Estradiol induces immune dysfunction and reduced wound healing rates after tissue injury

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ABSTRACT

• Estradiol and LPS-induced immune dysfunction leads to reduced wound healing rates after tissue injury.

BACKGROUND

Burn injury is a significant cause of mortality, with ~450,000 people suffering burn injury each year in the United States and ~5,000 people dying from it. Deaths from burn injury are commonly caused by immune dysfunction and subsequent opportunistic bacterial infection. Females tend to have longer hospital stays and higher mortality rates from burn infections. It has been shown previously that estradiol, the major female sex hormone, adversely alters production of serum interleukin-6 (IL-6), a cytokine protein that mediates immune responses. It would be useful to determine whether estradiol has this same effect on IL-6 in an in vivo wound model. Further, we would like to know how estradiol affects the rate of wound closure, both in the presence and absence of LPS (0.1 nm).

METHODS

1. Burn injury was simulated using an Ibidi 2D-cell culture insert (b). A 70% suspension of human airway epithelial cells (AECs) in respective medium was pipetted into each well and allowed to reach confluence over ~48 h.

2. The insert was removed, and the AECs were then submunged in medium. To the wells were then added one of the following:
   a. LPS (10 ng/mL)
   b. LPS (10 ng/mL) + estradiol (10 nm)
   c. LPS (10 ng/mL) + estradiol (100 nm)
   d. LPS (10 ng/mL) + estradiol (1.0 nm)
   e. Nothing

3. Photos were taken of wounds at 0, 6, 12, and 24 hours after removal of the insert (e.g., below).

4. After 24 hours incubation, the supernatant was removed, and the cells were analyzed using Nanostring for mRNA and immune gene expression.

5. The supernatant IL-6 content was quantified using an IL-6 EUSA.

RESULTS

• Differences in wound closure rate were observed upon introduction of estradiol and LPS in vitro, with estradiol-treatment wounds healing faster than controls.

• IL-6 protein content was also higher in the presence of estradiol and LPS.

• The presence of estradiol was also correlated with upregulation of up to 50 different genes.

• The direct response of the cells to the estradiol itself is further corroborated by the verified presence of estrogen receptors on the surface of these AECs.

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Figure 1: Increasing Concentrations of Estradiol Show Differences in Percent Wound Closure

Figure 2: IL-6 Protein Concentration Increases upon Addition of Estradiol and LPS

Figure 3: Nanocardi Genetic Barcoding Revealed Differential Expression of up to 50 Immune Genes after Exposure to Different Estradiol Concentrations

Figure 4: Increasing Concentrations of Estradiol Induce Increased IL-6 Gene Expression

Figure 5: Human Airway Epithelial Cells Express Genes for Estrrogen Receptor Proteins