Department of Dermatology

Emory University School of Medicine
WMB 5001
1639 Pierce Drive
Atlanta, GA 30322

Robert A. Swerlick, MD
Associate Professor
Tel 404-727-2893
Fax 404-727-5108
Email rswerli@emory.edu

Stephanie Shack
NIOSH Office of Extramural Programs

Re: FINAL REPORT - R21 OH07340-01

Dear Ms. Shack,

Attached is the final scientific report for the R21 exploratory grant entitled "Activation of skin cells and transcription factors by skin irritants." Our business office is compiling the final financial report and I plan to have it to your office immediately after the start of next month. The findings associated with this grant may be of substantial commercial value and a patent application is being filed. For that reason, the information in the scientific report is marked confidential and should not be released to the public. The final patent application should be filed early in 2003 and will allow the information to be released by Spring of 2003. I will forward a hard copy with the final financial report the first week of January.

Sincerely

Robert A. Swerlick, MD

ACTIVATION OF SKIN CELLS AND TRANSCRIPTION FACTORS BY SKIN IRRITANTS – FINAL REPORT - R21 OH07340-01

Department of Dermatology
Emory University School of Medicine
WMB 5014
1639 Pierce Drive
Atlanta, GA 30322

Principal Investigator: Robert A. Swerlick, MD
Final Report
Grant number R21 OH07340-01
December 19, 2002

Table of Contents:

List of Abbreviations:

EMSA – electrophoretic mobility shift assay
HDMEC – human dermal microvascular endothelial cells
HDF – human dermal fibroblasts
HK – human keratinocytes
IL-6 – Interleukin 6
IL-8 - Interleukin 8
MAP kinase – mitogen activated protein
MEK1 – Mitogen activated protein kinase kinase
mRNA – messenger RNA
NF-kB – nuclear factor kappa B
SLS – sodium lauryl sulfate
TNFα - tumor necrosis factor alpha
VEGF – vascular endothelial growth factor

Figures:

Tables: None

Abstract:

Irritant dermatitis is a source of significant workplace injury and morbidity. Our understanding of how irritants interact with the skin is minimal and expansion of our knowledge base regarding mechanisms mediating irritant action will provide us with a rationale basis of new approaches to prevention and treatment. The hypothesis proposed in this exploratory grant was that irritants affect the function or expression of transcription factors linked to the activity of pro-inflammatory genes. Activation or induced expression of pro-inflammatory genes by irritants results in the clinical manifestations of inflammation in the tissues exposed to irritants. The studies done in the proposal demonstrated that a common model detergent irritant, sodium lauryl sulfate, induces the expression of vascular endothelial growth factor (vascular permeability factor; VEGF/VPF) in a keratinocytes cell line in vitro. This induction appears to be mediated via induction of early growth factor 1 (egr-1), a transcription factor linked to cellular stress and injury. The induction of egr-1 was mediated via activation of the MAP kinase MEK1, as evidenced by inhibition of both egr-1 and VEGF induction by the specific MEK1 inhibitor PD98059. These data are the first description of a molecular mechanism linking the edematous and erythematous response to detergent exposure to induction of agents which induce increases in vascular permeability and other elements of the inflammatory response. These data provide a rational framework for the development of large scale in vitro screening assays to assess irritancy of compounds. They also provide novel approaches for the prevention and treatment of irritant dermatitis.

Significant Findings:

Despite years of observation clearly linking certain agents such as detergents to development of inflammation in the skin, no clear molecular link has been identified. These studies demonstrate for the first time that exposure of skin epithelial cells to dilute concentrations of a model detergent irritant induce the expression of a protein (egr-1) that has been directly linked to the production and secretion of VEGF, a mediator which can produce most if not all of the cardinal features of the inflammatory response observed clinically in patients with dermatitis. Furthermore, we have begun to characterize the pathway by which detergents may induce expression of VEGF and defined inhibitors of this pathway that may be useful in treating or preventing the development of irritant dermatitis.

Usefulness of Findings:

These findings are useful for three reasons:

1) They provide us with novel insights into why individuals develop skin inflammation after exposure to certain chemicals or substances
2) They provide us with new targets to treat or prevent irritant dermatitis
3) They provide us with possible approaches to screen compounds for potential irritancy using non-animal based systems.

Scientific report:

Irritant dermatitis is a major source of morbidity in the workplace (1-3) (4). Occupational contact dermatitis constitutes 90-95% of all occupational skin disease. Of that, about 80% is irritant contact dermatitis and the remaining 20% allergic contact. In 1993, 12% of >480,000 occupational illnesses reported were skin related. It is estimated that this represents only 2-10% of actual incidents. The effects of skin injury are not trivial since 21% of the reported cases resulted in days off from work. Estimates as to costs incurred may be $1 billion or more.

The pathophysiologic mechanisms of irritant dermatitis have been the focus of study, mostly using in vivo models of skin irritants (5-10). Application of irritants to human or non-human skin can result in the development of all the cardinal features of inflammation. Not surprisingly, this irritation is associated with the expression of multiple different pro-inflammatory cytokines and expression of adhesion proteins that regulate leukocyte trafficking. Leukocyte infiltration of the skin follows as a consequence of activation of these two families of genes. The events linking exposure of the skin to the irritant with the expression of pro-inflammatory cytokines and adhesion proteins have not been clearly defined. Our hypothesis was that certain agents that irritate the skin do so by directly or indirectly activate critical regulators of cytokine gene expression and adhesion molecules in much the same manner as infectious agents in the environment trigger activity of innate immunity. Irritants may act upon or bypass cell surface receptors, but proximal events initiated in signaling pathways involve transcription factor activation that results in cytokine gene expression.

On particular target for irritant effect is the nuclear factor kappa B (NF-kB) family of transcription factors. These proteins were felt to be possible targets for irritant effect since they are linked to the activation of a number of pro-inflammatory genes in the skin and exist preformed in an inactive state in virtually all cells. Furthermore, previous studies have implicated NF-kB activation as the target of certain irritants such as caustic metal salts (11). Base upon this hypothesis, the original aims of this proposal were:

1) To examine whether irritants can activate NF-kB cultured skin cells in vitro using epithelial and endothelial cells as targets.
   a) To examine whether irritant treatment of cultured skin cells results in translocation of NF-kB complexes from the cytoplasm to the nucleus
   b) To examine whether irritant treatment of HDMEC, HK, or HDF induces the phosphorylation and ubiquitination of IkBα, necessary steps for proteosome-mediated degradation
   c) To examine whether translocated NF-kB complexes are capable of binding to relevant response elements of cytokine and cell adhesion molecule gene promoters

2) To examine whether irritants can activate specific genes cultured skin cells in vitro using epithelial and endothelial cells as targets.
   a) To examine whether irritants induce or upregulate the expression of adhesion molecules ICAM-1 and E-selectin, and pro-inflammatory cytokines IL-8 and VEGF-C
   b) To examine whether irritants increase steady state mRNA expression of ICAM-1, E-selectin, IL-8, or VEGF-C

c) To examine whether irritant treatment of cultured skin cells results in increase transcription of adhesion molecule or cytokine genes dependent upon NF-kB responsive elements

Work Accomplished:

The work of the proposal involved three basic phases. In the first phase, we examined the effects of various irritants on transformed human epithelial cell lines (HACAT and A431 cells). The purpose of these studies was to define doses and exposure times of specific irritants that were relevant and not associated with profound cell death. The second phase of the study was to identify activation of specific genes that were induced after exposure to the irritants. The third phase was to identify the signal transduction pathways and transcription factors that were activated after irritant exposure.

Viability Studies

Our initial approach to exposure to irritants was to use continuous exposure of cells to dilute concentrations of specific irritants. As irritants we used metal salts (cobalt and nickel), surfactants (SLS), and pH. We were unable to develop a robust model examining the effect of pH since we were not able to develop an appropriate buffer system where we could be certain of a stable pH. We were able to develop interesting data examining the effects of metal salts, which will be outlined below. However, the most interesting data was generated in a model examining the effect of detergents (SLS). We believe this information is novel and has tremendous biological relevance given the role that detergents play as irritants in the workplace. Given the minimal amount of information that is already know about how irritants such as surfactants and soaps cause irritation of the skin, we focused on this area.

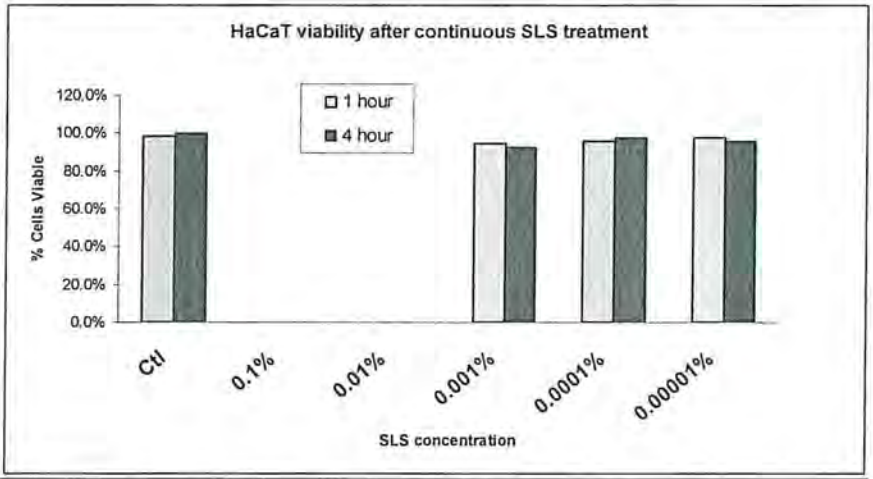


Figure 1: Dilute concentrations of SLS are do not cause cell death in HaCaT cells. HaCaT keratinocytes were treated with various concentrations of SLS (0.01% to 0.001%) for 1 to 24 hours, harvested and viability was assessed by trypan blue staining.

We first constructed dose and time studies to identify the optimal parameters for future studies using SLS. We were relatively certain that if we exposed cells to sufficient concentrations of SLS, cells would produce pro-inflammatory cytokines. However, if those concentrations induced cell death, the biological relevance of cytokine production in that context would be minimal. Therefore, we identified maximal concentrations under conditions of continuous exposure that were associated with minimal cell death using the HACAT cell line (figure 1). Exposure of HaCaT cells to SLS at concentrations of 0.001% or

lower was associated with excellent cell viability and subsequent studies were done with the concentration of 0.001%.

The original hypothesis put forth in the grant proposal was that key elements that may be important in irritant dermatitis were members of the NF-kB family of transcription factors. The rationale for this assumption was based upon a large literature linking NF-kB activation to a host of pro-inflammatory genes. We specifically examined the expression of genes linked to NF-kB activation, IL-8, IL-6, TNFα and VEGF-C mRNA after treatment of HACAT cells with continuous exposure to dilute concentrations of SLS. These genes were selected because they have been shown to be regulated by NF-kB and are expressed in skin inflammation. However, we did not consistently detect increases in the expression of these mediators after treatment with SLS as described above.

We then reassessed our model and considered changes in the nature of the irritants we used, the methods of exposure, and the genes that we examined. Since the skin is generally exposed to irritants in an episodic manner followed by removal of the irritant, we decided to modify our model to reflect this type of exposure. To this end, we repeated viability studies exposing HACAT cells to higher concentrations of SLS for shorter periods of time followed a washout and a one to four hour recovery time. Using this approach, we determined that HaCaT cells tolerated substantially higher concentrations without significant loss of viability. The optimal dose and time of exposure was 0.01% SLS for five minutes followed by assays as one and four hours (figure 2). Greater than 80% of cells treated in such a fashion remained viable.

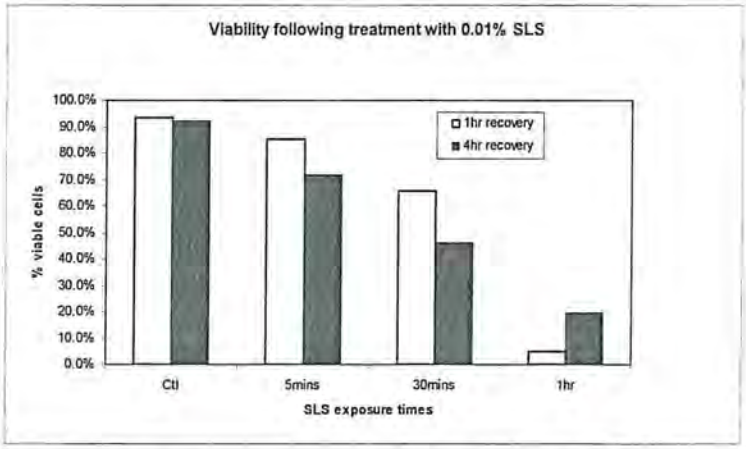


Figure 2: Short exposure of HaCaT cells to higher concentrations of SLS (0.01%) results in minimal cell toxicity. HaCaT cells were treated with 0.1% SLS for 5, 30, and 60 minutes and cell viability was assessed after one and four hours by trypan blue exclusion.

We then applied the modified exposure model to assess which genes were activated after SLS exposure. Our studies were still based upon the assumption that the key transcription factor family that would be activated was NF-kB and selected the genes to be examined based upon that assumption. Again, we found only minimal and inconsistent induction of TNFα, IL-8, and VEGF-C after treatment of HACAT cells with SLS. Using cytokines such as TNFα and other potential irritants such as cobalt chloride or nickel chloride, we were able to demonstrate that HACAT cells were capable of activation of NF-kB. From these data we concluded that SLS did not induce NF-kB activation in our model.

As an alternative, we examined whether other candidate genes induced via transcription factors other than NF-kB were activated by SLS treatment. We had previously examined the expression of VEGF-C, precisely because its expression had been associated with stimuli that activated NF-kB. Since NF-kB activation was not a characteristic of cells treated with SLS, we examined whether SLS induced other genes in the VEGF family of growth and permeability factors via a non-NF-kB mediated mechanism. Since VEGF-A (VEGF) has been associated with

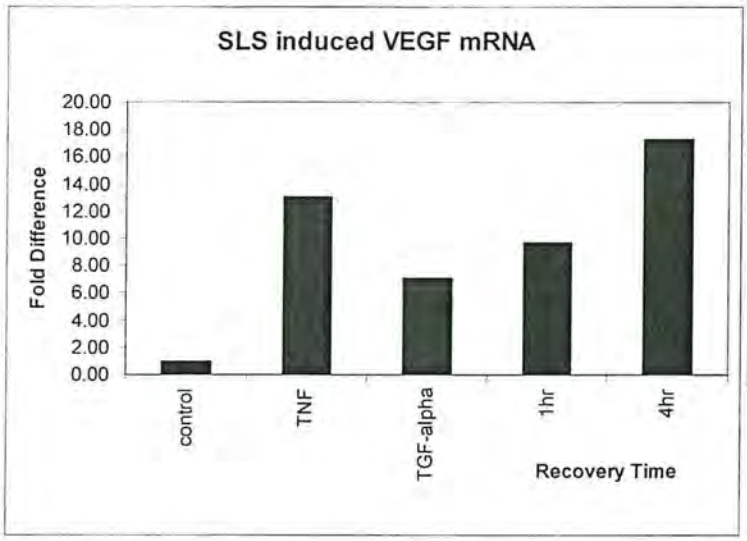
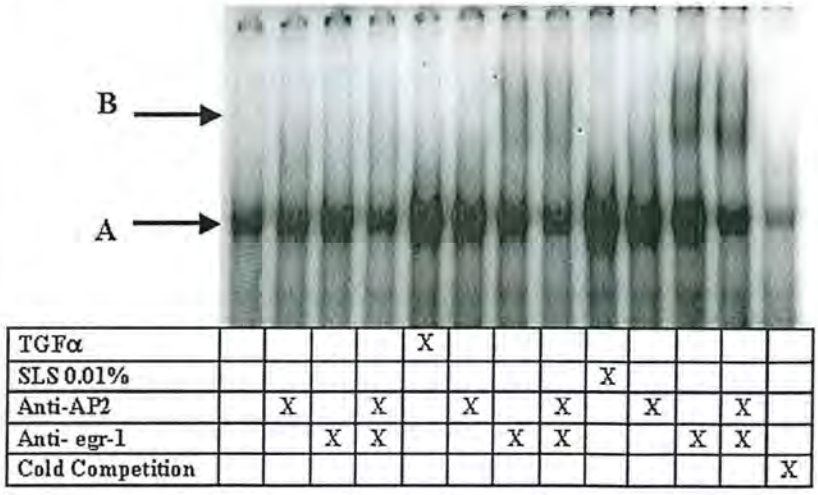


Figure 3: Short exposures of HaCaT cells to SLS (0.01%) induce VEGF mRNA expression. HaCaT cells were treated with TNFα (500 U/ml x 4 hours), TGFα (100 ng/ml x 4 hours), or with SLS (0.01%) for five minutes. The cells treated with SLS were then washed and allowed to recover for one or four hours. VEGF mRNA was measured by real time quantitative PCR.

a host of skin inflammatory disorders including irritant dermatitis, we examined whether SLS treatment induced VEGF mRNA expression. Treatment of HACAT cells with 0.01% SLS for 5 minutes resulted in induction of VEGF mRNA in a time dependent fashion. Induction of VEGF mRNA was modest (5-30 fold), but roughly equivalent to the level of induction seen after treatment with potent inducers of VEGF, TGFα or TNFα (Figure 3). The induction of VEGF mRNA was rapid but transient. After brief treatment with SLS (five minutes), expression of VEGF mRNA was maximal after one hour and substantially decreased after 4 hours. These data suggested that detergent irritants might mediate disease by induction of VEGF, whose expression is associated with vascular leak (edema) and angiogenesis (erythema).

Figure 4: SLS treatment of HaCaT cells results in egr-1 binding to VEGF promoter oligonucleotides. HaCaT cells were treated with TGFα (100 U/ml x 1 hour) or SLS (0.01%) for five minutes followed by one-hour recovery. Nuclear extracts were isolated and assessed for DNA binding to a VEGF based promoter oligonucleotide by EMSA.



In order to define the mechanisms involved in VEGF induction by SLS, we examined the promoter of the VEGF gene for regions that may confer SLS responsiveness. Previous studies in our laboratory have defined the TGFα response element within the VEGF promoter. This region may bind one of two transcription factors, AP-2 or egr-1. We proposed that if SLS induced VEGF gene transcription via activation via this portion of the VEGF promoter, we could further define this using oligonucleotides from this region to use in electrophoretic mobility shift assays (EMSA) (Figure 4). Treatment of HaCaT with TGFα resulted in complex formation, which was primarily shifted with antibody to egr-1, consistent with previous studies linking TGFα signaling

to egr-1 induction and VEGF gene transcription. In addition, treatment of HaCaT cells with SLS resulted in almost identical complex formation. The SLS induced complex was also shifted primarily with anti-egr-1 antibody. These data provided evidence that VEGF induction by SLS was mediated via induction of egr-1.

Egr-1 in a transcription factor that is not constitutively expressed, but may be induced in response to a variety of stresses, cytokines, and growth factors (12). Egr-1 expression may be linked to a number of pro-inflammatory genes, including TNFα, VEGF and tissue factor. The data presented above clearly suggested that SLS might induce dermatitis via induction of egr-1, leading to VEGF expression, erythema, and capillary leak. To further support this hypothesis, we examined whether SLS treatment resulted in activation of the egr-1 gene. HaCaT cells were treated briefly with SLS and cells were harvested after 1 and 4 hours. SLS treatment resulted in marked upregulation of egr-1 mRNA, which like VEGF induction, was transient, maximal after one hour (Figure 5).

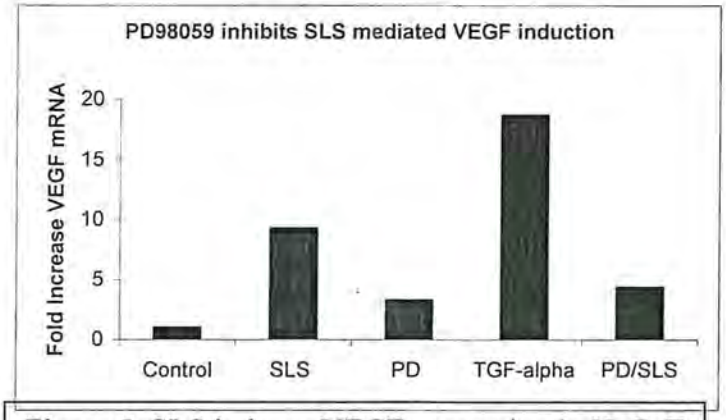


Figure 5: SLS induces VEGF expression in HaCaT cells via an MEK1 dependent mechanism. HaCaT cells were treated with SLS (0.01%) for five minutes, with or without PD98059 (50 uM) pretreatment. VEGF mRNA was assayed by real time quantitative PCR.

These data provided an additional defining the mechanism between SLS exposure and VEGF induction. Activation of egr-1 expression in endothelial and epithelial cells has been associated with a variety of stimuli which traffic through pathways, which involve the MAP kinase MEK1 that activates p42/44 by phosphorylation. If SLS induced egr-1 expression via a MEK1-dependent pathway, agents that block this pathway should inhibit induction of egr-1 and VEGF. In order to test this hypothesis, we pretreated HaCaT cells with PD98059, a specific inhibitor of MEK1 prior to SLS treatment. Pretreatment with PD98059 inhibited egr-1 induction (Figure 6) and induction VEGF mRNA by SLS (Figure 5). In contrast, pretreatment with the proteasome inhibitor MG-132 did not inhibit egr-1 or VEGF induction (data not shown). These data suggest that activation of MEK1 is an essential step in the induction of VEGF by SLS.

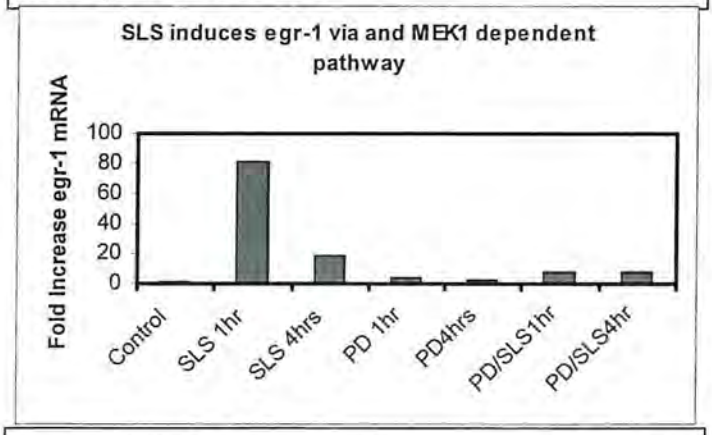


Figure 6: SLS induces egr-1 expression in HaCaT cells via an MEK1 dependent mechanism. HaCaT cells were treated with SLS (0.01%) for five minutes, with or without PD98059 (50 uM) pretreatment. Egr-1 mRNA was assayed by real time quantitative PCR.

Similar studies were also undertaken using metal salts including nickel chloride and cobalt chloride. Both of these agents induced expression of both VEGF and IL-8 in a dose-dependent fashion at non-toxic concentrations (data not shown). These data are consistent with previous studies demonstrating metal salts induce activation of NF-kB. In addition, our studies also suggest that these agents induce expression of egr-1. These findings are consistent with clinical observations that metal salts are strong irritants, potentially because they may activate multiple pro-inflammatory pathways. Detergents such as SLS are weaker irritants, perhaps because they primarily induce inflammation via induction of the egr-1 pathway.

Discussion:

Irritant injury to the skin is a major public health issue. Our ability to predict, prevent, and treat irritant dermatitis is limited by an only partial understanding of how agents actually act as irritants. The clinical presentation of irritant dermatitis can be reproduced using irritants under controlled conditions either in humans or animal models. However, these methods are cumbersome and do not lend themselves to the development of predictive models or to studies to define molecule mechanisms driving irritant dermatitis. In order to get away from expensive and controversial animals models used to screen irritants, attempts have been made to develop in vitro models using skin equivalents. However, these models are also expensive and readouts used have not been based on defined molecular events, which are characteristic of authentic irritant dermatitis.

Our observations are important because they link perhaps the most common class of irritants in the workplace, detergents, to the activation of a specific class of transcription factors, early growth response genes, and identify links to genes whose activation results in the cardinal signs of inflammation which characterize irritant dermatitis. Furthermore, our data suggest that egr-1 induction may be a characteristic of exposure of skin cells to a wide range of common irritants. This observation can serve as the framework to develop rapid in vitro screening assays which can use immortalized cell lines growth under common tissue culture conditions instead of expensive skin equivalents. A patent application is pending on this concept.

The identification of the importance of a pathway induced by irritants resulting in VEGF induction via induction of egr-1 through MEK1 also provides us with novel targets for treatment and prevention of irritant dermatitis. Agents that specifically block MEK1 activity may be useful in the treatment of irritant dermatitis. Furthermore, additional studies to define how detergents activate MEK1 in skin will also provide additional targets for the treatment and prevention of irritant dermatitis.

Methods:

Cell Culture: HaCaT cells were obtained from Norbert E. Fusenig (Division of Carcinogenesis and Differentiation, German Cancer Research Center (Deutsches Krebsforschungszentrum), Heidelberg, Germany) and were cultured in DMEM with L-glutamine, penicillin/streptomycin/antimycotic and 10-20% FBS.

Measurement of mRNA expression by real time quantitative PCR: Total RNA was isolated with TriReagent (Sigma) from HaCaT cells according to the manufacturer's instructions. To ensure that no contaminating genomic DNA was present, all samples were incubated for 1hr at room temperature with Rnase-free DNAse followed by heat inactivation of the enzyme for 30min at 75-80°C. cDNA will be prepared from 2ug of total RNA using the Superscript II Preamplification System for 1st Strand cDNA Synthesis (GIBCO/BRL, Carlsbad, CA) using the random primer protocol. All PCR primers were synthesized by the Microchemical Facility of the Winship Cancer Center of the Emory University School of Medicine. Real-time PCR was performed using SYBR green technology with the Applied Biosystems 5700 Gene-Amp 5700 Sequence Detection System. The intensity of fluorescence, a direct measure of the amount of product amplified was measured with each cycle. The threshold at which significant amplification is first detected will be determined, and all samples will be evaluated by determining how quickly each reaches this threshold. The cycle at which this threshold is achieved is recorded as the C_T value. Relative quantities will be determined by generating a standard curve from dilutions of cDNA containing the sequence of interest and correlating each dilution to C_T value. Data will be normalized with primers for a housekeeping gene and expressed as fold increase over an unstimulated control according to the manufacturer's instructions (Applied Biosystems Relative Quantitation of Gene Expression: ABI Prism 7700 Sequence Dection System: User Bulletin #2: Rev B (http://docs.appliedbiosystems.com/pebiodocs/04303859.pdf), December 11, 1997). Amplification controls include non-reverse transcribed RNA to ensure there is no contaminating genomic DNA, and a sample with no template to ensure no primer-dimer is present. A dissociation curve will be performed following each experiment to verify that a single product is present and that this product had the expected T_M. In addition, each product will be cloned into the TOPO TA cloning vector (Invitrogen) and sequenced.

Electophoretic mobility shift assay (EMSA): EMSA using nuclear extracts from cytokine stimulated HDMEC, HK, or HDF will be done as described previously (37,40,41). Radiolabeled double-stranded DNA will be synthesized by annealing an oligonucleotide complementary to the 3' end of the sequence and by extending the second strand with Klenow fragment, 50 μCi of [α-^{32}P] dCTP (Amersham), unlabeled dATP, dGTP, and dTTP (Pharmacia Biotech, Piscataway, NJ). All oligonucleotides will be synthesized by the Emory University Microchemistry Facility. To determine if tyrosine phosphorylation is required for complexes to be activated and to bind to oligonucleotides, lysates will be treated with specific tyrosine phosphatase (PTPase) and PTPase activity will be inhibited by the addition of sodium orthovanadate. For UV crosslinking/SDS-PAGE analysis, probes and competitor oligonucleotides will be prepared as for EMSA, but substituting 3 mM bromodeoxyuridine (BrdU, Sigma Chemical Co.) for dTTP. Binding reactions will be performed as described above. The samples will be exposed to UV irradiation in a Stratalinker (240 nm, 15 min, on ice). The binding reactions will be electrophoresed on 10% SDS-PAGE.

Publications:

1) Abstract submitted: Society of Investigative Dermatology 2003 Meetings

TITLE:
Sodium lauryl sulfate induces VEGF expression in immortalized keratinocytes via an egr-1, MEK1-dependent mechanism

AUTHORS (ALL): O'Reilly, F M.[1, 2]; Casper, K A.[1]; Newton-West, M[1]; Swerlick, R A.[1, 2]

1. Dickel, H., O. Kuss, A. Schmidt, J. Kretz, and T. L. Diepgen. 2002. Importance of irritant contact dermatitis in occupational skin disease. *Am J Clin Dermatol 3:283.*
2. Dickel, H., T. Bruckner, C. Bernhard-Klimt, T. Koch, R. Scheidt, and T. L. Diepgen. 2002. Surveillance scheme for occupational skin disease in the Saarland, FRG. First report from BKH-S. *Contact Dermatitis 46:197.*
3. Meyer, J. D., Y. Chen, D. L. Holt, M. H. Beck, and N. M. Cherry. 2000. Occupational contact dermatitis in the UK: a surveillance report from EPIDERM and OPRA. *Occup Med (Lond) 50:265.*
4. Elsner, P. 1994. Irritant dermatitis in the workplace. *Dermatol Clin 12:461.*
5. Lee, C. H., and H. I. Maibach. 1995. The sodium lauryl sulfate model: an overview. *Contact Dermatitis 33:1.*
6. Lee, C. H., and H. I. Maibach. 1994. Study of cumulative irritant contact dermatitis in man utilizing open application on subclinically irritated skin. *Contact Dermatitis 30:271.*
7. Lee, C. H., Y. Kawasaki, and H. I. Maibach. 1994. Effect of surfactant mixtures on irritant contact dermatitis potential in man: sodium lauroyl glutamate and sodium lauryl sulphate. *Contact Dermatitis 30:205.*
8. di Nardo, A., K. Sugino, P. Wertz, J. Ademola, and H. I. Maibach. 1996. Sodium lauryl sulfate (SLS) induced irritant contact dermatitis: a correlation study between ceramides and in vivo parameters of irritation. *Contact Dermatitis 35:86.*
9. Reiche, L., C. Willis, J. Wilkinson, S. Shaw, and O. de Lacharriere. 1998. Clinical morphology of sodium lauryl sulfate (SLS) and nonanoic acid (NAA) irritant patch test reactions at 48 h and 96 h in 152 subjects. *Contact Dermatitis 39:240.*
10. Tupker, R. A., E. E. Bunte, V. Fidler, J. W. Wiechers, and P. J. Coenraads. 1999. Irritancy ranking of anionic detergents using one-time occlusive, repeated occlusive and repeated open tests. *Contact Dermatitis 40:316.*
11. Goebeler, M., J. Roth, E. B. Brocker, C. Sorg, and K. Schulze-Osthoff. 1995. Activation of nuclear factor-kappa B and gene expression in human endothelial cells by the common haptens nickel and cobalt. *J Immunol 155:2459.*
12. Yan, S. F., T. Fujita, J. Lu, K. Okada, Y. Shan Zou, N. Mackman, D. J. Pinsky, and D. M. Stern. 2000. Egr-1, a master switch coordinating upregulation of divergent gene families underlying ischemic stress. *Nat Med 6:1355.*

Memorandum

Date: November 4, 2003

From: Michael J. Galvin, Ph.D., Program Official
Office of Extramural Programs, NIOSH, E-74

Subject: Final Report Submitted for Entry into NTIS for Grant 5 R21 OH007340-02.

To: William D. Bennett
Data Systems Team, Information Resources Branch, EID, NIOSH, P03/C18

The attached final report has been received from the principal investigator on the subject NIOSH grant. If this document is forwarded to the National Technical Information Service, please let us know when a document number is known so that we can inform anyone who inquires about this final report.

Any publications that are included with this report are highlighted on the list below.

Attachment
cc: Sherri Diana, EID, P03/C13

List of Publications

Title:	Activation of Skin Cells and Transcription Factors by Skin Irritants
Investigator:	Robert A Swerlick, M.D.
Affiliation:	Emory University
City & State:	GA
Telephone:	(404) 727-2893
Award Number:	5 R21 OH007340-02
Start & End Date:	9/30/2000–9/29/2002
Total Project Cost:	$228,750
Program Area:	Other Occupational Safety and Health
Key Words:	allergies, skin, dermatitis

Final Report Abstract:
Irritant dermatitis is a source of significant workplace injury and morbidity. Our understanding of how irritants interact with the skin is minimal and expansion of our knowledge base regarding mechanisms mediating irritant action will provide us with a rationale basis of new approaches to prevention and treatment. The hypothesis proposed in this exploratory grant was that irritants affect the function or expression of transcription factors linked to the activity of pro-inflammatory genes. Activation or induced expression of pro-inflammatory genes by irritants results in the clinical manifestations of inflammation in the tissues exposed to irritants. The studies done in the proposal demonstrated that a common model detergent irritant, sodium lauryl sulfate, induces the expression of vascular endothelial growth factor (vascular permeability factor; VEGF/VPF) in a keratinocytes cell line in vitro. This induction appears to be mediated via induction of early growth factor 1 (egr-1), a transcription factor linked to cellular stress and injury. The induction of egr- 1 was mediated via activation of the MAP kinase MEK1, as evidenced by inhibition of both egr-1 and VEGF induction by the specific MEK1 inhibitor PD98059. These data are the first description of a molecular mechanism linking the edematous and erythematous response to detergent exposure to induction of agents which induce increases in vascular permeability and other elements of the inflammatory response. These data provide a rational framework for the development of large scale in vitro screening assays to assess irritancy of compounds. They also provide novel approaches for the prevention and treatment of irritant dermatitis.

Publications:
No publications to date.

Title:	Activation of Skin Cells and Transcription Factors by Skin Irritants
Investigator:	Robert A Swerlick, M.D.
Affiliation:	Emory University
City & State:	GA
Telephone:	(404) 727-2893
Award Number:	5 R21 OH007340-02
Start & End Date:	9/30/2000–9/29/2002
Total Project Cost:	$228,750
Program Area:	Other Occupational Safety and Health
Key Words:	allergies, skin, dermatitis

Final Report Abstract:
Irritant dermatitis is a source of significant workplace injury and morbidity. Our understanding of how irritants interact with the skin is minimal and expansion of our knowledge base regarding mechanisms mediating irritant action will provide us with a rationale basis of new approaches to prevention and treatment. The hypothesis proposed in this exploratory grant was that irritants affect the function or expression of transcription factors linked to the activity of pro-inflammatory genes. Activation or induced expression of pro-inflammatory genes by irritants results in the clinical manifestations of inflammation in the tissues exposed to irritants. The studies done in the proposal demonstrated that a common model detergent irritant, sodium lauryl sulfate, induces the expression of vascular endothelial growth factor (vascular permeability factor; VEGF/VPF) in a keratinocytes cell line in vitro. This induction appears to be mediated via induction of early growth factor 1 (egr-1), a transcription factor linked to cellular stress and injury. The induction of egr- 1 was mediated via activation of the MAP kinase MEK1, as evidenced by inhibition of both egr-1 and VEGF induction by the specific MEK1 inhibitor PD98059. These data are the first description of a molecular mechanism linking the edematous and erythematous response to detergent exposure to induction of agents which induce increases in vascular permeability and other elements of the inflammatory response. These data provide a rational framework for the development of large scale in vitro screening assays to assess irritancy of compounds. They also provide novel approaches for the prevention and treatment of irritant dermatitis.

Publications:
No publications to date.