

Estradiol Levels Influence Neutrophil Responses Against HIV in Women

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Introduction: The population is aging, and older women are more susceptible to infections compared to younger women. This fact has been linked to impaired immune responses and changes in hormone levels after menopause. Neutrophils play a crucial role in the innate immune response against viral infections, such as human immunodeficiency virus (HIV), recognizing, trapping, and inactivating the virus using a process known as NETosis. In this response, neutrophils release DNA strands coated with antimicrobial peptides, known as Neutrophil Extracellular Traps (NETs). Interestingly, estradiol (E2), a sex hormone that decreases after menopause, has been shown to decrease susceptibility to HIV infection of T cells and macrophages *in vitro*, but the role of E2 in neutrophil response to HIV is largely unknown. We hypothesize that low levels of E2 due to menopause can impact neutrophil activity and susceptibility to HIV infection in women.

Methods: Blood neutrophils were isolated from both pre- and postmenopausal women and pre-treated with a concentration of 50 nM E2 for 1 h *in vitro*. Neutrophils were then stimulated with GFP-labelled HIV viral-like particles (HIV-VLPs) or Calcium ionophore, a known NET-inducer, for 30, 60 and 90 min. To quantify NETosis in real-time, time-lapse microscope images were taken every 3 min and analyzed using the Incucyte platform. NETs were separated from supernatants by centrifugation and α -Defensin (α Def) and myeloperoxidase (MPO) were measured in each by ELISA. E2 concentration in plasma was also measured by ELISA.

Results: Neutrophils from postmenopausal women showed a delayed NETosis response to HIV over 3 h compared to neutrophils from premenopausal women. Whereas increased NETosis with HIV is clear via Incucyte at later time points, after only 30 min of stimulation with HIV, no changes in concentration of α Def were observed in NETs or supernatants. However, neutrophils significantly increased α Def secretion 30 min after Ca^{2+} ionophore stimulation, and significantly more so in premenopausal women compared to postmenopausal. Additionally, the Ca^{2+} ionophore-stimulated neutrophils from premenopausal women had increased levels of MPO present in the NETs compared to postmenopausal women. Ongoing experiments will determine

antimicrobial peptide production 60 and 90 min post-stimulation. We wanted to determine if these changes in neutrophil response were due to changes in E2 levels associated with menopausal status and aging. Interestingly, pre-treating neutrophils from postmenopausal women with E2 improved their response to HIV after 30 min, showing increase release of NETs compared to untreated neutrophils. E2-treated neutrophils from young women, however, did not modify their response to HIV.

Conclusion: Aging delays neutrophil response to HIV stimulation and this was improved when neutrophils were treated with E2, suggesting that circulating E2 levels may play a role in neutrophil function against viral pathogens as women age.