AC-11® as it relates to NF-kappaB - an additional pathway for why AC-11® improves our natural DNA Repair Processes –

DNA is "coiled" (don't confuse this with the helix structure – two entirely different situations) around proteins called histones that give the chromosomes their physical shape (3-dimensional; X-chromosome, Y-chromosome etc.) and helps protect genes (in the cell's nucleus) from damage by also forming a protective coating that absorbs free radicals. A common source of breaks in a DNA strand (DNA damage) occurs when free radicals penetrate the protective histone coating.

In order for the natural DNA repair process to occur, DNA has to be uncoiled to allow access to damaged base pairs/areas. For example, a break in a DNA strand causes the release of a compound ADPRT (adenosine diphosphate ribosyl transferase) which "uncoils" DNA. This is commonly referred to as "opening up the chromatin." Once the chromatin is opened the various natural DNA repair mechanisms, such as incision repair, cut out the damaged section and insert a DNA replacement base pair(s).

NF-kappaB (nuclear factor kappa B), which is present in the intracellular fluid, is activated by oxidative stress as well as glycation (altered proteins caused by elevated and poorly controlled blood glucose levels, inflammation and methylation). NF-kappaB inhibits ADPRT. If ADPRT can't open (uncoil) the Chromatin, the DNA Repair process does not proceed. Also, the inhibition of NF-kappaB (by inhibition of a transcription factor) blocks cells from dividing, allowing more time for the DNA repair process to proceed.

Thus chemicals or phytochemicals that support the normal and healthy expression of NF-kappaB and TNF-alpha, such as AC-11® based upon its patented actives –CAE's™, indirectly improve the rate (ability, efficiency and capacity) of DNA Repair and help maintain the integrity of the genetic code.

Some additional comments about NF-kappa beta and reducing DNA Damage:

NF-kappa, when activated, also increases TNF-alpha (tissue necrosis factor-alpha) which in turn increases IL-1a, IL-1b, IL-6 and IL-8; these are the pro-inflammatory cytokines and leukotrienes. Because the pro-inflammatory cytokines (along with increasing inflammation) activate NF-kappaB, a self-reinforcing cycle is established yielding increased TNF-alpha, more pro-inflammatory cytokines and inflammation, which in conjunction with oxidative stress continues the activation of NF-kappaB – and so on and so on. (Excessive inflammation in the body is a primary cause of oxidative stress and premature aging and disease.)

Increased levels of TNF-alpha, besides inhibiting immune function, increase oxidative stress which results in increased DNA Damage. So phytochemicals that support the normal and healthy expression of NF-kappaB and TNF-alpha, such as AC-11®, decrease oxidative stress and its related DNA Damage.

The NF-κB Inhibitory Effect of AC-11

The transcription factor NF-κB has been shown to influence a variety of biological functions, one of which is inflammation. NF-κB is mostly contained in the cytoplasm of cells in combination with the inhibitor protein, IκB (Baldwin, 1996). Once separated, NF-κB migrates into the nucleus (nuclear translocation) where it activates genes encoding immunologically relevant proteins (such as TNF-α, IL-1β, IL-6, and IL-8; Baeuerle and Henkel, 1994; Tak and Firestein, 2001). The activation of NF-κB occurs under a variety of disparate conditions, including exposure to bacterial toxins, viruses and viral products, mitogens, UV light, γ-radiation, and oxidative stress (Baeuerle and Henkel, 1994). Most of the stimuli that result in NF-κB activation reflect exposure to pathologic conditions.

NF-κB has been associated with inflammatory diseases such as rheumatoid arthritis, asthma, and inflammatory bowel disease (Baldwin, 2001; Tak and Firestein, 2001). Additionally, it has been shown to be activated in the synovia of arthritics and can be inhibited by anti-arthritic therapies, including aspirin, prednisone, and gold (Baldwin, 1996).

Three separate *in vitro* experiments were conducted to assess the effect of AC-11 on the nuclear transcription factor, NF-κB (Table 1; Åkesson et al, 2003). In Experiment A, mouse pre-B lymphocyte cells were pre-treated with 0, 0.156, 0.312, 0.625, and 1.25 mg/ml of AC-11 for 3 or 4 hours then stimulated with lipopolysaccharide before staining to identify Igκ antibodies. The investigators reported that Igκ positive cells were inhibited in a dose-dependent manner. NF-κB binds to an enhancer element in the Igκ gene (Zhang and Ghosh, 2001). Thus, inhibition of NF-κB would be reflected in Igκ levels. These data are presented in Figure 1.

Table 1. The *in vitro* effect of AC-11 on NF-κB (Åkesson et al, 2003)

Experiment	Cell Type	AC-11 Concentration(s)*	Outcome
A	70Z/3 mouse lymphocyte	0, 0.156, 0.312, 0.625, 1.25	Igκ† was inhibited
B	Jurkat T-cells	0, 0.5, 1.0	NF-κB activity decreased
C	70Z/3 mouse lymphocyte	1.0	No inhibition of LPS-induced‡ IκBα degradation

*Concentrations reported in mg/ml.
†NF-κB inhibition would be expected to decrease Igκ levels.
‡LPS = lipopolysaccharide.

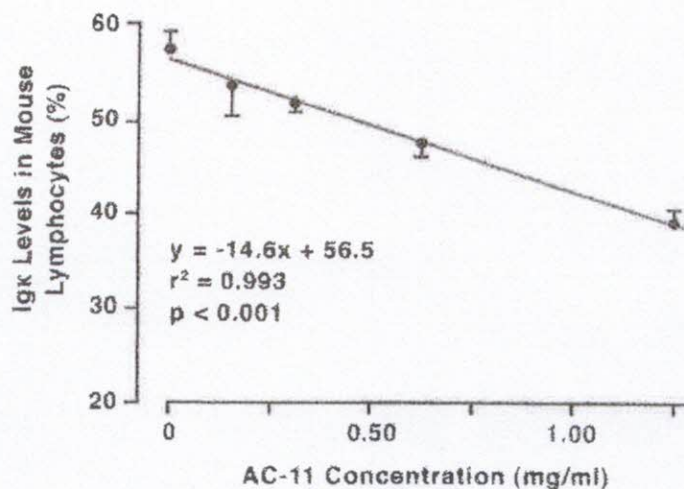$$y = -14.6x + 56.5$$
$$r^2 = 0.993$$
$$p < 0.001$$

Figure 1. The effect of AC-11 at various concentrations on Igκ expression in mouse lymphocytes (adapted from Åkesson et al, 2003).

In Experiment B, Jurkat human acute T-cell leukemia cells, transfected with a construct carrying NF-κB activated luciferase reporter gene, were pre-treated with 0, 0.5, and

1.0 mg/ml of AC-11 for 2 hours and then stimulated for 6 hours before measuring luciferase activity. At the highest concentration tested, AC-11 was found to reduce NF-κB activity by almost 50% (Figure 2).
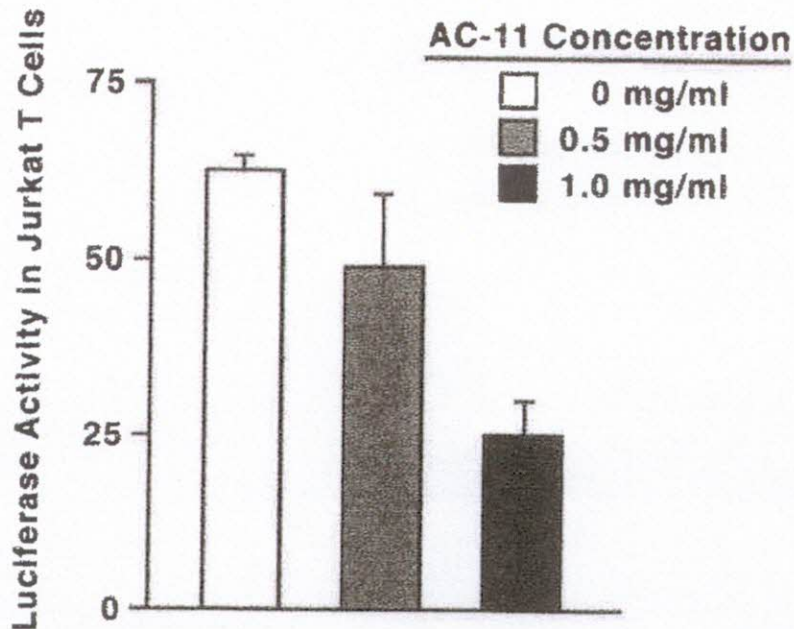

Figure 2. Luciferase activity in human Jurkat T cells transfected with an NF-κB reporter construct (adapted from Åkesson et al, 2003).

One of the experiments (Experiment C; Table 1), revealed that AC-11 did not promote the degradation of the NF-κB inhibitory protein, IκB. Otherwise, there are no data which provide an insight into how AC-11 inhibits NF-κB, nor are there data which correlate AC-11 intake (dose) and the degree of NF-κB inhibition produced. Because IκBα (the specific inhibitory protein measured in Experiment C) is one of a family of IκB proteins (Tak and Firestein, 2001), it could be argued that the concentration of one of the other inhibitory proteins or kinases may be significantly altered in the presence of AC-11 under physiological conditions. However, the effect of AC-11 on IκBβ, IκBε, or any of the IK kinases (IKK-1, IKK-2, IKK-γ, IKKi) have not been investigated.

Another possible mechanism by which AC-11 might inhibit NF-κB (which has not been investigated) is by binding to (or

otherwise activating) the glucocorticoid receptor (Auphan et al, 1995). As has been demonstrated (with dexamethasone) activation of the glucocorticoid receptor results in the synthesis of IκB which in turn binds to NF-κB in the cytoplasm. Once NF-κB is bound to IκB, translocation into the nucleus is prevented as well as the activation of genes that would release chemicals that propagate the inflammatory process.

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