Isolation of mononuclear cells from human tonsil using the gentleMACS™ Dissociator

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**Background**
Natural killer (NK) cells are an important component of the innate immune system. Without prior activation, NK cells can kill infected or malignant cells and produce cytokines that contribute to shaping both innate and adaptive immune responses. Recent studies have defined five main stages of human NK cell development. Very early phases of development, such as the generation of stage 1 NK precursor cells (NKPs) from HSCs, occurs in the bone marrow whereas later phases may occur in peripheral organs, as shown for secondary lymphoid tissues. Hepatic natural killer cells constitute approximately 40% of hepatic lymphocytes and are phenotypically and functionally distinct from blood NK cells. Whether hepatic NK cells derive from precursors in the bone marrow or develop locally from hepatic progenitors was still unknown. Moroso et al.¹ identified all five known sequential stages of NK cell development in the adult human liver and demonstrate that CD34⁺ hepatic progenitors can generate functional NK cells. Early NK cell precursors were similar in liver and blood. To analyze and demonstrate the immunophenotypical differences of hepatic stage 3 NKPs, expression of transcription factors and cytokines were compared with NPKs isolated from human tonsils.

This protocol describes the procedure used by Moroso et al.¹ to isolate mononuclear cells from human tonsils using the gentleMACS™ Dissociator.

**Materials and methods**

**Materials**
- gentleMACS Dissociator or gentleMACS Octo Dissociator
- gentleMACS C Tubes
- Centrifuge
- Waterbath (37 °C)
- Pasteur pipette
- Collagenase D in phosphate-buffered saline (PBS)
- DNase I
- Complete DMEM medium (10% fetal bovine serum, 1% penicillin/streptomycin)
- Nylon filter (100 µm mesh size)
- Ficoll™
- Trypan blue

**Methods**
1. Remove the fatty tissue from tonsil.
2. Cut tonsil in smaller fragments and transfer it in 2–3 gentleMACS C Tubes (up to 4000 mg of tissue per C Tube).
3. Add 8 mL of pre-warmed Collagenase D (0.5 mg/mL) and DNase I (3000 U/mL) in each tube.
4. Tightly close the C Tubes and attach it upside down onto the sleeve of the gentleMACS Dissociator.
5. Run the gentleMACS Program C.
6. Transfer C Tubes to a waterbath (37 °C) and incubate sample for 15 minutes.
7. Attach C Tubes upside down onto the sleeve of the gentleMACS Dissociator and run gentleMACS Program C.
8. Add freshly prepared complete DMEM medium (equal volume as Collagenase D/DNase I) to the tube and pass the sample over a nylon filter in a 50 mL tube.
9. Centrifuge at 1300 rpm for 6 minutes.
10. Resuspend cell pellet in 10 mL complete DMEM medium.
11. Carefully add 14 mL Ficoll density medium.
12. Centrifuge at 2000 rpm for 20 minutes at room temperature.
13. Aspirate the upper layer leaving the mononuclear cell layer undisturbed at the cell interface.
14. Harvest cell interface with Pasteur pipette.
15. Dilute cells 1:2 with cold (4 °C) complete DMEM medium and centrifuge at 1700 rpm for 10 minutes at 4 °C.

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*The content of this publication has not been verified by Miltenyi Biotec.*
Results

Moroso et al.¹ elaborated the following model for the NK cell development in the adult liver: early NKPs (stage 1 and 2) are continuously recruited from peripheral blood into the liver, where they differentiate, under the influence of the local microenvironment, into the so called “liver-specific” NK cells.

Conclusion

The isolation of mononuclear cells from human tonsil can be accomplished with ease using the the gentleMACS Dissociator.

Reference


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