Role of Nuclear Factor kappa B (NF-*k*B) in Dermatology

Protocol for Essay

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? List Of Abbreviations

AIDS	Acquired immune deficiency syndrome
ARD	Ankyrin repeat domain
ATA	Aurine tricarboxylic acid
ATP	Adenosine triphosphate
BAFF	B-cell activating factor belonging to the tumor necrosis factor (TNF) family
Bcl-2	B-cell lymphoma 2
Bcl-3	B-cell lymphoma 3-encoded protein
Bcl-2 L2	Bcl-2-like protein 2
Bcl-xl	B-cell lymphoma-extra large
BMS	Bristol-Myers Squibb
cDNA	Circular deoxyribonucleic acid
CLP	Cutaneous lichen planus
cIAPs	Cellular inhibitors of apoptosis proteins
c-MYC	Avian myelocytomatosis viral oncogene homolog
Cox-2	Cyclooxygenase 2
c-Rel	Cellular form of avian reticuloendotheliosis viral oncogene homolog
DNA	Deoxyribonucleic acid
DsDNA	Duble stranded Deoxyribonucleic acid
EBV	Epstein–barr virus
ED	Ectodermal dysplasia
EDA	Anhidrotic ectodermal dysplasia
EDA-ID	Anhidrotic ectodermal dysplasia with immunodeficiency
EGCG	Epigallocatechin-3-gallate
ELAM-1	Endothelial leukocyte adhesion molecule 1
HBV	Hepatitis B virus
HCV	Hepatitis C virus

HIV-1	Human immunodeficiency virus type 1
HSV-1	Herpes simplex virus type 1
HTLV-1	Human T-cell leukemia virus type 1
ICAM	Intercellular adhesion molecule
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
I*κ*B	Inhibitory-kappa B
IKK	Inhibitory-kappa B (I*κ*B) kinase
iNOS	The inducible form of nitric oxidase synthase
IP	Incontinentia pigmenti
IP-10	Immune protein-10
K	kappa
kDa	Kilodalton
KS	Kaposi's sarcoma
LMP1	Latent membrane protein 1
LPS	Lipopolysaccharide
MHC	Major histocompatability complex
MIP-1-α	Macrophage inflammatory protein 1 alpha
MMPs	Matrix metalloproteinases
mRNA	Messenger ribonucleic acid (RNA)
Nef	Negative regulatory factor
NEMO	Nuclear factor-kappa B (NF-*κ*B) essential modifier
NES	nuclear-export signal
NF-*k*B	Nuclear factor-kappa B
NIK	Nuclear factor-kappa B (NF-*κ*B) binding kinase
NLS	Nuclear localization sequence
NSAIDs	Nonsteroidal anti-inflammatory drugs

OL-EDA-ID	Anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID) with osteopetrosis and lymphoedema
OLP	Oral lichen planus
OspA	Outer surface protein A
PDTC	Pyrrolidinedithiocarbamate
PEST	Proline, glutamic acid, serine, and threonine
PGA1	Prostaglandin A1
PTEN	Phosphatase and tensin homolog
RA	Rheumatoid arthritis
RANK	Receptor activator of Nuclear factor-kappa B (NF-κB)
RelA	v-Rel homolog A
RelB	v-Rel homolog B
RHD	Rel homology domain
RNA	ribonucleic acid
ROS	Reactive oxygen species
SLE	Systemic lupus erythematosus
SRD	Signal receiving domain
TAD	Transcriptional activation domain
Tat	Transactivator of transcription
TNF-α	Tumor necrosis factor-alpha
TRAF	TNF receptor-associated factor
UV	Ultraviolet
VCAM-1	Vascular cell adhesion molecule 1
Vpr	Viral protein R
v-Rel	Avian reticuloendotheliosis viral oncogene homolog
VZV	Varicella zoster virus
XL-EDA–ID	X-linked anhidrotic ectodermal dysplasia with immunodeficiency

@ Introduction

Transcription is an important regulatory event in the pathway leading to gene expression. Transcription factors regulate transcription by binding to specific sequences present within the regulatory regions of deoxyribonucleic acid (DNA). Hundreds of transcription factors with functionally separable domains, essential for DNA-binding and activation, have been identified and characterized in several organisms. One such transcription factor, nuclear factor-kappa B (NF-*k*B), has been the subject of intense study based on the implications of its role as a key mediator of a wide variety of cellular responses **(Garg and Aggarwal, 2002)**. NF-*k*B is implicated in multiple physiological and pathological processes, including cell proliferation and differentiation, inflammatory and immune responses, cell survival and apoptosis, cellular stress reactions and tumorigenesis **(Yu et al, 2009).**

NF-*k*B is a nuclear transcription factor that was first identified in 1986 **(Sen and Baltimore, 1986).** It was so named because it was found in the nucleus bound to the immunoglobulin (Ig) kappa (*k*) light -chain gene in B cells. It was initially considered to be a B-cell–specific transcription factor but was later shown to be present in many different cells **(Aggarwal, 2004).**

The NF-*k*B transcription factor family is composed of several structurally related proteins that exist in organisms ranging from insects to humans. The vertebrate family includes five cellular proteins that revealed a surprising homology to the oncogene avian reticuloendotheliosis viral

oncogene homolog (v-Re1): **p65** which is also known as v-Rel homolog A **(RelA)**, v-Rel homolog B **(RelB)**, cellular form of v-Rel **(c-Rel)**, **p50 (NF-*κ*B1)**, and **p52 (NF-*κ*B2)**. These proteins form homodimers or heterodimers which bind to DNA target sites known as *k*B sites, where they directly regulate gene expression (**Ahn and Aggarwal, 2005).** In human skin, the NF-*k*B subunits, p50, p65, RelB, and c-Rel, were detected in both basal and suprabasal keratinocytes and in normal epidermis at massenger ribonucleic acid (mRNA) and protein levels (**Bell et al, 2002).**

NF-*κ*B is normally sequestered in the cytoplasm by a family of inhibitory proteins known as inhibitory-kappa B (I*k*B) proteins that retain NF-*κ*B in an inactive, thus preventing NF-*κ*B uptake into the nucleus.**I*κ*B-α**, **I*κ*B-β**, B-cell lymphoma 3-encoded protein **(Bcl-3), I*κ*B-γ**, **I*κ*B-ε**, **p100**, and **p105** comprise the seven members of the I*κ*B family**(Wan and Lenardo, 2010).** NF-*k*B is activated by many stimuli, including proinflammatory cytokines such as tumor necrosis factor-alpha(TNF-α), interleukin-1 beta (IL-1β), bacteria, lipopolysaccharides (LPS), viruses, viral proteins, physical, and chemical stresses, also, cellular stresses such as ionizing radiation and chemotherapeutic agents activate NF-*k*B **(Aggarwal et al, 2006).**

Upon receiving a signal, I*k*B proteins are phosphorylated by the I*k*B kinase (IKK) complex which consists of **IKK-α** and **IKK-β** serving as kinases and NF-*κ*B essential modifier **(NEMO) or IKK-γ** functioning as a regulatory subunit, and targeted for degradation by the 26s proteosome allowing translocation of the NF-*k*B dimer to the nucleus where it can activate certain genes which mediate cell proliferation as well as inflammatory and immune responses **(Hacker and Karin, 2006).**

Activation of NF-*k*B is a tightly regulated and transient event in normal cells as one of the first genes that NF-*k*B activates is I*k*B-α itself, which transports activated NF-*k*B from the nucleus to the cytoplasm **(Sethi et al, 2008).**

Normal activation of NF-*k*B is required for cell survival and immunity. NF-*k*B is a master transcriptional regulator critical for ectodermal development, differentiation, keratinocytes regulation and proliferation and immune function. In addition to an apparent cell-cycle arrest effect, NF-*k*B in the epidermis also protects against apoptosis **(Lizzul et al, 2005).**

In pathological conditions, different types of molecular alterations may result in impaired regulation of NF-*k*B activation. In such cases, NF-*k*B loses its transient nature of activation and becomes constitutively activated. This leads to deregulated expression of NF-*k*B–controlled genes **(Sethi et al, 2008)**. Aberrant regulation of NF-*k*B leads to development of many pathological states including proliferative and inflammatory disorders, such as psoriasis, lichen planus, atopic dermatitis, incontinentia pigmenti (IP), sunburn, Lyme disease, and autoimmune diseases such as systemic lupus erythematosus (SLE), as well as in skin carcinogenesis and several inherited diseases **(Bell et al, 2002).**

NF-*κ*B is highly activated at sites of inflammation in diverse diseases and can induce transcription of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6, chemokines such as macrophage inflammatory protein 1 alpha (MIP-1-α) and immune protein-10 (IP-10), adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule (ICAM), and endothelial leukocyte adhesion molecule 1 (ELAM-

1) matrix metalloproteinases (MMPs), cyclooxygenase 2 (Cox-2), and inducible nitric oxide synthase (iNOS). The identification of NF-κB as a key player in the pathogenesis of inflammation suggests that NF-κB–targeted therapeutics might be effective in inflammatory diseases. A variety of drugs used to treat human inflammatory disease have effects on NF-κB activity **(Yamamoto and Gaynor, 2001).**

NF-*k*B has emerged as an important player in the development and progression of malignant tumors, such as melanoma and squamous cell carcinoma. Aberrant regulation of NF-κB and the signaling pathways that control its activity are involved in cancer development and progression, as well as in resistance to chemo- and radiotherapy **(Baud and Karin, 2009)**. NF-*k*B targets genes that promote tumor cell proliferation, survival, metastasis, inflammation, invasion, and angiogenesis **(Sethi et al, 2008).** As NF-*k*B is involved in cancer development, modulating NF-*k*B activation pathways has important implications in cancer prevention and therapy **(Lin et al, 2010).**

The important involvement of NF-*k*B in the onset of autoimmune diseases and different types of cancer makes it an important drug target for the adjuvant therapy of these diseases. Great efforts have been made for the development of highly specific NF-*k*B inhibitors **(Calzado et al, 2007).** Several strategies have been employed to block the activation of NF-*k*B. Many inhibitors have been developed and act by targeting the NF-*k*B pathway at several stages (**Garg and Aggarwal, 2002).**These compounds include antioxidants e.g.: gutathione and a-lipoic acid, I*k*B phosphorylation inhibitors e.g.: thalidomide and sulfasalazine, proteasome inhibitors e.g.: bortezomib; lactacystin and epoxomicin, and NF-*k*B-DNA binding

inhibitors e.g.: oligodeoxynucleotide and vasoactive intestinal peptide **(Pande and Ramos, 2005).**

Ø *Aim of the essay:*

The aim of this essay is to throw the high light on Nuclear Factor kappa B (NF-*k*B) and its role in normal skin, in the pathogenesis of skin dermatoses and its uses as a therapeutic option in different dermatologic diseases.

1. WHAT IS NF- *k*B?

NF-*κ*B is a family of the most-studied transcription factors which are involved in responses to environmental changes. NF-*k*B represents a group of structurally related proteins that are regulated via shuttling from the cytoplasm to the nucleus in response to cell stimulation **(Birbach et al, 2002).** NF-*k*B regulates the transcription of an exceptionally large number of genes, particularly those involved in immune and inflammatory responses as well as those promote cell growth and survival **(Tripathi and Aggarwal, 2006).**

NF-*κ*B was originally discovered as a factor in the nucleus of murine B-lymphocytes that binds to immunoglobulin *κ* light chain gene **(Sen and Baltimore, 1986).** It was initially considered to be a B-cell–specific transcription factor but was later shown to be present in many different cells **(Aggarwal, 2004).**

Since its discovery, intense investigations have shown NF-*κ*B to have a role in a variety of biological processes including the immune response, inflammation, proliferation and apoptosis. Also, some studies have begun associating the NF-*κ*B transcription factors with numerous diseases including inherited syndromes such as incontinentia pigmenti, and cystic fibrosis as well as cancer, inflammatory diseases, asthma, neurodegenerative diseases, and immunodeficiency disorders **(Courtois, 2005& Xiao, 2004).**

1.1. Structure of NF-*κ*B:

There are five members of the mammalian NF-*κ*B family which form homo- and heterodimeric DNA-binding complexes and revealed a surprising homology to the oncogene v-Rel; **p65** which is also known as **RelA**, **RelB**, **c-Rel**, **p50(NF-*κ*B1)**, and **p52(NF-*κ*B2)**. In mammals, the genes rela, relb, c-rel, nfkb1, and nfkb2 encode the five NF-*κ*B protein family members RelA (p65), RelB, c-Rel, p50, and p52, respectively **(O'Dea and Hoffmann, 2009).** All members of NF-*κ*B family (except RelB) form a variety of homo-and heterodimers. RelB does not homodimerize but it forms heterodimers with either p50 or p52 **(Schmitz et al, 2004).**

Each member of this family contains a Rel homology domain (RHD) which is a 300-amino-acid region, including DNA-binding domain, dimerization domain, and the nuclear localisation sequence (NLS). The RHD is responsible for dimerization, DNA-binding, and it contains the NLS which is most likely the site for binding of I*k*B proteins which act as NF-*k*B inhibitors **(Ghosh et al, 1998).**

Whereas c-Rel, RelB and p65 each contains a transcriptional activation domain (TAD) that is responsible for activation of target genes, p50 and p52 do not have TAD and they function as repressors of NF-*κ*B transcriptions **(Yates and Górecki, 2006).** P50 and p52 are synthesized as inactive cytoplasmic precursors p105 and p100, which perform I*k*B functions in preventing p50 and p52 from binding to *k*B sites**.** Proteolytic generation of the active p50 and p52 from their precursors is signal dependent and requires adenosine triphosphate (ATP) **(Lang et al, 2003).**

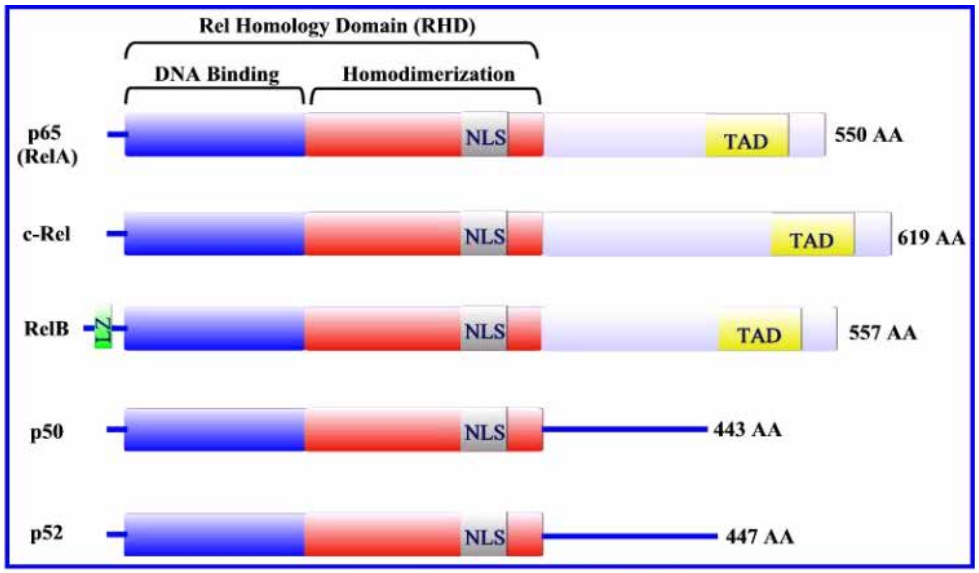

Figure 1 Members of NF-kB family

Figure (1): Members of NF-κB family. NF-κB family consists of five members, including p65 (RelA), c-Rel, RelB, p50 and p52. It is characterized as containing a Rel-homology domain (RHD), which includes DNA-binding and dimerization domains, and a nuclear localization sequence (NLS). Three members, p65, c-Rel and RelB, contain the transactivation domain (TAD) that is required for the transcriptional activation activities, but the TAD is lacked in other two members, p50 and p52 and thereby p50:p52 homodimer function as repressors of NF-κB transcriptions **(Xiao, 2004).**

1.2. Inhibitory-kappa B (IκB) proteins:

NF-κB is normally sequestered in the cytoplasm by a family of inhibitory proteins known as IκBs that retain NF-κB in an inactive form in the cytoplasm by masking the NLS within the RHD thereby preventing NF-κB uptake into the nucleus.Only when NF-κB has been released, it will translocate to the nucleus and bind specific κB sequences in the regulatory regions of target genes where they directly regulate gene expression **(Wan and Lenardo, 2010).**

IκB-α, **IκB-β**, **BCL-3**, **IκB-γ**, **IκB-ε** along with the precursor NF-κB proteins **p100** and **p105** comprise the seven members of the IκB family **(Hayden and Ghosh, 2004).** P100 and p105 function as IκBs due to the presence of several ankyrin repeats responsible for binding the dimerisation domain of NF-κB dimmers. Except Bcl-3, containing TAD

and functional in nucleus, all the other members of I*κ*B family lack the TAD and are functional in cytoplasm **(Xiao, 2004).** Only I*κ*B-α contains a nuclear-export signal (NES) at its amino terminus, which is essential for shuttling the NF-*κ*B–I*κ*B-α complex out of the nucleus **(Li and Verma, 2002).**

I*κ*B-α contains a centrally located ankyrin repeat domain (ARD). Ankyrin repeats are 33 amino acid modules originally identified in the human erythrocyte protein ankyrin then they have been identified in numerous proteins with diverse functions**.** Ankyrin repeats are protein-protein interaction domains that bind to the RHD to block the NLS of NF-*κ*B (**Almawi and Melemedjian, 2002).** Amino-terminal to the ARD, the signal receiving domain (SRD) contains amino acid residues that accept the phosphorylation and ubiquitination activation signals.Though a vital component of NF-*κ*B activation, the SRD does not appear to physically participate in I*κ*B/NF-*κ*B complex formation.The segment carboxy-terminal to the ARD is rich in the amino acids; proline, glutamic acid, serine, and threonine (PEST), and his region becomes phosphorylated at serine and threonine residues by casein kinase II and is associated with basal protein degradation**,** so the PEST sequence is important for basal degradation of free I*κ*B-α. PEST region of I*κ*B-α also participates with the ARD in forming the I*κ*B-α/NF-*κ*B complex **(Huxford et al, 1998).**

A number of studies revealed that separate I*κ*B molecules preferentially inhibited distinct NF-*κ*B protein dimers. For example, I*κ*B-α and I*κ*B-β preferentially interacted with dimers containing p65 and had been shown to be the main functional modulators of the ‘classical’ NF-*κ*B p65/p50 heterodimer. Interestingly, the only I*κ*B molecules, which could

associate with ReIB are p100, and p105 which all could effectively inhibit p52/ReIB heterodimers **(Schmitz et al, 2004).**

I*κ*B-α is the most widely studied I*κ*B family member and regulates the activity of the NF-*κ*B p50–p65 heterodimer. In addition to being present in the cytoplasm, I*k*B-α and I*k*B-β can affect NF-*k*B by binding to *k*B sites in the nucleus**.** In particular, I*k*B-α, and probably also I*k*B-β, interact with p50/p65 NF-*k*B heterodimers in the nucleus as it does in the cytoplasm and can free p50/p65 heterodimers from *k*B sites and then induce transport of these bound heterodimers from the nucleus into the cytoplasm, resulting in inhibition of NF-*k*B-dependent transcriptional **(Lang et al, 2003& Beinke and Ley, 2004).**

Bcl-3 is a protein that in humans is encoded by the Bcl-3 gene which is a proto-oncogene candidate identified in some cases of B-cell leukemia. Bcl-3, through its binding to p50/p50 homodimers, can enhance the expression of genes whose transcription is positively regulated by NF-*k*B **(Lenardo and Siebenlist, 1994).** In particular, p50/p50 homodimers can occupy NF-*k*B binding sites and act as transcriptional repressors by preventing transactivating NF-*k*B heterodimers such as p50/p65 or p50/c-Rel from binding to these sites. Bcl-3, when it binds to p50/p50 homodimers in the nucleus, it results in their release from the NF-*k*B binding sites, allowing these sites to be occupied by transactivating NF-*k*B heterodimers such as p50/p65 to bind and enhance transcription. Therefore, increased nuclear levels of Bcl-3 may enhance NF-*k*B-dependent gene expression **(Sun and Andersson, 2002).**

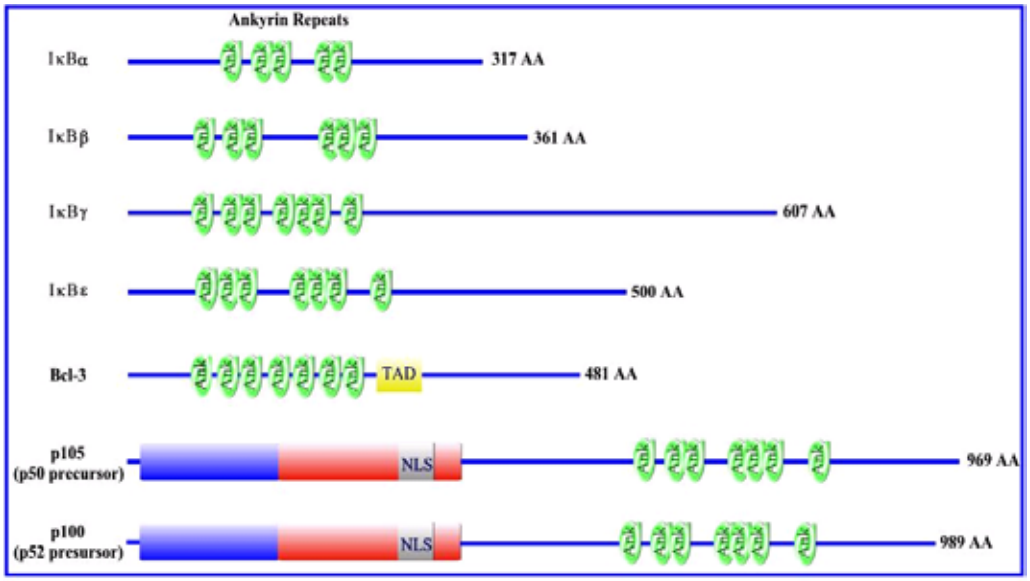

Figure 2 Members of IκB family

¡ **Figure (2): Members of I*k*B family.** The inhibitory *κ*B (I*κ*B) family are sharing the conserved ankyrin repeat motifs (labeled as Ank) that are essential for I*κ*B activity and binding to the RHD of NF-*κ*B proteins, and consist of seven members: I*κ*B-α, I*κ*B-β, I*κ*B-γ, I*κ*B-ε, Bcl-3, p105 and p100. The number of ankryin repeats varies from five (I*κ*B-α), six (I*κ*B-β) and seven (Bcl-3, I*κ*B-ε, I*κ*B-γ, p100 and p105). Except Bcl-3, containing the transcriptional activation domain (TAD) and functional in nucleus, all the other members of I*κ*B family lack the TAD and functional in cytoplasmic. P105 and p100 are the precursors for p50 and p52, respectively **(Xiao, 2004).**

1.3. Function of NF-*k*B:

NF-*κ*B family forms a variety of homo- and heterodimers which are capable of regulating a different gene complement. NF-*κ*B regulates hundreds of targets genes, either directly through binding to the promoter, or indirectly through the transcriptional regulation of intermediate factors, so NF-*κ*B plays a critical role in diverse cellular processes associated with proliferation, cell death, development, as well as innate and adaptive immune responses **(Wan and Lenardo, 2010).**

The specific NF-*k*B dimers have distinct DNA-binding site specificities for the related 9-10 base pair DNA sites (called *κ*B sites) which are present in the regulatory regions of target genes, and this allows for the regulation of distinct sets of genes. NF-*k*B dimers could act as a transcriptional activators or repressors at *k*B sites, depending on whether