Differential regulation of nuclear factor-kappa B subunits on epidermal keratinocytes by ultraviolet B and tacrolimus

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Original Article

Abstract  Modulation of nuclear factor-kappa B (NF-κB) expression has important clinical implications including anti-inflammation. Recently, we have shown that direct regulation of NF-κB/p65 subunit may account for tacrolimus ointment’s remarkable clinical efficacy on treating inflammatory dermatoses. However, NF-κB is a dimeric transcription factor formed by hetero- or homodimeration of the five Rel family proteins. The complete operational scheme of different NF-κB subunits remains obscure. It has been shown that homodimers consisting of NF-κB/p50 may serve an inhibitory role in suppressing inflammation while dimers consisting of NF-κB/p65 activate inflammatory pathway. Our current study aimed to explore the effects of ultraviolet B (UVB) on epidermal keratinocytes in terms of specific NF-κB subunits NF-κB/p50 and NF-κB/p65. Additionally, the effects of tacrolimus on differential regulation of NF-κB subunits of UVB irradiated keratinocytes were also investigated. Our result showed that UVB sequentially regulated the activities of different subunits of NF-κB: the activity of NF-κB/p50 was downregulated in the early stage (6 hours), followed by upregulation of NF-κB/p65 in the later stage (12 hours). The results from immunofluorescence, immunocytochemical, and immunohistochemical analyses indicated that the nuclear expression of NF-κB/p50 could be seen constitutively while the nuclear expression of NF-κB/p65 could only be seen after UVB irradiation. Furthermore, treatment with tacrolimus didn’t affect the nuclear activation and translocation of NF-κB/p50, while the UVB induced NF-κB/p65 nuclear expression was suppressed by tacrolimus. In summary, we have shown that UVB irradiation sequentially regulated different NF-κB subunits. The clinical efficacy of tacrolimus may be...
Introduction

Many inflammatory dermatoses require prolonged use of topical agents to suppress on-going inflammation. Therefore, much effort has been made to control skin inflammation with least side effects. Recently, topical tacrolimus (FK506) has shown remarkable therapeutic efficacy for treating various inflammatory dermatoses. Since epidermal keratinocytes are immune competent cells with capacities to synthesize and secrete important inflammatory cytokines [1], the direct effect of tacrolimus on lesional skin requires thorough investigation. Recently, we have shown that the anti-inflammatory property of topical tacrolimus involves down regulation of inflammatory cytokine tumor necrosis factor alpha (TNF-α) via modulation of nuclear factor kappa B (NF-κB), particularly by suppressing the activation and translocation of NF-κB/p65 subunit [2].

Nuclear factor kappa B (NF-κB) is an important regulator of immune and inflammatory responses, controlling the expression of genes encoding cytokines, chemokines, adhesion molecules, and regulators of the cell cycle and apoptosis [3]. Modulation of NF-κB expression has important clinical implications including anti-inflammatory [4,5]. Nuclear factor kappa B is retained in the cytosol by an inhibitor of kappa B (IκB) in most cell types. It is a dimeric transcription factor formed by hetero- or homodimeration of the five Rel family proteins that comprise RelA (p65), RelB, cRel, p52, and p50 [6]. The classical NF-κB heterodimer, composed of p50 and p65 subunits, is a potent activator of gene expression [7]. Upon activation by various extracellular stimuli, IκB are phosphorylated and degraded through the 26S proteasome [8]. This event leads to translocation of the p50/p65 dimer to the nucleus and binds to its responsive elements present in a variety of genes such as TNF-α.

The complete operational scheme of NF-κB is by far more complicated than the classical mechanism described above. There are at least 10 potential homodimeric and heterodimeric combinations of Rel subunits. It has been reported that individual Rel protein may have distinct biological activities, both in vitro and in vivo [9]. More importantly, NF-κB/p50 homodimers and heterodimers containing RelB may inhibit transcription activity normally activated by p50/p65 dimer [10–12]. In resting T lymphocytes, NF-κB/p50 homodimers have been found to inhibit interleukin-2 gene expression [13,14]. It has been shown that TNF-α transcription in macrophages is attenuated by a mechanism that involves NF-κB/p50 induction [10,15]. Therefore, different combinations of NF-κB subunits may serve to orchestrate the complicated regulatory pathway of NF-κB. Tumor necrosis factor alpha (TNF-α) is an important mediator of inflammatory reactions, and ultraviolet B (UVB) is known to induce skin inflammation. It has been reported that keratinocytes produce and secrete TNF-α after UVB exposure [1]. The mechanisms proposed to participate in this particular UVB induced response include: direct DNA damage [16–18], reactive oxygen intermediates [19–21], and activation of NF-κB [2,22]. Using TNF-α as a marker for inflammatory response, we have previously shown that tacrolimus imparted its anti-inflammatory action on UVB irradiated keratinocytes via inhibition of NF-κB/p65 subunit. However, since other NF-κB dimers, such as p50 homodimers, may also be involved in the anti-inflammatory process brought about by tacrolimus on UVB irradiated keratinocytes, the possibility of differential regulation of different NF-κB subunits requires further investigation. Our current study aimed to explore the effects of UVB on epidermal keratinocytes in terms of specific NF-κB subunits: NF-κB/p50 and NF-κB/p65. In addition, the effect of tacrolimus, a topical immune modulator for treatment of inflammatory dermatoses, on differential regulation of NF-κB subunits of UVB irradiated keratinocytes were also investigated.

Materials and methods

Cell culture for keratinocytes

Cell cultures for keratinocytes were performed as described elsewhere [23]. Healthy adult foreskin was the source for cell cultures. The adipose tissues were removed by a sterilized scissor, and the remaining epidermis were sectioned into 5 mm × 5 mm blocks and placed into 0.25% trypsin-EDTA for 24 hours. The epidermis was then washed with 0.25 mg/ml soybean and PBS, followed by 1200 rpm centrifugation at 4 °C for 5 minutes. After removal of excess suspensions, appropriate amount of keratinocytes was cultured onto Petri dishes and placed into a 5% CO2 incubator of 37 °C. The culture medium for keratinocytes (serum-free medium (SFM) complete medium, supplemented with 2 ng/ml of recombinant human epidermal growth factor and 25 µg/ml of bovine pituitary extracts; Gibco, New York, USA) was changed every two days. When primary cultures reached 80% confluence, they were passaged to expand the culture. Cells at passages 3–5 were used for experiments.

Treatments of keratinocytes

Cultured human keratinocytes were irradiated with a UVB lamp. For UVB irradiation, a specific mercury/vapor UVB lamp made under low pressure by Vilber Loumat (Marne-la-Vallee, France) emitted a peak wavelength of 312 nm with intensity of 1.28 mW/cm² was used. The dose of irradiation was measured by a UVP radiometer (Ultra-Violet Products (UVP), San Gabriel, CA, USA). During UVB irradiation, the lids of culture dishes or plates were removed and the culture medium was replaced with phosphate buffered
Expression of NF-κB by UVB and tacrolimus

saline (PBS) to avoid formation of medium-derived toxic photoproducts induced by UV exposure. Keratinocytes were treated with or without 50 mJ/cm² UVB irradiation and incubated for the indicated time frame. This particular UVB usage was used since our previous study has indicated that, at 50 mJ/cm², UVBs were able to elicit predictable and reproducible inflammatory response from cultured keratinocytes. To determine the effect of tacrolimus on UVB irradiated keratinocyte, cultured cells were irradiated with 50 mJ/cm² UVB as described above and followed by addition of tacrolimus (Calbiochem-Novabiochem, San Diego, CA, USA) at indicated concentration (0, 10, 100, and 1000 nM) and incubated for the indicated time frame. For immunochemical analysis, only tacrolimus at concentration of 1000 nm was added. The control cells were treated with equivalent amount of Dimethyl sulfoxide (DMSO). The experiments were performed as described below.

Preparation of nuclear protein lysates

Since NF-κB is a transcription factor that only becomes active once it translocates to the nucleus and binds to its responsive element, the nuclear protein lysates were obtained for evaluation of NF-κB activities. A commercial kit (Nuclear Extract Kit, Active Motif North America, Carlsbad, CA, USA) was used to extract nuclear protein lysates according to the manufacturer’s instructions. The treated cells were washed with 5 ml of ice-cold PBS/phosphatase inhibitors. Three ml of ice-cold PBS/phosphatase inhibitors was added to each dish. The cells were removed from the dish by gently scraping with a cell lifter and followed by centrifugation at 500 rpm for 5 minutes. After removing the supernatants, the cell pellets were resuspended in 500 μl of hypotonic buffer and incubated for 15 minutes on ice. Twenty-five μl of detergent was added and vortex for 10 seconds at highest setting. The suspensions were then centrifuged for 30 seconds at 14,000 × g. The supernatants were removed and the remaining pellets were resuspended in 50 μl of complete lysis buffer for 30 minutes on ice, followed by centrifugation for 10 minutes at 14,000 × g. The supernatants (nuclear proteins) were stored at −80 °C for further use. The protein concentrations were measured by a protein quantitation kit (Bio-Rad, Hercules, CA, USA).

Assessment of nuclear NF-κB/p50 and NF-κB/p65 activation and translocation using Enzyme-linked immunosorbent assay (ELISA)

The nuclear binding NF-κB/p50 and NF-κB/p65 in cultured keratinocytes were assessed by Trans-AM NF-κB Enzyme-linked immunosorbent assay (ELISA) kit (Active Motif North America, Carlsbad, CA, USA) according to the manufacturer’s instructions. The commercially available Trans-AM kit was employed for the assay of NF-κB/p50 and NF-κB/p65 using an common oligonucleotide containing an NF-κB consensus site (5′-GGGACTTTCC-3′) that binds to the nuclear extract and can detect NF-κB, which can recognize an epitope on NF-κB/p50 and NF-κB/p65 activated and bound to its target DNA. Nuclear protein lysates (4 μg) of each sample diluted in 20 μl complete lysis buffer were loaded per well in duplicate. For positive control, 20 μl of complete lysis buffer containing 1 μl of positive control cell extract per well was used. For negative control, 20 μl of complete lysis buffer per well was used. The plate was sealed with the adhesive film and incubated for 1h at room temperature with mild agitation (100 rpm on a rocking platform), after which the wells were washed three times with 200 μl of wash buffer, and 100 μl of diluted primary antibody against NF-κB/p50 or NF-κB/p65 (1:1,000 dilution in antibody binding buffer) was added to each well and incubated at room temperature for 1h without agitation. The wells were washed again three times with wash buffer, and 100 μl of diluted HRP-conjugated antibody (1:1,000 dilution in antibody binding buffer) was added to each well and incubated for 1h at room temperature. The wells were washed again four times with wash buffer followed by the addition of 100 μl of developing solution. The wells were incubated for 10 minutes at room temperature shielded from direct light. This was followed by the addition of 100 μl of stop solution to each well, and the absorbance was read within 5 minutes at 450 nm.

Immunofluorescence staining

Keratinocytes were seeded on glass coverslips with a density of 2 × 10⁴ cells/well in 24-well plate for 24 hours until cell attachment. Six hours after 50 mJ/cm² UVB irradiation, they were washed with PBS and were then fixed with 100 % methanol at −20 °C for 20 minutes. Cells were blocked with 10 % bovine serum albumin (Gibco) in PBS for 30 minutes at room temperature to eliminate non-specific binding. After washing briefly with PBS, the cells were incubated overnight at 4 °C with 200 μl of anti-NF-κB/p50 or anti-NF-κB/p65 mouse monoclonal antibody (1:100 dilution; Santa Cruz Biotechnology, Santa Cruz, CA). After three washings with PBS, the cells were incubated for 1 h at room temperature with 200 μl of biotin-conjugated goat antimouse antibody (1:100 dilution; Santa Cruz Biotechnology, Santa Cruz, CA). After three washings with PBS, the cells were incubated for 1 h at room temperature with 200 μl of fluorescein isothiocyanate (FITC)-conjugated streptavidin (1:100 dilution, Immunoresearch, Baltimore, PA, USA). After three washings with PBS, the cells were incubated for 1 h at 4 °C with fluorescein isothiocyanate (FITC)-conjugated streptavidin (1:100 dilution, Immunoresearch). After washing with PBS, the cells on the glass coverslips were embedded in mounting medium (Aquamount; Lerner Laboratories, Pittsburgh, PA, USA), and then the cells were photographed with a Zeiss fluorescence microscope (Axioskop 2 plus, Gottingen, Germany).

Immunocytochemical staining

Cultured keratinocytes were seeded on glass coverslips with a density of 2 × 10⁴ cells/well in 24-well plate for 24 hours until cell attachment. Twelve hours after indicated treatment, they were washed with PBS and then fixed in acetone, blocked with 3% H₂O₂, and then incubated with 10% bovine serum albumin (BSA) (Sigma) in PBS for 60 minutes at room temperature to eliminate nonspecific binding. After washing briefly with PBS-Tween 20: Sigma, USA (PBS-T), the cells were incubated with primary antibody against NF-κB/p50 or NF-κB/p65 mouse monoclonal antibody (1:100 dilution; Santa Cruz Biotechnology, Santa Cruz, USA) at room
temperature for 60 minutes. After three washings with PBST, the cells were incubated for 10 minutes at room temperature with 200 µl of biotinylated link antimouse antibody (Dako, Carpinteria, CA, USA). Following three washings with PBST, the cells were incubated for 15 minutes at room temperature with 200 µl of streptavidin-horseradish peroxidase (HRP) (Dako). Finally, PBST was used to remove excess streptavidin-HRP, and the color was developed using DAB substrate-chromogen solution (Dako). The cells were counterstained with hematoxylin and viewed under light microscope. The NF-κB/p50 or NF-κB/p65 positive cells were analyzed quantitatively by selecting six random fields and counting the stained and total cells.

**Animal model**

Pathogen-free female C3H/HeN mice were purchased from the National Laboratory Animal Center in Taipei, Taiwan. All mice were kept in groups of five per cage and housed at 24 ± 2°C and 50 ± 10% relative humidity. Six-week-old mice were separated into two groups (n = 5 for each group): the control and UVB irradiated groups.

**UVB irradiation of mice and skin biopsies**

The protocol has been described previously elsewhere [2]. Briefly, the dorsal skin of the mice was shaved with an electric clipper 48 hours before UVB irradiation. The UVB irradiation was administered through an UVB lamp with intensity of 1.28 mW/cm² (Marne-la-Vallée, Vilber Lourmat, France). A single dose of 90 mJ/cm² UVB was delivered to the dorsal skin of each mouse in the experimental group. No UVB irradiation was given to the control group. A thin layer of tacrolimus ointment (Protopic ointment, 0.1%; Astellas Pharma US, Deerfield, Illinois, USA) was applied to the left half of the dorsal skin immediately after UVB irradiation. Those without UVB irradiation also received same treatment. All mice were sacrificed 48 hours after UVB exposure. Skin punch biopsy 5mm in size were collected on the left (tacrolimus treated) and right (no treatment) dorsal skin for immunohistochemical studies. The skin samples were collected 48 hours after 90 mJ/cm² UVB irradiation because this regimen has been shown to induce reproducible inflammation in C3H/HeN mouse skin [24].

**Immunohistochemical staining**

Three-micron of paraffin sections from mouse skins were deparaffinized in xylene and rehydrated in graded alcohol dilutions. Endogenous peroxidase activity was blocked by incubating with 3% H₂O₂ for 5 minutes. Antigen retrieval was performed by pressure-cooking for 10 minutes (121°C, 1.2 kg/cm²) in 0.01 M citrate buffer (pH 6.0). The slides were then incubated with primary antibody against NF-κB/p50 or NF-κB/p65 mouse monoclonal antibody (1:100 dilution; Santa Cruz Biotechnology) for 1 hour at room temperature. Antibody reactions were detected with biotinylated link anti-rabbit antibody (Dako) for 20 minutes at room temperature, followed by incubation with streptavidin-HRP (Dako) for 20 minutes at room temperature. The color was developed using DAB substrate-chromogen solution (Dako). The slides were then counterstained with hematoxylin.

**Keratinocyte viability assay**

For determination of keratinocytes viability after UVB irradiation, the method previously described by Scudiero and colleagues [25] was used. Briefly, the keratinocytes were seeded on a 96-well plate at the cell density of 2 × 10⁴ cells per well. Six hours after 0 and 50 mJ/cm² UVB irradiation, a commercially available kit (CellTiter 96 aqueous proliferation assay kit, Promega, Madison, WI, USA) was used to estimate the cells’ viability via mitochondrial activity according to the manufacturer’s instructions.

**Statistical analysis**

The results expressed as mean ± standard deviation. Differences among groups were analyzed by Student t test.
A p value of less than 0.05 was considered to be statistically significant.

**Results**

UVB irradiation sequentially suppressed nuclear activation and translocation of NF-κB/p50 while induced nuclear activation and translocation of NF-κB/p65 on cultured keratinocytes

As demonstrated in Fig. 1, the nuclear activation and translocation of NF-κB/p50 in cultured human keratinocytes was significantly inhibited 6 hours after UVB irradiation. \((p < 0.05)\) In contrast, nuclear activation and translocation of NF-κB/p65 was not significantly altered as this time point. At 12 hours after UVB irradiation, cultured keratinocytes showed significant increase in NF-κB/p65 nuclear activation and translocation \((p < 0.05)\) while the changes seen in NF-κB/p50 were insignificant.

Immunofluorescence staining showed decreased nuclear expression of NF-κB/p50 on cultured human keratinocytes after UVB irradiation

The results from immunofluorescence analysis demonstrated that there were no significant nuclear expressions of NF-κB/p65 at 6 hours with or without UVB irradiation (Fig. 2A). However, UVB irradiation significantly suppressed the constitutive expression of NF-κB/p50 in keratinocytes in the early stage (6 hours). These results are shown in Fig. 2B. These findings further confirmed the results shown in Fig. 1.

Immunocytochemical staining showed constitutive nuclear expression of NF-κB/p50 on cultured human keratinocytes

Little nuclear expression of NF-κB/p65 was observed constitutively. Twelve hours after UVB irradiation, nuclear expression of NF-κB/p65 was observed constitutively. Twelve hours after UVB irradiation, nuclear expression of NF-κB/p65 was observed constitutively.

**Figure 2.** Immunofluorescence staining for different NF-κB subunits 6h after UVB irradiation. (A) Top: the cultured keratinocytes showed no constitutive NF-κB/p65 nuclear expression; bottom: UVB irradiation did not affect the nuclear expression of NF-κB/p65 (NF-κB/p65, 400×); (B) top: constitutive nuclear expression of NF-κB/p50 was noted in keratinocytes; bottom: significant suppression of nuclear NF-κB/p50 expression was observed after UVB irradiation (NF-κB/p50, 400×). The figures shown represented typical immunocytochemical findings in one of two independent experiments. NK-κB = nuclear factor kappa B; UVB = ultraviolet B.
expression of both NF-κB/p50 and NF-κB/p65 was observed. While no obvious change in nuclear expression of NF-κB/p50 was found after tacrolimus treatment, the NF-κB/p65 nuclear expression was suppressed, demonstrating the inhibitory effect of tacrolimus on nuclear expression of NF-κB/p65 after UVB irradiation and corroborating with our previous finding [2]. Representative fields of our immunohistochemical staining were demonstrated in Fig. 3. 

Immunohistochemical staining showed constitutive nuclear expression of NF-κB/p50 on the mouse skin

The NF-κB/p65 expression on the biopsy specimens obtained without UVB irradiation was mostly confined to the cytoplasm of epidermal cells. Topical tacrolimus treatment did not influence the nuclear expression of NF-κB subunits on the non-UVB irradiated group. On the other hand, biopsy specimens obtained after UVB treatment showed nuclear expression of both NF-κB/p50 and NF-κB/p65. Topical tacrolimus treatment immediately after UVB exposure suppressed nuclear expression of NF-κB/p65 on mouse skin while the nuclear expression of NF-κB/p50 was not significantly affected. Representative fields of our immunohistochemical staining were demonstrated in Fig. 4.

Tacrolimus did not significantly alter the nuclear activation and translocation of NF-κB/p50 subunit

Since the nuclear activation and translocation NF-κB/p50 was significantly suppressed 6h after UVB irradiation, we set out to determine the impact of tacrolimus on UVB irradiated keratinocyte at this time point in terms of NF-κB/p50. As shown in Fig. 5, no significant changes were induced by different concentrations of tacrolimus.
Tacrolimus did not significantly alter keratinocyte viability 6h after UVB irradiations

To ensure that our analyses of NF-κB/p50 subunits in UVB irradiated keratinocytes were valid, we evaluated the cell viability of UVB irradiated keratinocyte after treating with various concentrations of tacrolimus for 6h. As demonstrated in Fig. 6, tacrolimus treatment did not significantly alter keratinocyte viability, confirming that our previous analyses were valid.

Discussion

It is known that UV exposure elicits skin inflammation, and activation of the NF-κB transcription factor is a clear molecular effect imparted by UVB irradiation [26,27]. The NF-κB family transcription factors play an important role in the inflammatory process [28–30]. Adachi and colleagues [31] has previously shown that, in epidermal keratinocytes, different UV lights elicited specific signal transduction pathway. Although it was known that UVB irradiations were capable of inducing NF-κB activity [32], little has been done to explore the regulation of UVB irradiation on specific NF-κB subunits. We have previously shown that TNF-α secretion by keratinocytes was significantly increased after UVB irradiation [2]. Moreover, it has been reported that TNF-α expression is negatively regulated by certain NF-κB subunits, such as NF-κB/p50 and Rel B [10–12]. Although the precise mechanism by how Rel B regulates TNF-α gene transcription remains poorly defined [11,12], more insights have been gained on how NF-κB/p50 may affect TNF-α production. It is known that the distal region of TNF-α promoters carry κB sites that preferentially bind with NF-κB/p50 homodimers, a phenomenon shared by human and murine cells [10,33]. Murine macrophages...
have been shown to secrete certain factors that could suppress TNF-α production via enhancing the expression of NF-κB/p50 [10]. This inhibitory effect was eliminated if the preferential NF-κB/p50 binding sites were removed. Recently, Oakley and colleagues [34] has shown that NF-κB/p50 subunits play a critical role in the injured liver by limiting the expression of TNF-α in a mouse model. It also has been reported that NF-κB/p50 lacks transcriptional activation domains and has been found to have neutral or inhibitory effects on other promoters [14,35,36]. Baer and colleagues [10] has shown that the inhibition at distal NF-κB promoter sequences via NF-κB/p50 induction appeared to dominant over the positive effect imposed by NF-κB/p65 on the proximal region, suggesting an active repression mechanism by NF-κB/p50 at the distal promoter site.

One important feature that distinguished NF-κB/p50 homodimer from p50/p65 heterodimer is that contrasting cytoplasmic confinement of p50/p65 heterodimers, p50/p50 homodimers are found in the nuclei of most resting cells and are capable of DNA binding [13–15,37–39].

The result from our immunofluorescence, immunocytochemical, and immunohistochemical studies showing constitutive nuclear NF-κB/p50 expression but not NF-κB/p65 expression corroborated with their findings. In addition, our result also indicated that UVB sequentially regulate the activities of different subunits of NF-κB: the activity of NF-κB/p50 was down regulated in the early stage (6 hours), followed by up regulation of NF-κB/p65 in the later stage (12 hours). Since the unstimulated cells expressed constitutive nuclear NF-κB/p50 expression, downregulation of suppressive NF-κB/p50 appears to be reasonable prior to the induction of NF-κB/p65 activator.

Tacrolimus, a topical immunomodulator used for treating inflammatory dermatoses, has demonstrated remarkable effects on atopic dermatitis [40]. It is believed that cytokine TNF-α is a major mediator of inflammation in various inflammatory skin conditions. After UVB irradiation, cultured keratinocytes showed marked elevation of TNF-α secretion [1,2]. Although several possibilities have been proposed to involve in the mechanisms of UVB induced TNF-α secretion, previous studies have suggested that UVB induces TNF-α expression via activation of NF-κB [2,22]. Although the results from the immunochemical studies and our previous report showed that tacrolimus suppressed NF-κB/p65 subunit activity [2], our current result also indicated that tacrolimus has little effect on the nuclear activation and translocation of NF-κB/p50. Therefore, the capacity of tacrolimus on inhibiting NF-κB activity may be enhanced if dual inhibitory function could be exerted: more specifically, by inducing nuclear translocation of the repressive NF-κB/p50 homodimers while inhibiting translocation of the classical p50/p65 heterodimers simultaneously.

Taken together, we have shown that UVB irradiation sequentially regulates different NF-κB subunits. Moreover, the clinical efficacy of tacrolimus may be attributed to its specific regulatory effect on NF-κB/p65 but not NF-κB/p50. Since inhibition of inflammatory cytokine promoter by NF-κB/p50 appears to dominate over activation imposed by NF-κB/p65, the potential therapeutic roles of long standing specific NF-κB/p50 inducer await further exploration.

References


Expression of NF-kB by UVB and tacrolimus


