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Customer protocol

Dissociation of cervical explants into single-cell suspensions using the gentleMACS™ Dissociator

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Background

The immune cells present in the human genital tract are important for protection from sexually transmitted infections and also can be a target of viral infections, such as HIV. Therefore knowledge about the distribution and variability of the different immune cell types is important for understanding the susceptibility to infections and the development of prevention strategies against infection. Most of the data about the immune cells in genital tract is based on immunhistochemistry and quantitative data for all immune cells including lymphocytes and antigen presenting subtypes is insufficient. Also, the traditional protocols for enzymatic digestion of tissue for flow cytometry analysis leads to cleavage and loss of some immune cell markers, such as the NK cells marker CD56.

Materials and methods

Materials

- gentleMACS Dissociator, gentleMACS Octo Dissociator, or gentleMACS Octo Dissociator with Heaters
- gentleMACS C Tubes
- Centrifuge
- Incubator (37 °C)
- Digestion buffer (10 mg/mL collagenase IV in RPMI)
- Cell strainer (100 µm mesh size)
- (Optional) Red Blood Cell Lysis Solution (10×)

Methods

1. Transfer 8–10 pieces of polarized cervical explants (approximately 50–200 mg/piece) into the gentleMACS C Tube. Add equal volume of digestion buffer.
2. Tightly close the C Tube and attach it upside down onto the sleeve of the gentleMACS Dissociator.
3. Run gentleMACS Program **m_spleen_01**.
4. After termination of the program, detach C Tube from the gentleMACS Dissociator.
5. Incubate the sample for 30 minutes at 37 °C on a shaker platform at low speed (approximately 120 rpm).
6. Attach C Tube upside down onto the sleeve of the gentleMACS Dissociator.
7. Run gentleMACS Program **m_spleen_01**.
8. (Optional) Centrifuge sample at 2000×g for 1 minute.
9. Apply the cell suspension to a cell strainer (100 µm mesh size) placed on a 50 mL tube.
10. Rinse strainer with 30 mL RPMI medium and collect the flow-through.
11. Centrifuge at 1500×g and 4 °C for 5 minutes.
12. (Optional) Perform red blood cell lysis.
13. Resuspend cells to the required volume for further applications.

Results

Trifonova, R. T. *et al.* developed an optimized protocol for isolation of immune cells from the epithelium and stroma allowing simultaneous detection by flow cytometry of different cell subsets including lympocytes and myeloid cells.[1] This protocol combines enzymatic digestion and mechanical dissociation which give a good yield of immune cells and also preserves immune cell markers such as CD4, CD56, and CD209 which could be cleaved after harsh and prolonged enzymatic treatment. This method has been used to determine differences in immune cells in human cervix based on localization, age, and menopausal status. We are also interested in the development of topical agents for manipulation of susceptibility to viral infections such as HIV and HSV using siRNA to silence host and viral genes. The described protocol has been used to isolate immune cells and determine gene knock-down after treatment with CD4aptamer-siRNA chimeras both in human cervical explants model and in genital tract from 'humanized' mice.[2]

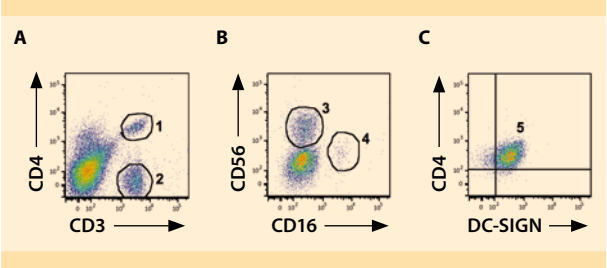

Figure 1: Flow cytometry analysis of immune cells in human cervix: A: $CD3^+$ T cells including $CD4^+$ (1) and $CD4^-$ (2). B: $CD56^{high}$ $CD16^-$ (3) and $CD56^{dim}$ $CD16^+$ (4) NK cell subsets detectable in the $CD3^-$ population of immune cells. C: DC-SIGN $(CD209)^+$ $CD4^{dim}$ antigen presenting cells (5).

Conclusion

Generation of single-cell suspensions from human cervical explants and mouse vaginal tissue can be accomplished with ease using the gentleMACS Dissociator.

References

1. Trifonova, R. T. *et al.* (2014) Distribution of immune cells in the human cervix and implications for HIV transmission. Am. J. Reprod. Immunol. 71(3): 252–264.

2. Wheeler, L. A. *et al.* (2011) Inhibition of HIV transmission in human cervicovaginal explants and humanized mice using CD4 aptamer-siRNA chimeras. J. Clin. Invest. 121(6): 2401–2412.

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