

The Role of Oxidation in FSL-1 Induced Signaling Pathways of an Atopic Dermatitis Model in HaCaT Keratinocytes

I. Koren Carmi, R. Haj, H. Yehuda, S. Tamir,
and Abraham Z. Reznick

Abstract

Oxidative stress (OS) is common in inflammatory conditions and may be important in atopic dermatitis (AD) etiology. The aim of this project was to study the involvement of oxidation in FSL-1 (deacylated lipoprotein)-triggered signaling pathways leading to AD-typical cytokine expression in HaCaT keratinocytes. HaCaT keratinocytes, pretreated with the inhibitor to OS N-acetylcysteine (NAC), were exposed to FSL-1, a stimulator of AD-related cytokines. Cytokines expression was studied by real time polymerase chain reaction (PCR); nuclear factor-kappa B (NF- κ B) and p38 mitogen activated protein kinase (MAPK) activities were studied by western blotting; and the oxidative state of cells was determined by the dichlorofluorescein (DCF) assay. We found that endogenous OS in keratinocytes appeared 4 h after FSL-1 administration. OS activated NF- κ B, but not p38 MAPK, and the inhibition of OS reduced FSL-1 induced interleukin (IL) 33, thymic stromal lymphopoietin (TSLP) and TNF α mRNA expression. We conclude that FSL-1 triggers an OS reaction in HaCaT keratinocytes, which is probably a secondary event affecting the expression of specific AD typical cytokines, possibly through the NF- κ B pathways. This role of OS in the inflammatory response in AD is worth further investigating.

Keywords

Inflammation • N-acetylcysteine • Nuclear factor-kappa B • p38 mitogen activated protein kinase • Thymic stromal lymphopoietin

I. Koren Carmi
Department of Anatomy and Cell Biology, Rappaport
Faculty of Medicine, Technion – Israel Institute of
Technology, P.O. Box 9649, Haifa, Israel

Laboratory of Human Health and Nutrition Sciences,
MIGAL – Galilee Research Institute, Kiryat-Shmona,
Israel
e-mail: ifat.koren@gmail.com

R. Haj and A.Z. Reznick (✉)
Department of Anatomy and Cell Biology, Rappaport
Faculty of Medicine, Technion – Israel Institute of
Technology, P.O. Box 9649, Haifa, Israel
e-mail: reznick@technion.ac.il

H. Yehuda and S. Tamir
Laboratory of Human Health and Nutrition Sciences,
MIGAL – Galilee Research Institute, Kiryat-Shmona,
Israel

1 Introduction

1.1 Atopic Dermatitis (AD)

AD is a chronic and relapsing inflammatory skin disease characterized by dry, itchy, eczematous skin lesions which are susceptible to cutaneous bacterial, viral, and fungal infections. In the past decades, its prevalence has increased in the developed countries affecting up to 25 % of all children and 1–10 % of adults worldwide (Novak and Simon 2011). AD causes a significant impairment in the patient's quality of life and treatments approaches are limited to symptomatic, unspecific anti-inflammatory or immunosuppressive agents (Boguniewicz and Leung 2010).

The homogenous symptoms of AD imply a shared common disease, but recent research efforts have revealed the complex nature of this fascinating disease, which nonetheless cannot be considered to be a uniform entity. AD frequently starts in early infancy, termed early-onset AD, as opposed to late-onset AD, which develops in adulthood (Terui 2009). In about 70–80 % of patients, AD takes on the 'extrinsic' form, which is associated with increased IgE and eosinophil levels in the serum, while in the remaining patients AD has the 'intrinsic' form, characterized by normal IgE levels. Several studies have shown that, at the age of 5 years, 50 % of the children with AD have developed other atopic and allergic respiratory diseases – asthma, allergic rhinitis, and sensitization to inhalant allergens (Patrizi et al. 2011). Thus, AD is often the initial step in the so-called "atopic march" (Lenung et al. 2004).

Two hypotheses propose the cause underlying the disease. One suggests that the primary defect is genetic immunologic disturbance, with an epidermal dysfunction which is induced by local inflammation. This hypothesis is supported by the development of AD in a bone marrow recipient after the engraftment of hematopoietic stem cells from an atopic donor (as he receives the 'atopic' immune cells of the atopic donor). The

second hypothesis, which is supported by strong evidence in recent studies, claims that a primary structural abnormality of the stratum corneum (SC) underlies the pathogenesis of AD (Terui 2009).

Data that has accumulated over the past years has indicated the ability of epithelial cells to initiate and direct the immune response in allergic skin diseases. Upon activation, keratinocytes will initiate a process of alerting and activating the immune system in a way that will predominantly drive a T_H2 response. Thymic stromal lymphopoietin (TSLP), IL25 and IL33 constitute a keratinocyte-derived triad of cytokines, which collectively drives T_H2 polarization through complimentary and sometimes synergistic mechanisms.

TSLP was found to be highly expressed in epithelial cells, especially keratinocytes from patients with AD (Carmi-Levy et al. 2011) as well as in AD lesions and bronchial epithelial cell of asthmatic patients (Tuan Vu et al. 2010). Tissue-specific overexpression of TSLP in mouse skin induced an AD-like phenotype and variants of TSLP and its receptor have been found to be associated with the risk of AD (Carmi-Levy et al. 2010). TSLP was recently found to be produced following skin tape stripping, which induces one of the first features of AD, namely the disruption of the skin epithelial barrier (Carmi-Levy et al. 2011) and may be the link between epithelial barrier dysfunction and T_H2 immune polarization in AD.

1.2 Oxidative State

Oxidative stress has a major role in AD. Due to the skin's function as an interface between the body and the environment, it is chronically exposed to both endogenous and environmental pro-oxidant agents, leading to the harmful generation of reactive oxygen species (ROS). ROS are involved in the damage of cellular constituents, such as DNA, cell membrane lipids, or proteins and can act as secondary messengers in the induction of several biological responses, such

as the activation of the NF- κ B signal transduction pathway, the generation of cytokines, and the modulation of other signaling pathways (Tsukahara et al. 2003). Peroxides have been shown to be produced in keratinocytes following UVB activated MAPK signal transduction pathways (Peus et al. 1999). In another study, UVA stimulation was found to induce ROS production by activation of NADPH oxidase probably in the mitochondria (Valencia and Kochevar 2008).

1.3 P38 MAPK

The MAPKs, which integrate and process various extracellular signals, are primary components of the intracellular signaling circuitry. Four variants of p38 MAPK have been identified (α , β , γ , and δ). P38 α is perhaps the most physiologically relevant kinase involved in inflammatory responses, with a central role in the expression of pro-inflammatory cytokines and in immune cell proliferation and differentiation.

1.4 NF- κ B

The master regulator of inflammation is the transcription factor NF- κ B. It is also considered to be a prototypical example of sensitivity to oxidative stress. Non-activated NF- κ B is composed of p50 or p52, p65 (REL-A, REL-B, c-REL) and the inhibitor I κ B (I κ B α , I κ B β , I κ B ϵ , bcl-3), while the complex p50-REL-A and the inhibitor I κ B α are most common. In the classical pathway, NF- κ B is activated *via* phosphorylation of I κ B at two conserved serine residues in the N-terminus (Ser32, Ser36) by IKK (I κ B kinases – composed of IKK1, IKK2 and NEMO subunits), followed by ubiquitination and proteolysis of its bound inhibitor – I κ B. This allows for the migration of the liberated NF- κ B into the nucleus and its activation as a transcription factor. In the canonic activation mechanism, phosphorylation of I κ B, its degradation and the activation of NF- κ B results in the transcription

of the inhibitor I κ B, thus I κ B mRNA expression indicates NF- κ B activation (Perkins 2006).

In the present research, HaCaT human keratinocytes were treated with FSL-1, a diacylated lipoprotein that has been found to upregulate the expression and secretion of TSLP in human keratinocytes (Tuan Vu et al. 2010). TSLP expression in keratinocytes is a phenomenon that is specific to atopic dermatitis (Carmi-Levy et al. 2010; Tuan Vu et al. 2010). Therefore, this system was chosen to study the involvement of ROS in AD-related cytokine expression in keratinocytes.

2 Methods

2.1 HaCaT Keratinocytes

The human immortal keratinocyte cell line, HaCaT, was grown in Dulbecco's Modified Eagle's Medium (DMEM, Biological Industries, Beit Haemek, Israel) supplemented with 2 mM L-glutamine (Biological Industries, Beit Haemek, Israel), 10 % fetal calf serum (Sigma-Aldrich, Rehovot, Israel), 100 U/ml penicillin and 0.1 mg/ml streptomycin (Biological Industries, Beit Haemek, Israel) at 37 °C, with 5 % CO₂. Viability was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Sigma-Aldrich, Rehovot, Israel). In experiments, cells were seeded in DMEM in six wells plates and were pretreated with/without 2 mM NAC (Sigma-Aldrich, Rehovot, Israel) for 60 min followed by the administration of 1 μ g/ml FSL-1 for indicated periods of time.

2.2 Induction of AD-Related Cytokine Reaction

Diacylated lipoprotein, FSL-1 (Fibroblast-Stimulating Lipopeptide-1, InvivoGen, Toulouse, France) is a synthetic lipoprotein that represents the N-terminal part of the 44-kDa lipoprotein LP44 of *Mycoplasma salivarium*.

Mycoplasmal lipoproteins, such as FSL-1 and MALP-2, contain a lipolyated N-terminal diacylated cysteine residue, whereas bacterial lipoproteins contain a triacylated one. This structural difference plays a crucial role in the initial recognition of microbial lipoprotein by the host innate immune system.

2.3 Real Time PCR

mRNA was isolated using TRI Reagent (Sigma, Rehovot, Israel) and cDNA was prepared using the Verso cDNA kit (Thermo Scientific, US) and programmed 42 °C for 60 min, 50 °C for 30 min, and 95 °C for 2 min. The cDNA of the mRNA transcript was amplified by spectrofluorometric thermal cycler (Rotor-GeneTM 6000, Corbett research, Mortlake, Australia) using KAPA Sybr fast (KAPA Biosystems, Cape Town, South Africa) with specific primers for: GAPDH (housekeeping – to which every gene of interest was normalized -5' → 3' CGACCACTTTGTCAAGCTCA, TGTGAG-GAGGGGAGATTTCAG), IkB α (5' → 3' CTG-TGATCACCAACCAGCCAGA, GTAGCCAT-GGATAGAGGCTAAG), TSLP (5' → 3' TGCCTGCGGCTCTAGCTTGC, AGCCCATGACTGCTCCCCT), IL33 (5' → 3' CCTCAAATGAATCAGGTGACGGTGT, ACAAAGAAGGCCTGGTCTGGCA), IL25 (5' → 3' CCTGCTAGGCCCAACCGCCA, GGGGTC-CATGTGGGAGCCTGT) and TNF α (5' → 3' GTGATCGGCCCCAGAGGGA, CACGCC-ATTGGCCAGGAGGG). Results were represented as fold change of the control (without treatment at each time point).

2.4 Protein Analysis

Proteins from cells lysed in a solution containing CellLytic Lysis Buffer (Sigma, Rehovot, Israel) and Protease Inhibitor Cocktail (Sigma, Rehovot, Israel), were separated on a standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blotted on

nitrocellulose membranes. Blots were exposed overnight to primary monoclonal antibodies (separately) for: α -tubulin (Santa Cruz Biotechnology, Dallas, Texas, US), p38 MAPK (R&D systems, Minneapolis, US), phospho-p38 MAPK (T180/Y182 R&D systems, Minneapolis, US), IkB α (Santa Cruz Biotechnology, Dallas, Texas, US) and phospho-IkB α (S32/S36 Cell Signaling, Danvers, Massachusetts, US) at room temperature. The secondary antibodies – anti goat or anti rabbit (according to the primary antibody used) conjugated to horse-radish peroxidase (Jackson Immuno-Research, West Grove, Pennsylvania, US) were used. Detection was performed by enzyme-linked chemiluminescence (ECL) (Biological Industries, Beit Haemek, Israel), using ImageQuant LAS 4000 Digital Imager System (GE Healthcare, Buckinghamshire, UK). Protein quantities were determined by densitometry and analyzed using Total Lab Software (version V2006C, Nonlinear Dynamics, University of Leicester, UK). Results are represented as fold change relative to the control (without treatment at time zero).

2.5 Dichlorofluorescein (DCF) Assay

The DCF assay measures the level of oxidative stress. Cells grown to 50 % confluence on cover glass in 24-well plates were detected with the nuclear DNA-staining fluorescent dye Hoechst (1.5 μ g/ml, incubation for 30 min). These cells were incubated with 10 μ M dichlorofluorescein diacetate (CM-H₂DCFDA, Invitrogen, Toulouse, France) for 60 min at 37 °C in the dark for the internalization of this chemical, which fluoresces upon oxidation. Subsequently, cells were pre-exposed to the OS inhibitor, NAC, for 60 min before the stimulation with 1 μ g/ml FSL-1. The difference in the fluorescence was quantitated by fluorescence microscopy and the average spectrometric optical density (OD) values were calculated. Results are represented as fold-change from the control (without treatment at each time point).

3 Results

3.1 FSL-1 Stimulates ROS Production Which Is Reduced by OS Inhibition

The induction of ROS by FSL-1 treatment of keratinocytes was verified by a DCF assay. After HaCaT cells were stimulated with 1 $\mu\text{g/ml}$ FSL-1, ROS levels increased (Fig. 1a) by 13 %, but only at 4 h (tested in 2 h and 6 h as well) following FSL-1 treatment, in comparison to the negative control (without treatment at each time point). At 4 h, when the keratinocytes were pretreated with 2 mM NAC, the FSL-1 induced ROS (at 4 h) decreased by 27 %, as compared to the negative control (Fig. 1a, b).

3.2 FSL-1 Does Not Induce Phosphorylation of p38 MAPK Through OS

In order to learn if FSL-1 affects the p38 MAPK pathway by means of OS, HaCaT cells were

pretreated with the antioxidant NAC (2 mM) for 60 min before exposure to FSL-1 (1 $\mu\text{g/ml}$ 10–120 min). The western blot showed that FSL-1 induced p38 MAPK phosphorylation, but this induction was not significantly inhibited by NAC (Fig. 2a, b), implying that OS is not involved in FSL-1 triggered phosphorylation of p38 MAPK.

3.3 OS Is Not Involved in FSL-1 Induced NF- κ B Activity Immediately After FSL-1 Administration

The participation of OS in FSL-1 induced NF- κ B activity was investigated. Western blots of NF- κ B activation revealed that FSL-1 (1 $\mu\text{g/ml}$) induced NF- κ B activity, which was not inhibited by NAC (mM).

Although lower levels of phospho-I κ B α at 30 min, lower production *de novo* of I κ B α at 120 min and lower I κ B α mRNA expression, as compared to levels obtained from FSL-1 alone, were observed (Fig. 3a–d), no inhibition of

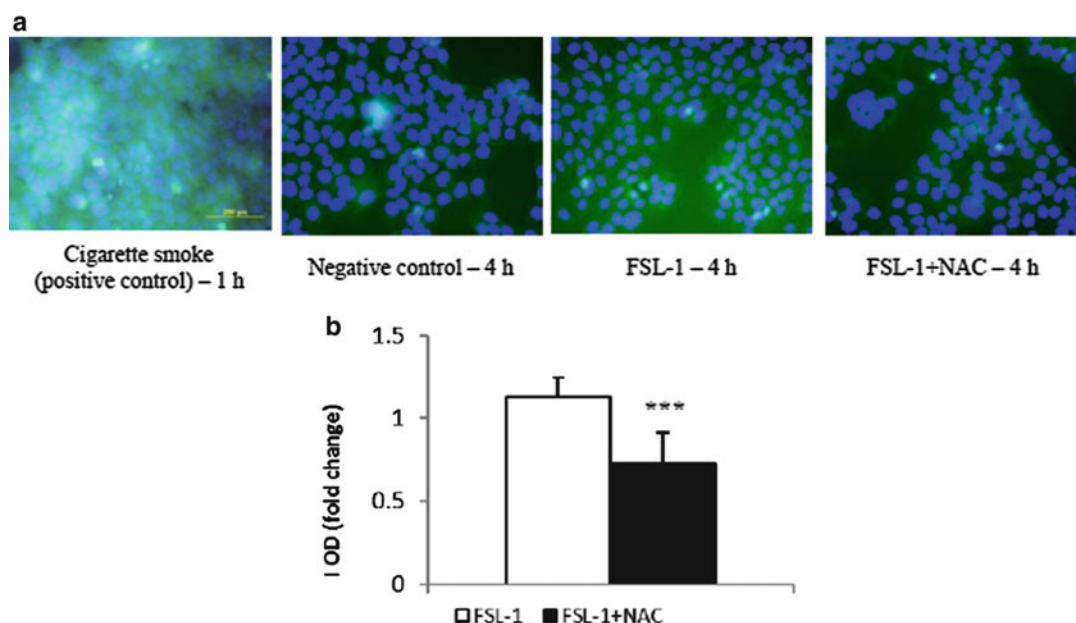
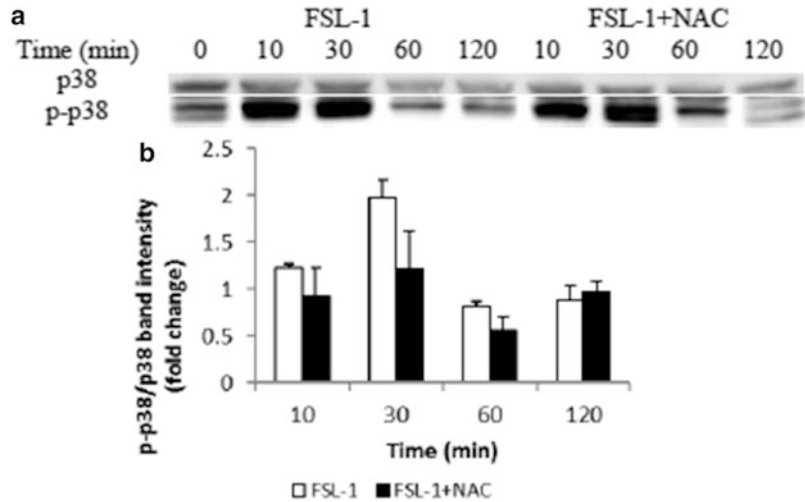


Fig. 1 FSL-1 induces ROS which is inhibited by NAC. HaCaT keratinocytes were pretreated with 2 mM NAC 1 h prior to stimulation with FSL-1. ROS levels were determined using DCF, microscopy imaging (a) and

computerized quantifying, presented relatively to control without treatment (b). Number of cells tested: FSL-1 = 350; FSL-1 + NAC = 180. ***p < 0.0005; Data are means $\pm$ SE

Fig. 2 The antioxidant NAC does not inhibit FSL-1-induced phosphorylation of p38 MAPK. HaCaT

keratinocytes were treated with 2 mM NAC prior to stimulation with FSL-1 (1 μ g/ml). Representative western blot (a) and calculated phospho-p38/p38 ratios (b) of time dependent p38 and phospho-p38. Number of wells tested: FSL-1 = 6–8; FSL-1 + NAC = 2–3. Data are means $\pm$ SE



NF- κ B activation by NAC took place. This is concluded from the lower levels of I κ B α 10–30 min after FSL-1 administration (Fig. 3b), which should have been higher in case of NF- κ B activation inhibition. NAC is able to decrease the level of phospho-I κ B α by its reduction and not only by sequestering OS. In summary, these results indicate that OS was not apparent immediately after FSL-1 administration.

3.4 Inhibition of OS Reduced FSL-1 Induced TSLP, TNF α and IL33 mRNA Expression

The effect of OS on the induction of AD-related cytokines triggered by FSL-1 was also studied. Keratinocytes were tested for mRNA expression of the AD typical cytokines by real time PCR at different time periods, following FSL-1 stimulation, with and without 2 mM NAC pretreatment (Fig. 4).

NAC had no significant effect on FSL-1 induced IL25, but it did have an inhibitory effect on FSL-1-induced TSLP, IL33 mRNA (at 4 h) and TNF α expression (at 2 and 6 h) after FSL-1 administration, indicating the participation of OS in the mRNA expression of these AD-related cytokines.

4 Discussion

The aim of this research was to study the role of oxidative stress in the signal transduction pathways of AD. The *in vitro* model chosen was HaCaT keratinocytes stimulated by FSL-1, a diacylated lipoprotein, which was found to upregulate the expression and secretion of TSLP in primary human keratinocytes through ligation to TLR2 or TLR6 (Tuan Vu et al. 2010). Furthermore, FSL-1 stimulation induces a MyD88-dependent signaling cascade leading to the activation of NF- κ B and the production of proinflammatory cytokines. FSL-1 has also been found to rapidly activate I κ B phosphorylation in keratinocytes and to bring about the high expression of TNF α in macrophages and NF- κ B in TLR2/TLR6 transfected HEK293 cells (Zhao et al. 2010).

In this study, it was found that FSL-1 induced the production of endogenic oxidative species, besides activating NF- κ B, p38 MAPK and brought about an increase in the expression of IL33, TSLP, and TNF α cytokines. The induction of cytokine expression was inhibited by an OS inhibitor, NAC, implying the involvement of OS in certain AD-related pathways in keratinocytes.

FSL-1 induced the production of ROS in keratinocytes. Since the time period required for a significant elevation of the oxidation level

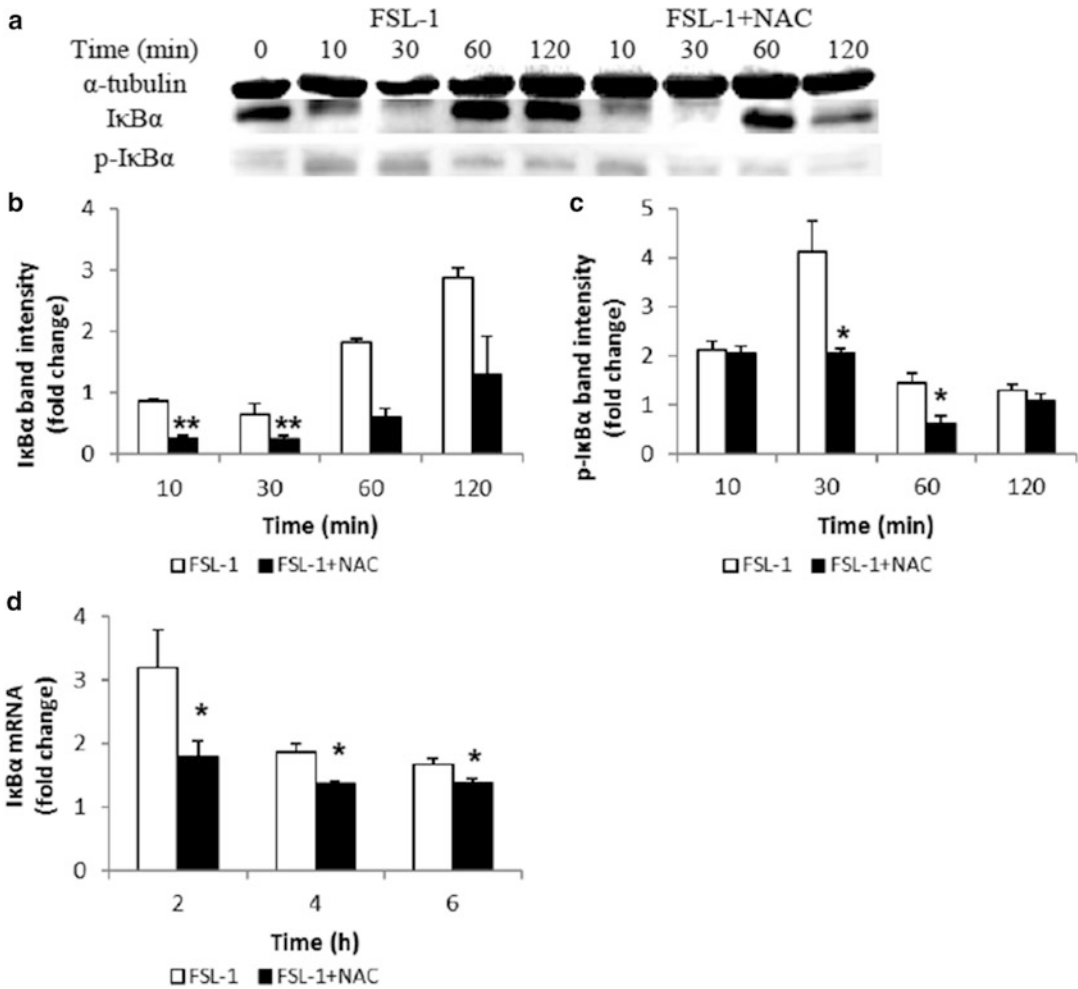


Fig. 3 NAC inhibits phosphorylation, expression and resynthesis of IκBα in FSL-1 by means of its reduction and not by OS sequestering. HaCaT keratinocytes were treated with and without 2 mM NAC prior to stimulation with FSL-1 (1 μg/ml). Time dependent IκBα and phospho-IκBα levels are presented in representative western blot (a); and protein levels were quantified by densitometry and presented relative to negative control (without treatment). Relative

protein levels of IκBα (b) and phospho-IκBα (c). Number of wells tested: FSL-1 = 4–6; FSL-1 + NAC = 2–3. Time dependent IκBα mRNA expression was measured using real time PCR and is presented normalized to the housekeeping gene (GAPDH) and relative to negative control (without treatment) at each time point (D). Number of wells tested: FSL-1 = 9–12; FSL-1 + NAC = 3. * $p < 0.05$, ** $p < 0.005$ relative to FSL-1 alone. Data are means $\pm$ SE

is longer (4 h) than that observed after stimulation with other triggers of OS, e.g. cigarette smoke, the reaction to FSL-1 is probably a secondary reaction. It is well known that OS is related to inflammation of all types, as cells of the immune system produce and secrete different kind of enzymes, ROS, and nitric oxide species (NOS) to fight intruders, but also in the case of

sterile inflammation, meaning when no pathogens are involved (Kim et al. 2011). Keratinocytes, which are the body's first line of defense and barrier from the outside world, are part of the innate immune system and are also able to produce ROS. In the case of AD, oxidation may be the fuel of the vicious cycle that is the basis of the disease chronicity.

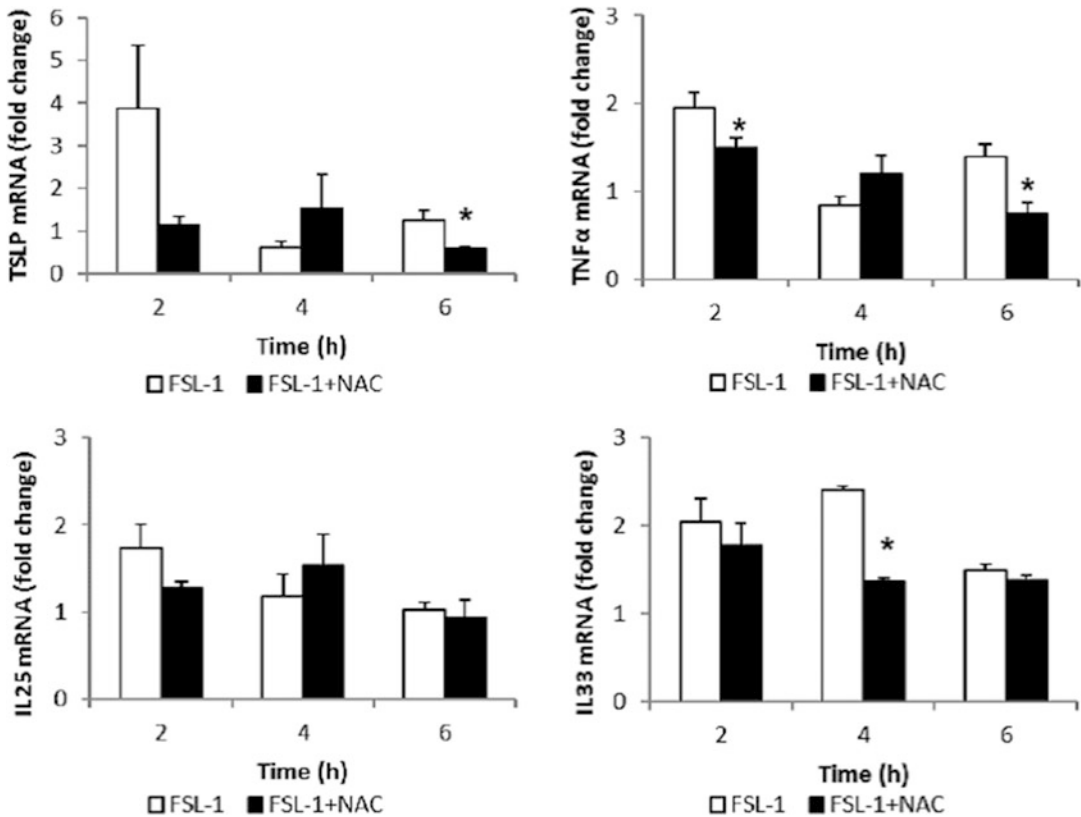


Fig. 4 NAC decreases FSL-1-induced mRNA expression of TSLP, TNFα, and IL33. HaCaT cells were pretreated with 2 mM NAC before stimulation with FSL-1 (1 μg/ml). Time dependent mRNA expression was measured using real time PCR and is presented as

normalized to the housekeeping gene (GAPDH) and relative to the negative control (without treatment) at each time point. Number of wells tested: FSL-1 = 3–14; FSL-1 + NAC = 3. * $p < 0.05$ relative to FSL-1 alone. Data expressed as means $\pm$ SE

The observed rise in the prevalence of AD cannot be attributed to genetics and it has been hypothesized that it may be due to the increase in ROS generated from environmental pollution and solar radiation. In Nagoya City, where nitrogen oxide concentration is the highest in Japan, an increase in the incidence of AD patients was detected. It was also reported that there is a greater number of AD patients in urban areas than in rural districts, again pointing to the possible connection between environmental oxidation and AD (Niwa et al. 2003).

FSL-1 does not induce phosphorylation of p38 MAPK through OS shortly after its administration; meaning, p38 MAPK does not recruit NF-κB, which in turn leads to cytokine production in the cascade resulting from OS, as was

hypothesized and indicated in literature (Kumar et al. 2003; Gochman et al. 2011; Kaisari et al. 2013).

OS does not show any apparent relation to the NF-κB pathways immediately after FSL-1 administration, probably since no rise in OS was observed shortly after FSL-1 administration. A series of investigations showed that oxidative stress activates NF-κB in different types of cells, but not in all. In addition, several recent studies have indicated that oxidants, such as hydrogen peroxide or peroxynitrite, repressed NF-κB activation in several cell systems *in vitro* (Loukili et al. 2010).

An indication for NF-κB relevance to AD is exemplified in a research that administered topical NF-κB decoy oligodeoxynucleotides in NC/Nga

atopic mouse model twice a month and found a significant reduction in clinical skin condition and a marked improvement of histological findings (Morishita et al. 2004). Another indication of the relevance of NF- κ B in AD is the finding that many cytokines related to inflammation in general and to AD in particular, seem to be regulated by NF- κ B (it promotes the transcription of TNF α , T_H2 cytokines, and TSLP which is known to be an important factor in atopic diseases) (Moon and Kim 2011). In this study, it was found that the antioxidant NAC was apparently able to sequester the induced ROS, thus preventing the OS from causing up-regulation of TSLP, IL33, and TNF α (as mentioned previously, TSLP and IL33 are known AD-related cytokines, while TNF α is an inflammatory cytokine) mRNA expression. Possibly, NAC prevented the increase in cytokine expression by means of NF- κ B inhibition.

Expression of IL25 (which is related to the T_H2 immune response and was detected in human AD lesions and in a murine model for allergic airway disease) was not found to increase as a consequence of FSL-1 employment. The explanation, as was found upon further investigations, is that IL25 is produced by dendritic cells within the dermis of patients and not by keratinocytes (Hvid et al. 2011).

These triggered pathways may lead to the additional production of AD-typical cytokines in what may be a vicious cycle, in which cell components are damaged and chronic atopic disease develops. An example of this possibility is the immunomodulatory T_H2-associated cytokine, IL33, which activates NF- κ B in a murine cutaneous model (Pushparaj et al. 2009). Another possible reactivation pathway may be through TNF α and its receptor, which are known together to activate NF- κ B.

The role of OS in AD is important, since oxidation may be the instigator of the vicious cycle that is responsible for the chronicity of this disease. The elucidation of the OS-related processes contributing to AD may reveal targets that may be relevant in new, more efficient treatments, with fewer side effects. Such treatments could be alternatives to the

corticosteroids used today, or to the expensive, and yet to be approved biological therapy (Guttman-Yassky et al. 2013).

Acknowledgments This work was supported by Rappaport Institute and by MIGAL – Galilee Research Institute.

Conflicts of Interest The authors declare no conflicts of interest in relation to this manuscript.

References

- Boguniewicz M, Leung DY (2010) Recent insights into atopic dermatitis and implications for management of infectious complications. *J Allergy Clin Immunol* 125:4–13
- Carmi-Levy I, Homey B, Soumelis V (2011) A modular view of cytokines networks in atopic dermatitis. *Clin Rev Allerg Immunol* 41:245–253
- Gochman E, Mahajna J, Reznick AZ (2011) NF- κ B activation by peroxynitrite through I κ B α -dependent phosphorylation versus nitration in colon cancer cells. *Anticancer Res* 31:1607–1617
- Guttman-Yassky E, Dhingra N, Leung DYM (2013) New era of biologic therapeutics in atopic dermatitis. *Expert Opin* 13:549–561
- Hvid M, Vestergaard C, Kemp K, Chrisensen GB, Deleuran B, Deleuran M (2011) IL-25 in atopic dermatitis: a possible link between inflammation and skin barrier dysfunction. *J Invest Dermatol* 121:150–157
- Kaisari S, Rom O, Aizenbud D, Reznick AZ (2013) The involvement of NF- κ B and muscle specific E3 ubiquitin ligase MuRF1 in cigarette smoke induced catabolism in C2 myotubes. *Adv Exp Med Biol* 788:7–17
- Kim D, Byamba D, Wu WH, Kim T, Lee M (2011) Different characteristics of reactive oxygen species production by human keratinocytes cell line cells in response to allergens and irritants. *Exp Dermatol* 21:99–103
- Kumar S, Boehm J, Lee JC (2003) P38 MAP kinases: key signalling molecules as therapeutic targets for inflammatory diseases. *Nature Rev* 2:717–726
- Lenung DY, Boguniewicz M, Howell MD, Nomura I, Hamid QA (2004) New insights into atopic dermatitis. *J Clin Invest* 113:651–657
- Loukili N, Rosenblatt-Velin N, Rolli J, Levrard S, Feihl F, Waeber B, Pacher P, Liaudet L (2010) Oxidants positively or negatively regulate nuclear factor κ B in a context-dependent manner. *J Biol Chem* 285:15746–15752
- Moon P, Kim H (2011) Thymic stromal lymphopoietin is expressed and produced by caspase-1/NF- κ B in mast cells. *Cytokine* 54:239–243

- Morishita R, Tomita N, Kaneda Y, Ogihara T (2004) Prevention and regression of atopic dermatitis by ointment containing NF- κ B decoy oligodeoxynucleotides in NC/Nga atopic mouse model. *Curr Opin Pharmacol* 4:139–146
- Niwa Y, Sumi H, Kawahira K, Terashima T, Nakamura T, Akamatsu H (2003) Protein oxidative damage to the stratum corneum: evidence for the link between environmental oxidation and changing prevalence and nature of atopic dermatitis in Japan. *Br J Dermatol* 149:248–254
- Novak N, Simon D (2011) Atopic dermatitis – from new pathophysiologic insights to individualized therapy. *Allergy* 66:830–839
- Patrizi A, Pileri A, Bellini F, Raone B, Neri I, Ricci P (2011) Atopic dermatitis and the atopic march: what is new? *J Allergy (Cairo)*. doi:10.1155/2011/279425
- Perkins ND (2006) Post-translational modifications regulating the activity and function of the nuclear factor B pathway. *Oncogene* 25:6717–6730
- Peus D, Vasa RA, Beyerle A, Meves A, Krautmacher C, Pittelkow M (1999) UVB activates ERK1/2 and p38 signaling pathways *via* reactive oxygen species in cultured keratinocytes. *J Invest Dermatol* 112:751–756
- Pushparaj PN, Tay H, H'ng S, Pitman N, Xu D, McKenzie A, Liew FY, Melendez AJ (2009) The cytokine interleukin-33 mediates anaphylactic shock. *Proc Natl Acad Sci U S A* 104:9773–9778
- Terui T (2009) Analysis of the mechanism for the development of allergic skin inflammation and the application for its treatment: overview of the pathophysiology of atopic dermatitis. *J Pharmacol Sci* 110:232–236
- Tsukahara H, Shibata R, Ohta N, Sato S, Hiraoka M, Ito S, Noiri E, Mayumi M (2003) High levels of urinary pentosidine, an advanced glycation end product, in children with acute exacerbation of atopic dermatitis: relationship with oxidative stress. *Metab Clin Exp* 52:1601–1605
- Tuan Vu A, Baba T, Chen X, Le Anh T, Kinoshita H, Xie Y, Kamijo S, Hiramatsu K, Ikeda S, Ogawa H, Okumura K, Takai T (2010) *Staphylococcus aureus* membrane and diacylated lipopeptide induce thymic stromal lymphopoietin in keratinocytes through the Toll-like receptor 2-Toll-like receptor 6 pathway. *J Allergy Clin Immunol* 126:985–993
- Valencia A, Kochevar IE (2008) Nox1-based NADPH oxidase is the major source of UVA-induced reactive oxygen species in human keratinocytes. *J Invest Dermatol* 128:214–222
- Zhaoa J, Benakanakerea MR, Hosurb KB, Galiciaa JC, Martinc M, Kinane DF (2010) Mammalian target of rapamycin (mTOR) regulates TLR3 induced cytokines in human oral keratinocytes. *Mol Immunol* 48:294–304