NFκB translocation in macrophages after innate immune activation

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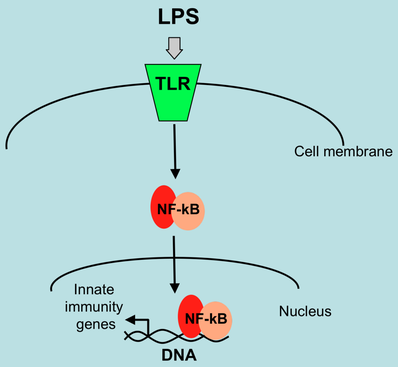
Introduction

The innate immune system, composed of cells and mechanisms that protect the host from invasion by other organisms, is the first line of defense against pathogens. Innate immunity provides immediate protection against infection, and one of the first cells to recognize potential danger to the body's health are macrophages.

Macrophages can engulf and kill pathogens. They can be activated by components of viruses or bacteria, such as bacterial lipopolysaccharide (LPS), which results in the increased ability to kill microbes. Nuclear factor kappa B (NFκB) is a transcription factor which plays a significant role in macrophages by activating hundreds of innate immune genes in response to LPS.

There are receptors that activate NFκB called toll-like receptors (TLR). These receptors activate the NFκB pathway, which links innate and adaptive immune response through the production of inflammatory cytokines. These receptors are a central element in the innate immune response, and are essential in recognizing and defending against invading pathogens.



Hypothesis

I hypothesize that when macrophages are stimulated by tumor necrosis factor-α (TNF-α), LPS or E. coli DH5-α, that LPS will activate NFκB p65 more quickly than the other stimuli. After LPS treatment, I predict that the p65-YFP will migrate to the nucleus and back out into the cytoplasm.

Methods

Cell Culture

Experiments were performed using Raw 264.7 macrophages expressing the NFκB p65-Yellow Fluorescent Protein (YFP) fusion-protein transgene, which had been artificially introduced into the macrophages.

Transformation

E. Coli strain DH5-α were thawed on ice in an eppendorf tube. A plasmid containing Green Fluorescent Protein (GFP) was added to the DH5-α cells. The bacteria were heat-shocked at 42°C and then incubated on an LB agar plate overnight with the antibiotic ampicillin. Ampicillin is a competitive inhibitor of the enzyme, transpeptidase, which is required by bacteria to make their cell walls. Only bacteria containing the GFP plasmid (with an antibiotic resistance gene for ampicillin) will survive. Next, one colony was selected and placed in LB broth and incubated overnight.

Stimuli

Three sets of macrophages were stimulated with lipopolysacchharide (LPS) at 200 ng/ML, Tumor Necrosis Factor-alpha (TNF-α), and the E. coli strain (DH5-α) with the GFP DNA plasmid.

Results

Figure 1

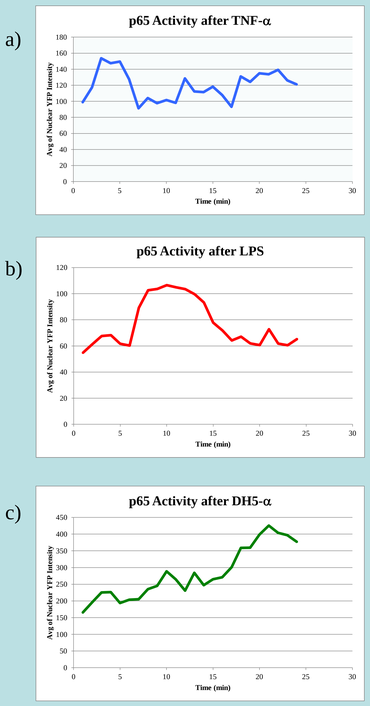


Figure 2

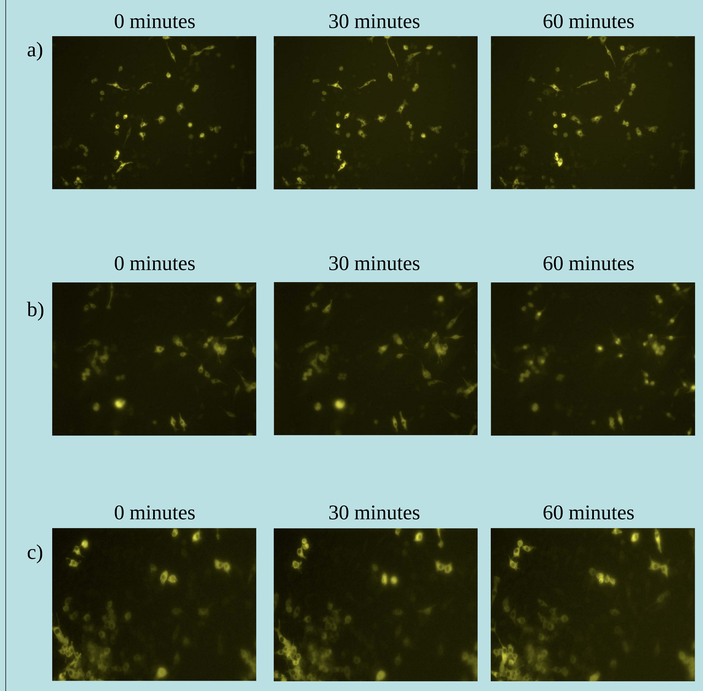


Figure 1. The average intensity of YFP within the nucleus after stimulus with a) TNF-α b) LPS or c) DH5-α.
Figure 2. Representative images of macrophages during the time course. p65-YFP macrophages were stimulated with a) TNF-α b) LPS or c) DH5-α.

Conclusions

According to the results, TNF-α translocates into the nucleus at 3 minutes at a low intensity, declining at 6 minutes with a lower but slightly elevated p65 activity until 25 minutes. In comparison, LPS stimulation induces low p65 activity until 5 minutes, at which point the nuclear activity begins to intensify, with its peak in the nucleus at 11 minutes, after which it declines slowly. Stimulation with DH5-α induced p65 activity with a slowly increasing slope, with a much later but higher peak at 22 minutes.

Surprisingly, stimulation with DH5-α induced the longest and highest amount of p65-YFP nuclear activity after treatment. The YFP intensity was also seen as the strongest throughout cytoplasm the cells, continuing to induce the activity of NFκB as late as 60 minutes after stimulation.

References

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