

Isolation and ex vivo culture of alveolar macrophages from the resected lungs of patients with pulmonary tuberculosis

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Abstract

Alveolar macrophages were obtained from the specimens of surgically resected lung tissue. Pieces of lung tissues (~10-30 g) obtained from lung parts resected from patients with treatment-refractory pulmonary tuberculosis. Lung tissue specimens were cut into small pieces and rubbed through a metal screen of sieve with pores of 0.5-2.0 mm diameter in phosphate-buffer saline (PBS, pH 7.4) for separating of cellular suspension containing alveolar macrophages from the closed granulomatous-fibrotic tissue. Cellular pellets after centrifugation at 400 g for 5 min at room temperature were placed to 24-well plates (Orange Scientific, Belgium) with cover glasses in the bottom and cultured in 0.5 ml complete growth RPMI 1640 medium containing 10% fetal bovine serum, 2 mM glutamine and 50 µg/ml gentamicin (BioloT, Russia) for 16-18 hours at +37 °C in atmosphere containing 5% CO₂.

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Protocol

Step 1.