

The Impact of Leukocyte-Derived Cytokines on Intracellular Signaling Cascades in Human Periovarian Granulosa Cells

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Mid-cycle gonadotropin-induced ovulation, a pivotal event in female reproductive physiology, shares many features with an acute inflammatory response, notably marked by a massive influx of leukocytes into the dominant follicle. Despite this, the precise impact of leukocytes on the ovulatory process remains largely elusive. Our previous finding, derived from single-cell RNA-sequencing (scRNA-seq) analysis using follicular aspirates obtained from in vitro fertilization (IVF) patients has revealed the presence of ten distinct leukocyte subpopulations. Based on the single-cell transcriptomic datasets, we found that inflammatory cytokines – e.g., *IL1B*, *IL10*, *IL16*, *IFNG*, *TNF*, *LTA*, and *LTB* – were specifically expressed in leukocytes, while the expression of their corresponding receptors – *IL1RI*, *IL1RAP*, *IL10RA*, *IL10RB*, *CD9*, *IFNGR1*, *IFNGR2*, *TNFRSF1A*, and *LTBR* – was abundantly detected in follicular (granulosa and theca) cells. To demonstrate the action of the leukocyte-derived cytokines and their cognate receptors in granulosa cells, primary human granulosa-lutein cells (hGLCs) were treated with \pm human chorionic gonadotropin (hCG, 1 IU/ml) \pm recombinant human (rh) protein of leukocyte-derived cytokines for 36 hours. Western blot analysis was performed using the cultured hGLCs to assess whether leukocyte-derived cytokines activate intracellular signaling pathways in periovarian granulosa cells. Treatment of rhIL1B (1, 5, 10 ng/ml), rhIFNG (1, 5, 10 ng/ml), rhTNF (1, 5, 10 ng/ml), and rh1LTA:2LTB (a heterotrimer of LTA and LTB; 10, 20, 50 ng/ml) increased the levels of phosphorylated IRAK4 (pIRAK4), pSTAT1, pIKK α/β , and pRelA, respectively, which are key mediators for MYD88, STAT1, and canonical NK κ B signaling pathways. These in vivo and in vitro findings showed that specific cytokines and their corresponding receptors are expressed in leukocytes and follicular cells, respectively. Furthermore, these cytokines activated specific intracellular signaling pathways in granulosa cells expressing their receptors. Collectively, this study suggests that leukocyte-derived cytokines regulate granulosa cell functions by activating specific signaling pathways, thereby potentially facilitating the ovulatory process in the human ovary. (Supported by P01HD71875, R03HD095098, and R01HD096077)