Single-cell dose dependent lipopolysaccharide response of NFκB nuclear translocation in macrophages

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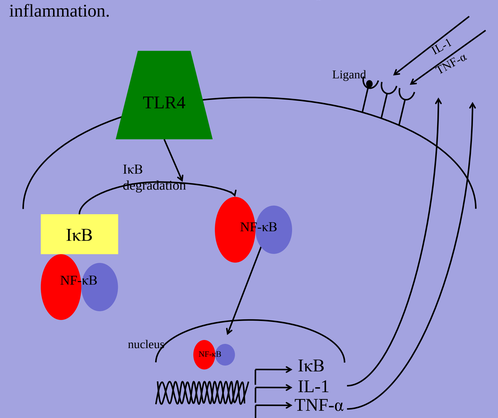
Abstract

Nuclear Factor kappa B (NFκB) is a transcription factor that plays a prevalent role in macrophages, activating hundreds of innate immune genes in response to Lipopolysaccharide (LPS). By stimulating RAW 264.7 macrophages expressing the NFκB p65-Enhanced Yellow Fluorescent Protein (EYFP) fusion-protein transgene with LPS, activation of the NFκB can be seen through nuclear translocation. Macrophages at different densities and different habitual condition were stimulated by low and high doses of LPS. The cells were plated at densities ranging from 16 thousand to 60 thousand cells per plate and were either in conditioned media or new media, then stimulated by the low LPS, 10ng/mL, or high LPS, 1μg/mL. The plated cells were then imaged at five-minute intervals for eleven hours, and the nuclear translocation of NFκB was tracked. In response to the stimulus, the high-density macrophages, which were clustered together, with the high dose of LPS, 1μg/mL, induced the greatest amount of p65-YFP nuclear activity, reactivation occurring at multiple intervals. The more dense cells responded better to the high amount of LPS in comparison to the less dense cells with the same stimulus because macrophages that are closer together have a higher induction rate of NFκB.

Background

The innate immune system is composed of cells and mechanisms that defend the host body from infection of other organisms, making it the body's first line of defense. This immunity system provides immediate protection against infection and bacteria. The cells that are the first to recognize the potential danger to the body's health are macrophages. These macrophages are cell types that absorb and consume pathogens. They can be activated by bacterial lipopolysaccharides (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.

Toll-like receptors (TLR) activate the NF- κB pathway, which links innate and adaptive immune response by production of inflammatory cytokines; such as TNF-α and IL-1. These receptors are a central element in the innate immune response, vital in recognizing and defending against invading pathogens. I-Kappa-B (IKB) initiates the activation of NF-κB by signal-induced degradation. With this degradation, the NF-κB is able to enter the nucleus and activate the specific DNA binding sites. The activation then leads to an immune response, such as inflammation.



Methods

Experiments were performed using Raw 264.7 macrophages expressing the NFκB p65-Enhanced Yellow Fluorescent Protein (EYFP) fusion-protein transgene, which had been artificially introduced into the macrophages. Macrophages were plated at various densities ranging from 16 thousand to 60 thousand cells per 35mm plate. The cells were then stimulated with either low or high doses of LPS; 10ng/mL and 1 μg/mL. Following the course of stimulation, the macrophages were left to be imaged under a Zeiss inverted light microscope at 5 minute intervals for 11 hours. The imaged cells were uploaded onto the program "Fiji (Just Image J)," where the nuclear intensity activity within the nucleus was tracked.

Figure 1

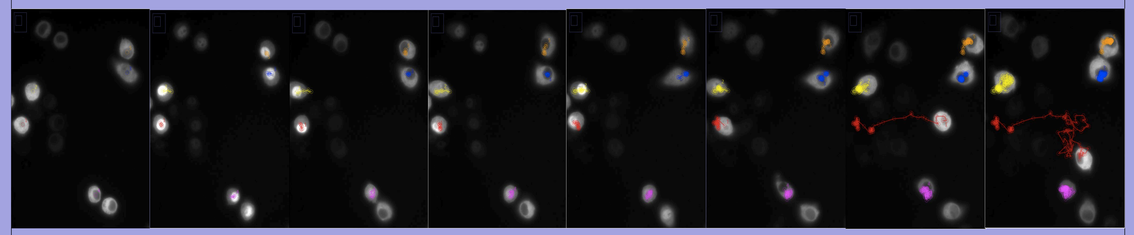
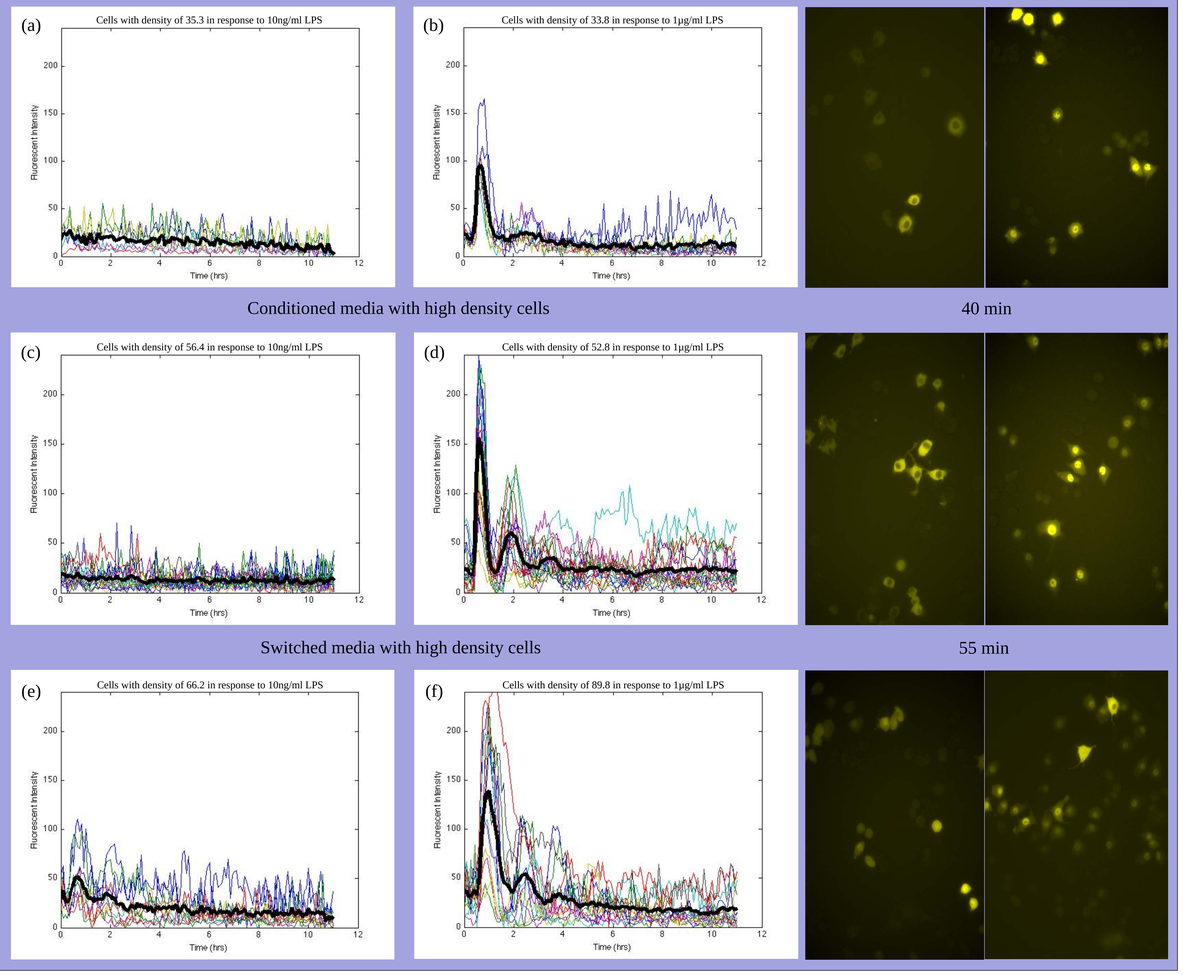


Figure 1. The imaged cells were uploaded onto the program "Fiji (Just Image J)," where the nuclear intensity activity within the nucleus was tracked.

Results



Analysis

Figure 2

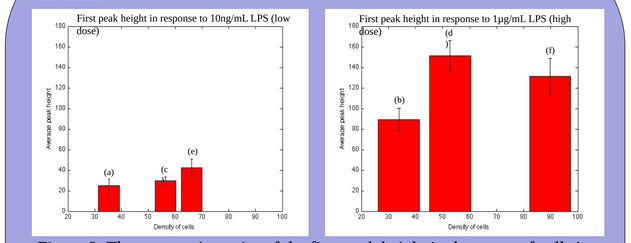
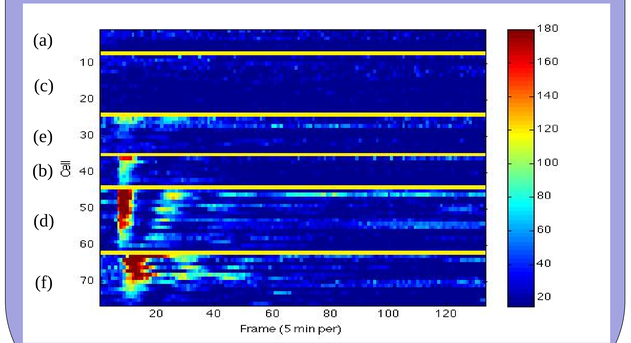


Figure 2. The average intensity of the first peak height in the range of cells in each condition. The first three conditions of low density; low LPS, high density; low LPS, and high density; new media; low LPS (a,c,e) depict that the NF-κB nuclear translocation was weaker. The second three conditions of low density; high LPS, high density; high LPS, and high density; new media; high LPS (b,d,f) show the strong response of NFκB.

Figure 3.

The colorbar illustrates the individual cell activity of NFκB in the each time frame of the eleven hour run.



Conclusion

According to the results, low-density macrophages stimulated with a low amount of LPS induced weak NFκB activity within the cell. The low-density cells with a high dose of LPS induced a uniform activation of NFκB within the nucleus with little reactivation. High-density cells stimulated with a low amount of LPS showed no nuclear translocation of NFκB, just as the low density cells with the same amount of LPS did. The high-density cells stimulated with the high dose of LPS showed stronger activation with oscillating reactivation, and were the strongest activation out of the three conditions with the high dose of LPS. The high-density macrophages stimulated in new media with the low dose of LPS showed the strongest activation of NFκB out of the three conditions with low LPS. The high-density macrophages in new media induced with the high dose of LPS showed oscillating activity of NFκB.

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