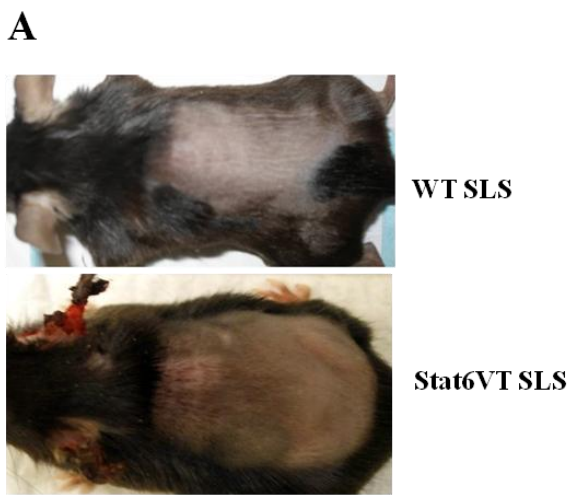
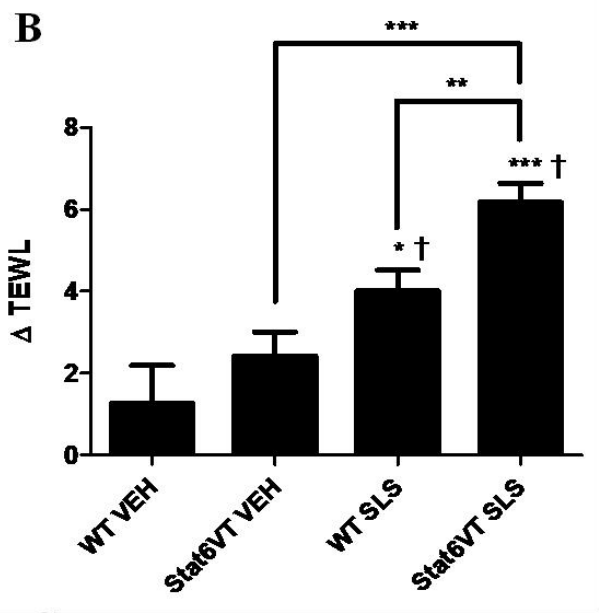


Fig. 1 TEWL is augmented in STAT6VT transgenic mice with repeated SLS irritation. STAT6VT transgenic and WT C57BL/6 sibling controls were treated for 3 six-day periods with 5% SLS followed by a single six-day period of 10% SLS. TEWL(g/hm^2) measurements were taken prior to the application of the topical irritant. The dates of topical irritation were: Days 1-6, 20-25, 39-44 and 48-53, as indicated. STAT6VT mice show a marked increase in TEWL (g/hm^2) during each of the application periods as compared to WT mice. Results are the mean ± SEM from samples of 5-8 mice. Results significantly different from WT, * p<0.05, ** p<0.01.





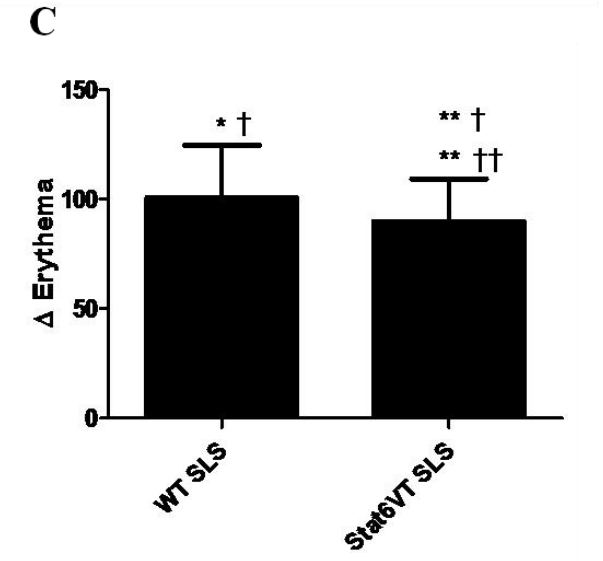


Fig. 2 Barrier disruption in WT versus STAT6VT transgenic mice following repeated SLS treatment. STAT6VT and littermate controls (10-14 weeks) were shaved and TEWL and erythema were measured with a Multi Probe Adapter fitted with either a Tewameter® TM 300 or Mexameter® MX18 (Courage-Khazaka, Germany) during the chronic treatment period. All measurements were taken prior to the application of the topical irritant. Results are the mean ± SEM from samples of 5-8 mice. *A,* Photographs of STAT6VT transgenic mice and littermate controls showing areas affected by skin inflammation. *B,* Barrier function is disrupted following chronic irritant treatment. *C, E*rythema is increased in STAT6VT and littermates following SLS treatment. In both *B* and *C,* results significantly different from WT, * $p<0.05$, ** $p<0.01$, † represents conditions compared to WT control, †† represents conditions compared to STAT6VT control.

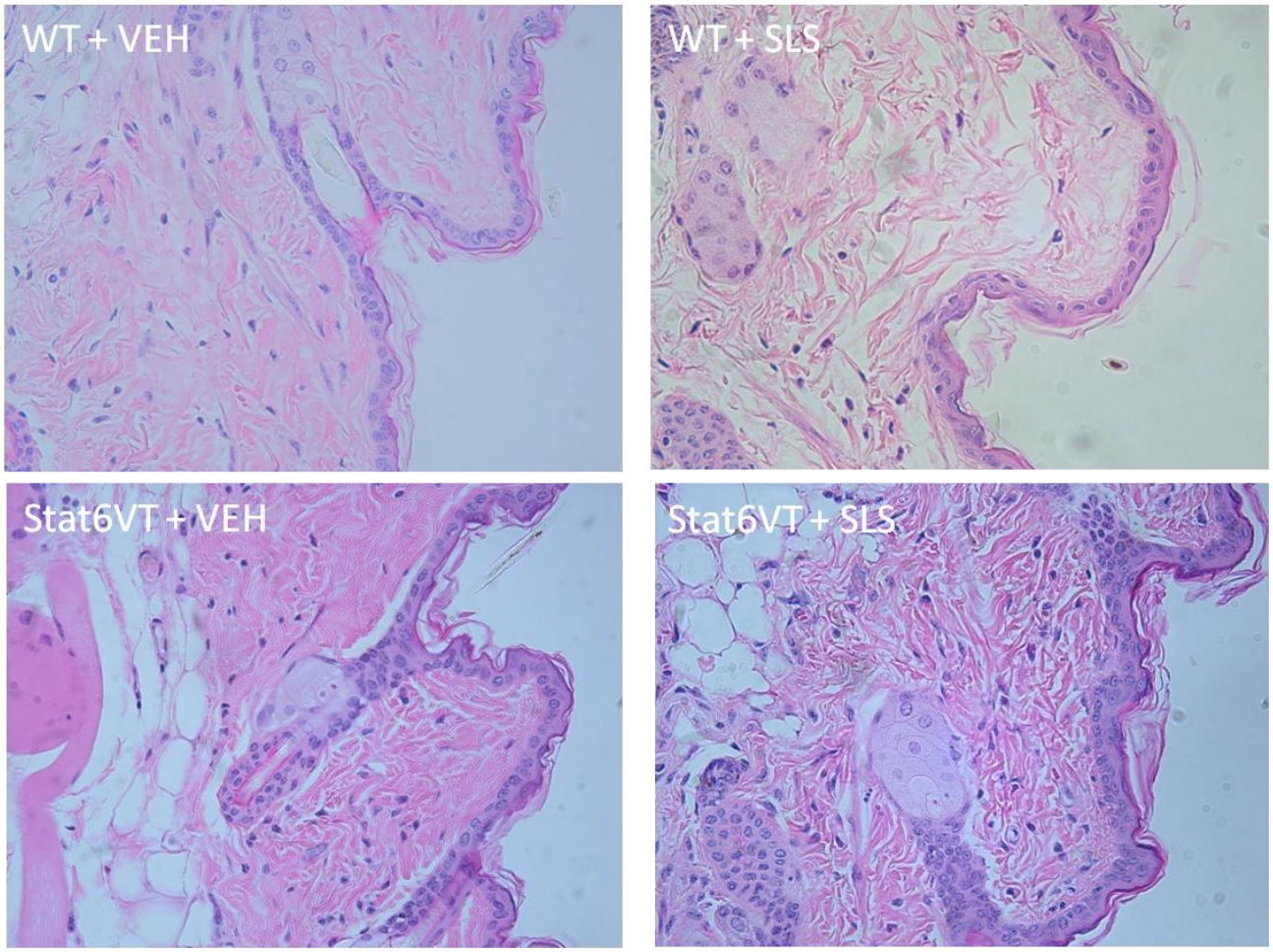


Fig. 3 STAT6VT mice exhibit enhanced inflammation in the shaved dorsal epidermis following repetitive SLS application. The dorsal epidermis of STAT6VT and littermate controls were chronically treated with vehicle or 5% SLS. A portion of the dorsal epidermis was fixed in 10% buffered formalin and H&E stained slides were produced. The images show inflammatory infiltration in STAT6VT mice, 400X.

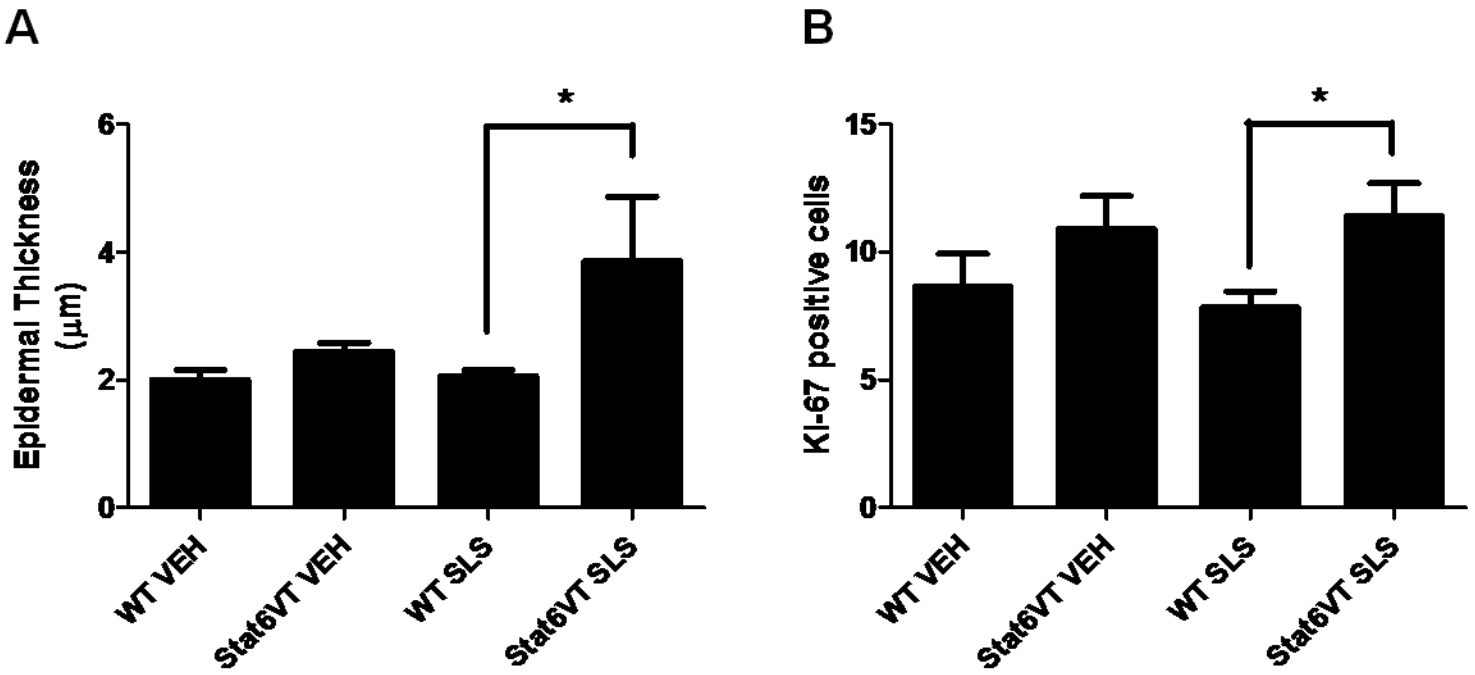


Fig. 4 STAT6VT transgenic mice feature augmented hyperplasia and active proliferation following repeated SLS treatment. The dorsal epidermis of STAT6VT and littermate controls were chronically treated with vehicle or 5% SLS. Results are the mean ± SEM from samples of 5-8 mice. *A*, STAT6VT transgenic mice have augmented epidermal thickness. A portion of the dorsal epidermis was excised, fixed in 10% buffered formalin, and paraffin-embedded. Epidermal thickness was then measured in H&E stained slides by light microscopy using a micrometer. *B*, Paraffin-embedded mouse skin excised were subjected to immunohistochemical analysis of Ki-67 as detailed in the methods sections. For each treatment group, Ki-67 positive cells were counted with MetaMorph Premium Offline software in 5 randomly chosen high power fields. In both *A* & *B*, slides were blinded prior to analysis, and results significantly different from WT, * $p<0.05$.

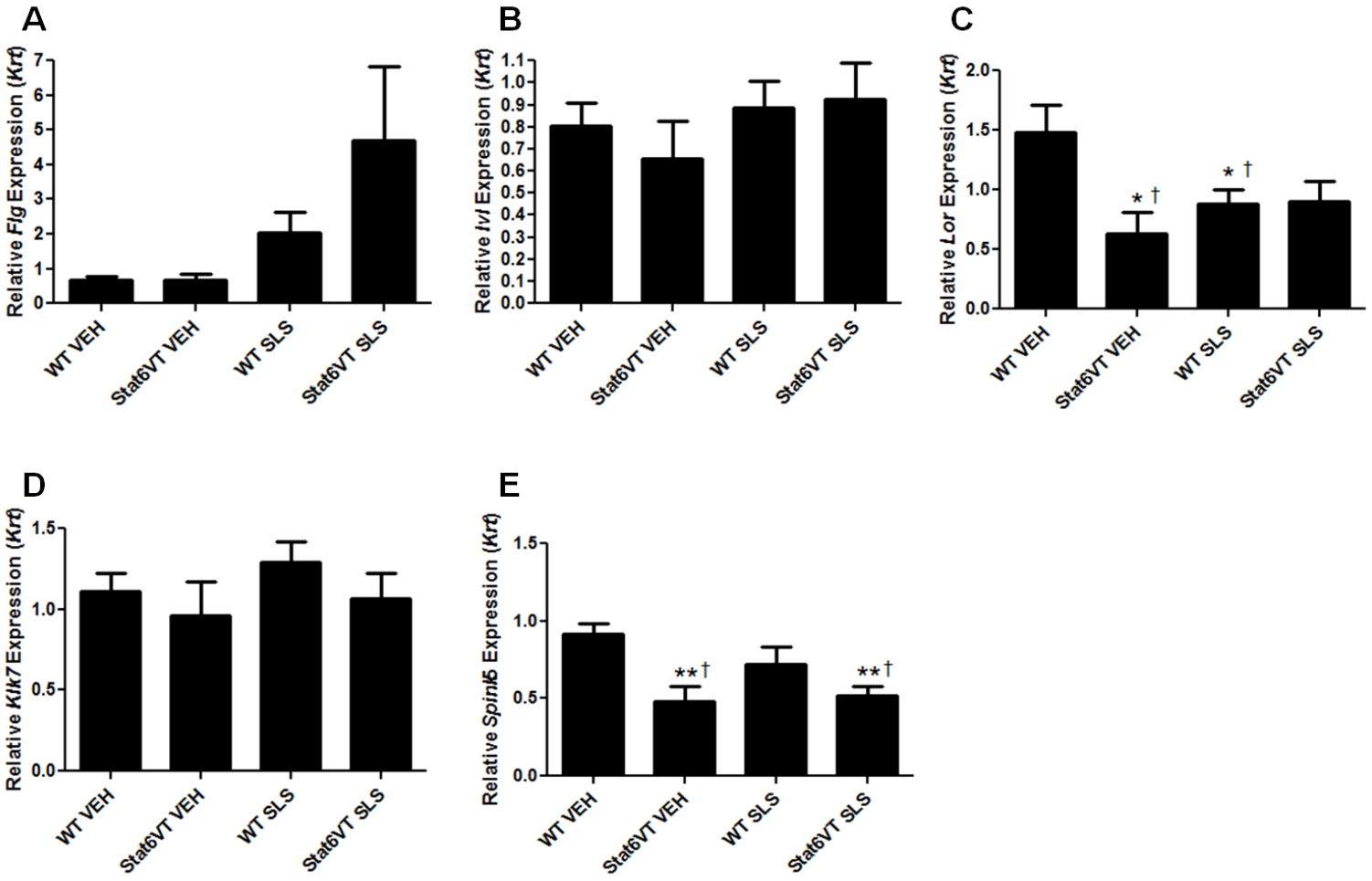


Fig. 5 Effect of SLS on EDC genes in WT versus STAT6VT transgenic mice. *A-E,* RNA was isolated from skin of WT and STAT6VT transgenic mice before analysis of EDC mRNA using qPCR. Each sample for qPCR was run in triplicate. Results are the mean ± SEM from samples of 5-8 mice. Bar graphs represent the relative expression normalized to expression of *Krt*14. Results significantly different from WT, * $p<0.05$; ** $p<0.01$, † represents conditions compared to WT vehicle control.

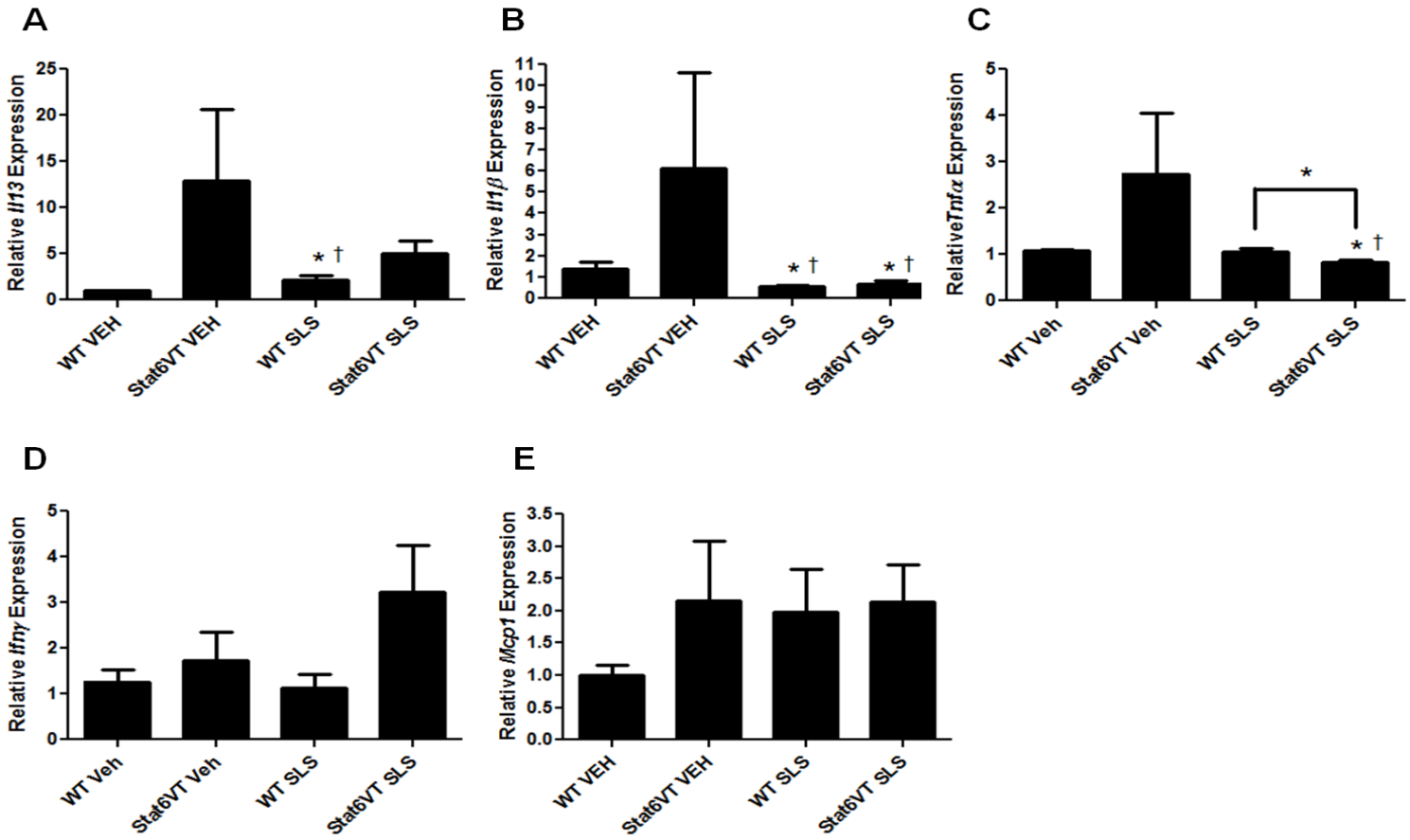


Fig. 6 Effect of SLS on inflammatory cytokine gene expression in WT versus STAT6VT transgenic mice. *A-E*, RNA was isolated from skin of WT and STAT6VT transgenic mice before analysis of inflammatory murine cytokine mRNA using qPCR. Each sample for qPCR was run in triplicate. Results are the mean ± SEM from samples of 4-7 mice. Bar graphs represent the relative expression normalized to expression of beta microglobulin. Results significantly different from WT, *$p<0.05$, † represents conditions compared to WT vehicle control.

Table 1 Inflammatory cytokine protein expression in STAT6VT transgenic mice treated with repeated SLS

	WT VEH	STAT6VT VEH	WT SLS	STAT6VT SLS
GM-CSF	1.230 ± 0.490	1.862 ± 0.604	1.508 ± 0.763	1.984 ± 1.155
IL-1ß	1.413 ± 0.533	1.764 ± 0.527	1.922 ± 0.800	2.699 ± 2.476
IL-2	0.292 ± 0.063	0.328 ± 0.104	0.409 ± 0.207	0.445 ± 0.155
IL-5	1.202	1.474 ± 0.522	1.389 ± 0.727	1.568 ± 0.394
IL-6	0.394 ± 0.240	0.413 ± 0.094	0.781 ± 0.630	1.272 ± 1.420
IL-10	ND	0.018	0.336 ± 0.356	0.264 ± 0.224
IL-13	0.318 ± 0.154	0.309 ± 0.103	0.498 ± 0.225	0.460 ± 0.179
IL-12	0.135 ± 0.064	0.165 ± 0.043	0.220 ± 0.100	0.289 ± 0.075
IFNγ	0.346 ± 0.157	0.526 ± 0.641	1.061 ± 0.430 *** †**	1.170 ± 0.681 *** †**
MCP-1	0.967 ± 0.276	1.497 ± 0.304	1.303 ± 0.487	1.525 ± 0.609

Cytokine analysis was done using the MILLIPLEX™ MAP system and total protein was normalized to BCA assay as described in the methods section. Results significantly different from WT, *$p<0.05$, † represents conditions compared to WT vehicle control.

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Werfel, T. (2009). The role of leukocytes, keratinocytes, and allergen-specific IgE in the development of atopic dermatitis. *J Invest Dermatol, 129*(8), 1878-1891.

Curriculum Vitae

Sonia Cristina DaSilva

Education

Master of Science in Biochemistry and Molecular Biology
Indiana University Indianapolis, IN November 2010

Graduate Certificate in Biotechnology
Indiana University Indianapolis, IN May 2009

Master of Arts in Administration & Supervision
St. Peters College Jersey City, NJ May 2001

Bachelor of Arts in Biological Sciences
Rutgers University New Brunswick, NJ May 1999

Work Experience

Laboratory Technician
Indiana University School of Medicine – Indianapolis, IN – Jan. 2008 to Nov. 2010

Teacher of Biological Sciences
Hunterdon Central Regional High School – Flemington, NJ – Sep. 2001 to June 2007

Life Science Teacher
Christa McAuliffe Middle School – Elizabeth, NJ – Sep. 1999 to June 2001

Lab Experience and Job Responsibilities

Research Experience

Thesis Research: Biochemistry Department, Indiana University School of Medicine, 2009-2010 (research advisor: Dr. Jeffrey Travers)

- Characterization of a murine model for the study of atopic dermatitis through biophysical measurement of skin barrier function
- Performed analysis of gene expression of cytokines and epidermal differentiation markers by carrying out total RNA isolation, cDNA synthesis and quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Laboratory Technician: Pathology and Laboratory Medicine Department, Indiana University School of Medicine, 2008-2010 (Supervisor: Dr. Raymond Konger)

- Maintenance of multiple murine lines involving breeding, weaning and genotyping
- Maintenance of a human skin cell line, including conducting transfection studies to determine the effect of various agonists and antagonists of the peroxisome proliferator activator receptor gamma (PPARγ)
- Preparation of research findings in a scientific seminar format

- Supervision of research students including instruction in laboratory techniques relevant to each respective study
- Facilitated collaboration with academic institutions, vendors and consultants as needed to complete investigative studies

Teaching Experience

- Designed and implemented presentations centered on state mandated learning objectives for daily 80-minute lessons to develop students that are informed citizens in science
- Continuously updated lessons to account for modifications and breakthroughs in all courses taught including *Honors*, *College Preparatory* and *Laboratory Biology* and *Honors Advanced Topics in Genetics*
- Supervised and coordinated multiple, simultaneous independent student scientific research projects
- Instructed and revised student development of scientific research paper paper following APA guidelines
- Created an environment for students to foster appreciation of biology through cooperative learning, manipulatives and inquiry based lessons
- Integrated technology into the classroom to emphasize and develop specific skill areas
- Instructed students in the conducting bacterial transformation of green fluorescent protein (GFP) from *Aequorea victoria* to *Escherichia coli*
- Designed and implemented student centered experiments using wildtype and UV-sensitive *S. cerevisiae* to study the effectiveness of various UV protection products. Provoked student interest in scientific inquiry through implementation of similar experiments in classroom
- Directed multiple student studies simultaneously, including characterization of mutations induced by alcohol and cigarette smoking on *Drosophila melanogaster*

Curriculum Development

- Developed and implemented *Honors Advanced Topics in Genetics* course at a nationally recognized *Blue Ribbon high school*
- Collaborated with local community college to organize student participation in the *Annual Life Science Career Awareness Day* where students experienced a “day in the life of a scientist” at leading pharmaceutical and biotechnology companies in the New Jersey area

Technology

- Statistical analysis tools including GraphPad Prism 4.0 and 5.0 and Microsoft Excel
- Internet databases including Protein Data Bank, BLAST design and primer design
- Utilization of internet resources to supplement presentations and instructional lectures such as streaming videos and sound files

Professional Strengths and Responsibilities

- Experience in the field of *in vivo* toxicology and *in vitro* toxicology
- High degree of professionalism, scientific rigor and understanding with consistent attention to detail
- Ability to handle multiple projects and meet deadlines
- Highly motivated team player with well developed communication skills and strong scientific writing
- Fluent in Spanish and Portuguese

Activities and Volunteering

- Instructor – *Mad Science INC* – Indianapolis, IN – 2007 to Present
- Volunteer – *Headcount voter registration* – Indianapolis, IN – 2007 to Present
- Ski Club Advisor – *Hunterdon Central Regional High School* – Flemington, NJ – 2005 to 2007
- Class Council Advisor – *Hunterdon Central Regional High School* – Flemington, NJ – 2001 to 2007
- Translator – *Medical Reserve Corp.* – Hunterdon County, NJ – 2005 to 2007
- Volunteer – *Leos Portuguese Youth Club* – Elizabeth, NJ – 2001
- Ecology Conservation Director – *Boy Scouts of America* – Forestburg, NY – 1999

Professional Training and Seminars

- Princeton University satellite center – Molecular Biology Outreach Program – Morris, NJ
    - Studied the functions of restriction enzymes and their use as a molecular biology tool by working with *Eco*RI, *Pst*I, and *Hin*dIII to digest bacteriophage lambda DNA
    - Performed PCR analysis of mitochondrial DNA isolated from human cheek cells. Used the resulting mtDNA polymorphisms to determine lineage by comparing ancestral relationships of modern populations
- Tri-State Biotechnology Educator's Day – Philadelphia, PA
- Earthwatch Expedition – Wolves and Moose expedition to Isle Royale, MI
- Choosen by Ortho-McNeil Pharmaceutical, INC to attend intensive, week-long Keys to Science Institute for Molecular Biology – Colorado Springs, CO

Memberships and Certificates

Society for Investigative Dermatology – Member
New Jersey Certificate – Teacher of Biological Sciences
New Jersey Provisional Certificate – Educational Leadership Administration and Supervision

Publications and Poster Sessions

S.C. DaSilva, et al., (2010) "Abnormal barrier function and allergic skin inflammation in mice over expressing Th2 cells via a constitutively active STAT6 gene". *The Society for Investigative Dermatology 2010 Annual Meeting,* Atlanta, Georgia, May 5-8, 2010.

Sahu, R.P., **DaSilva, S.** and Konger, R.L. (2010) "Loss of epidermal PPARγ in SKH-1 mice resulting in absent sebaceous glands, a defect in permeability barrier function, and augmented UVB-induced apoptosis and inflammatory responses". *The Society for Investigative Dermatology 2010 Annual Meeting,* Atlanta, Georgia, May 5-8, 2010.

Konger, R.L., A Kozman, R.P. Sahu, **S. DaSilva** and J.B. Travers. PAF receptor knockout mice exhibit increased chemical carcinogenesis and an increase inchronic PMA-induced inflammation. J. Invest. Dermatol. 130:S31, 2010.

Konger, R.L., Billings, S.D., Prall, N.C., Katona, T.M., **DaSilva, S.C.,** Kennedy, C.R.J., Badve, S., Perkins, S.M. and Lacelle, P.T. (2009) "The EP(1) subtype of prostaglandin E(2) receptor: Role in keratinocyte differentiation and expression in non-melanoma skin cancer". *Prostaglandins, Leukotrienes and Essential Fatty Acids* PMID 19625175