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The role of γδ T cells in the immunological control of the reproductive system in *in situ* and *ex vivo* studies, and their effect on the response to antigens administered intravaginally.

Abstract

In the literature, γδ T cells are often referred to as the "bridge" between innate and acquired immunity. These cells are most widely distributed in epithelial tissues, suggesting that they form the first line of defense against pathogens. Their main functions have been known primarily through studies of epidermal homeostasis in mice, but their role in the reproductive tract remains largely unexplored. This research attempts to explain the role of γδ T cells in the immune control of the female reproductive system.

Due to the lack of available protocols, the first stage of the research work was to develop methods for the analysis of γδ T cells in the murine vagina. The vaginal epithelium is relatively thick and dense, which makes it difficult to use standard imaging and cytometric techniques. Based on the CUBIC clearing method, an optical clearing protocol of vaginal tissues was developed, which enabled 3D visualization of γδ T cells and determination of their distribution. It was shown that γδ T cells sense sex hormone-induced changes of epithelium and reach the peak number in the progesterone-dominated diestrus phase. They are also located closer to the vaginal lumen during diestrus, compared to the estrus phase, which is dependent on estrogens. Their number in the vaginal epithelium is three times greater than in the vaginal stroma, which is independent of hormonal changes during the estrous cycle. The second approach of γδ T cell analysis in the reproductive system was to use flow cytometry after enzymatic digestion of the tissue. By comparing multiple enzymes, Liberase TL was selected as the most effective enzyme for the analysis of the cellular composition of the murine reproductive tract. The developed protocol allowed for the verification of microscopic data and to demonstrate that γδ T cells do not have a significant impact on the number and percentage of other immune cell populations at the steady-state and in the course of inflammation caused by stimulation of Toll-like receptors (TLR7 and TLR9), both in vagina and uterus. These conclusions were drawn based on a cytometric comparison of cells isolated from tissues obtained from wild-type mice and the genetically modified strain lacking γδ T cells (*Tcrd*$^{-/-}$). The obtained data were partially confirmed by studies of the role of γδ T lymphocytes in inducing immune responses after immunization with antigens applied onto the vaginal mucosa. In the absence of γδ T cells, the immune response to the protein antigen - ovalbumin, is similar to that in wild-type mice. At the same time, γδ T lymphocytes moderately enhance the response after vaginal vaccination with

the polysaccharide antigen from *Candida albicans* conjugated with tetanus toxoid, which suggests their protective role against mycological infections. The comparative analysis of the routes of antigen administration showed that only the vaginal route depends on the estrous cycle phase, and hormonal changes do not affect the subcutaneous and intranasal administration. The ambiguous influence of γδ T cells on the status and response of the immune system in mice of reproductive age inspired the study of their role in old mice suffering from the dysfunction of immune responses at multiple levels. The analysis of gene expression and inflammatory cytokine production in aged individuals showed that the aging process skews immune responses in the female reproductive system toward the proinflammatory type, which is very selectively dependent on γδ T cells. The lack of these cells in old females, on the one hand, weakens the anti-infective immunity, which was evidenced by the decrease in the lactoferrin gene expression in *Tcrd*$^{-/-}$ mice. On the other hand, γδ T cells deficiency increases the complement system gene expression and the GM-CSF factor production, which may ultimately contribute to a dysregulation of the mechanisms responsible for controlling the inflammation.

The results indicate that γδ T cells do not play a critical role in controlling immune responses in the epithelium of the reproductive tract. Instead, they are responsible for the fine-tuning of immunological processes. Undoubtedly, intraepithelial γδ T cells contribute to innate antimicrobial responses, and their role depends on the location and the cellular context of the tissue. It is possible that in the absence of γδ T cells, other populations of the immune system take over the homeostasis maintenance in the reproductive epithelium. Further research is necessary to characterize their function and interactions with other cells of the immune system, both in the vagina and in the uterus.