Exposures to the environmental toxicants pentachlorophenol (PCP) and dichlorodiphenyltrichloroethane (DDT) modify secretion of interleukin 1-beta (IL-1β) from human immune cells

Peripheral Blood

CASE STUDY

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Introduction

IL-1β is a pro-inflammatory cytokine that has been linked to a multitude of diseases when over-produced by immune cells. Exposure to environmental contaminants found in human blood may contribute to inflammation and dysregulation in immune cell function. This study examined the impact of the environmental toxicants pentachlorophenol (PCP) and dichlorodiphenyltrichloroethane (DDT) on the secretion of IL-1β in immune cells of increasing complexity. LymphoSep™ cell separation medium from MP Bio was used to prepare PBMCs, MD-PBMCs, and NK cells to test exposures to PCP and DDT on IL-1β secretion ex vivo.

Overview

- **Keyword:** Dichlorodiphenyltrichloroethane; Interleukin 1-β; MD-PBMCs; NK cells; PBMCs; Pentachlorophenol
- **Aim of the study:** To investigate the effects of ex vivo exposures to PCP and DDT on the secretion of IL-1β in human cells
- **Application:** Treating cells with PCP or DDT; assaying for IL-1β (ELISA)
- **Sample name:** Peripheral blood mononuclear cell (PBMC)
- **Material:** LymphoSep™ - Lymphocyte Separation Medium (SKU 0916922) from MP Bio

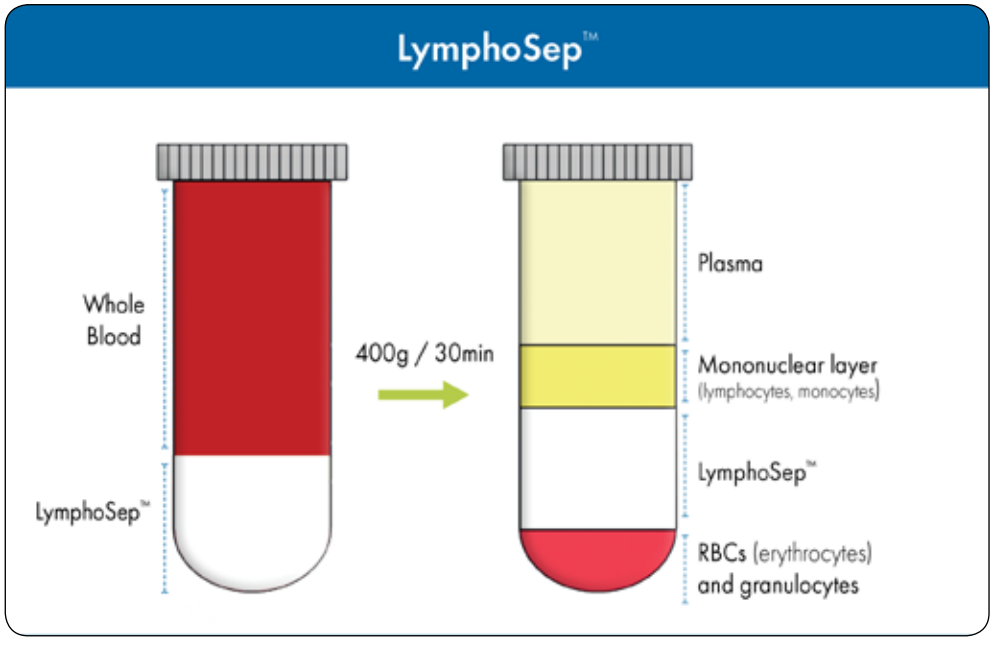

Figure 1.
Isolation of mononuclear cells from whole blood using LymphoSep™ density separation medium.

CASE STUDY

Protocol and Parameters

1. Leukocytes were isolated from filters obtained from the Red Cross Blood Bank by back-flushing with an elution medium (sterile phosphate buffered saline (PBS) containing 5 mM disodium EDTA and 2.5% [w/v] sucrose) and then collecting the eluent.
2. The eluent was layered onto LymphoSep™ cell separation medium (1.077g/mL) and centrifuged at 1200g for 30–50 min.
3. Following centrifuging and washing, cells were layered on bovine calf serum for platelet removal.
4. The cells were then suspended in RPMI-1640 complete medium (RPMI-1640 supplemented with 10% heat-inactivated BCS, 2 mM L-glutamine and 50 U penicillin G with 50 µg streptomycin/mL)
5. Monocyte-depleted (MD) PBMCs (10–20% CD16+, 10–20 % CD56+, 70–80% CD3+, 3–5% CD19+, 2–20% CD14+) were prepared by incubating PBMCs in glass Petri dishes (150 × 15 mm) at 37 °C and air/CO2, 19:1 for 1 h.

Conclusion

This study demonstrated that both PCP and DDT increased IL-1β secretion from all immune cell preparations tested. Immune cells from all donors showed compound-induced increases in IL-1β secretion at one or more concentrations and at one or more lengths of exposure. LymphoSep™ was used successfully to isolate peripheral blood mononuclear cells (PBMC) from leukocyte filters (PALL-RCPL or FLEX obtained from the Red Cross Blood Bank) with high yields and viability. LymphoSep™ was also used successfully to isolate NK cells from buffy coats in combination with a rosetting procedure.

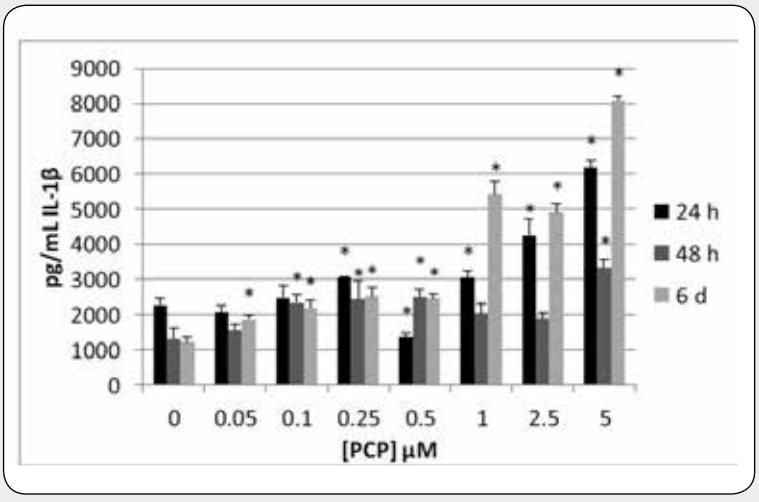

Figure 2.

Concentration of secreted IL-1β when PBMCs were exposed to 0.05-5 µM PCP (donor F197).

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