Sample aging profoundly reduces monocyte-, but not T-cell-driven innate immune responses in human whole blood cultures

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INTRODUCTION & AIM

Whole blood cultures are widely used for investigation of physiological pathways and drug effects *in vitro*. Whole blood cultures require minimal sample handling and mimic *in vivo* conditions as far as possible. However the time span between blood collection and start of the experiment may have a big influence on experiment outcome, and may vary substantially between and within experiments. In this study we test the effects of sample aging on cell viability and stress, and cellular reactivity to exogenous immune triggers.

METHODS

Experiments were started directly after blood draw and at 0.5h, 1h, 2h, 4h and 10h after blood draw.

Whole blood stimulation: heparinized whole blood from healthy volunteers was stimulated with LPS (2ng/ml), SEB (100ng/ml) or PMA (150ng/ml) + ionomycin (7.5µg/ml). Culture supernatants were assayed for TNFα, IFNγ, IL-1β, IL-2 and IL-6.

Flow cytometry: flow cytometry was done on whole blood after RBC lysis, and cells were stained for TLR4-APC (clone HTA125), Annexin V-FITC, CD14-VioBlue (REA599), CD3-VioGreen (REA613). After fixation and permeabilization intracellular staining was performed for IL-2PE (N7.48A), TNFα-PE (REA656), IFNγ-PE (REA600) or IL6-PE (MQ2-13A5) in the presence of FcR blocking reagent. Unstimulated samples and FMO controls were used for gate setting.

Mitochondrial function: mitochondrial membrane potential (MMP) was assessed in whole blood after RBC lysis. Leukocytes were incubated with 0.5µM JC-1, for 15 minutes. CCCP (10µM) was used as a positive control for impaired MMP.

RESULTS

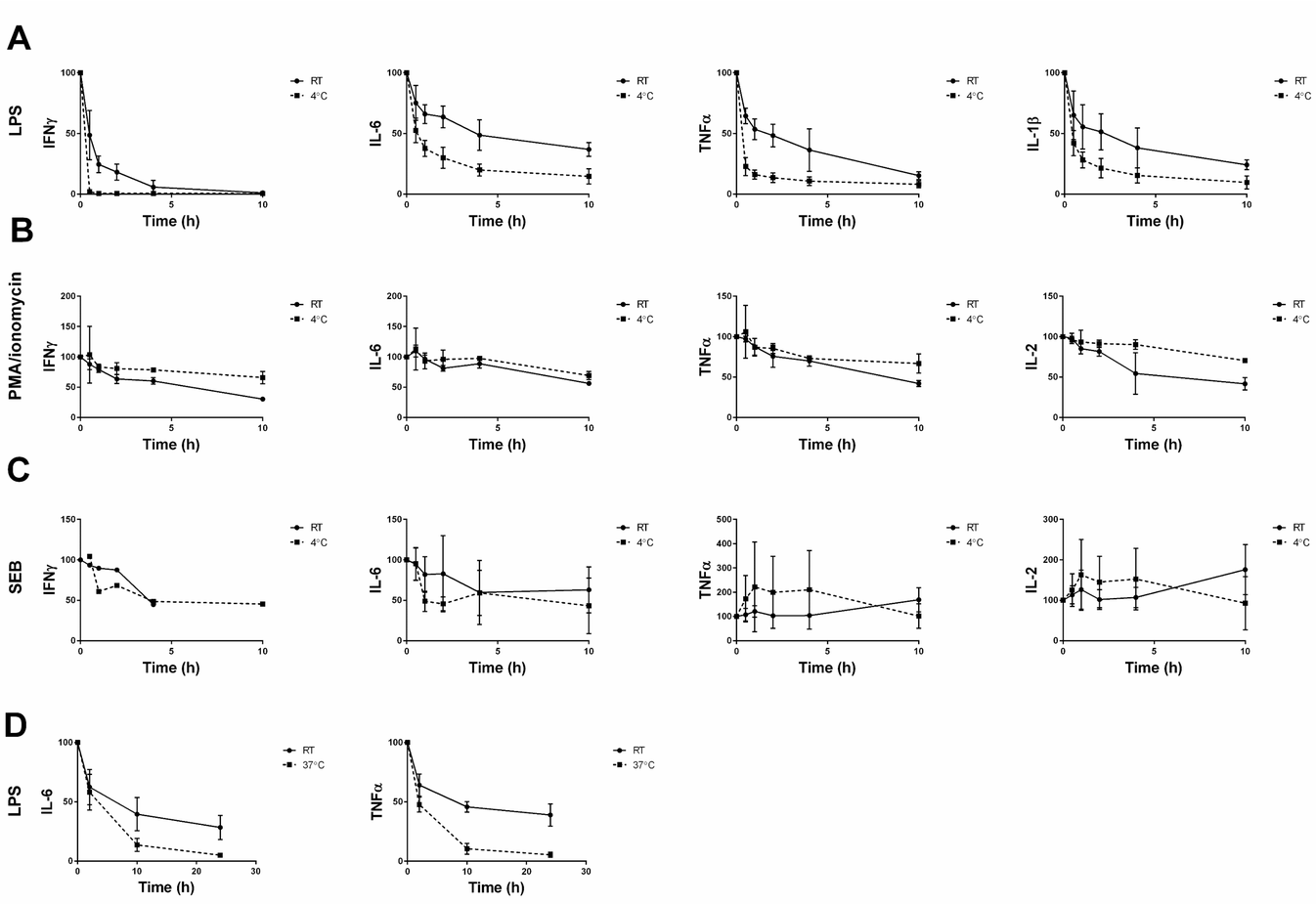

Figure 1: cytokine release in whole blood culture supernatants (n=5, average + SD), after blood aging of up to 10 hours.

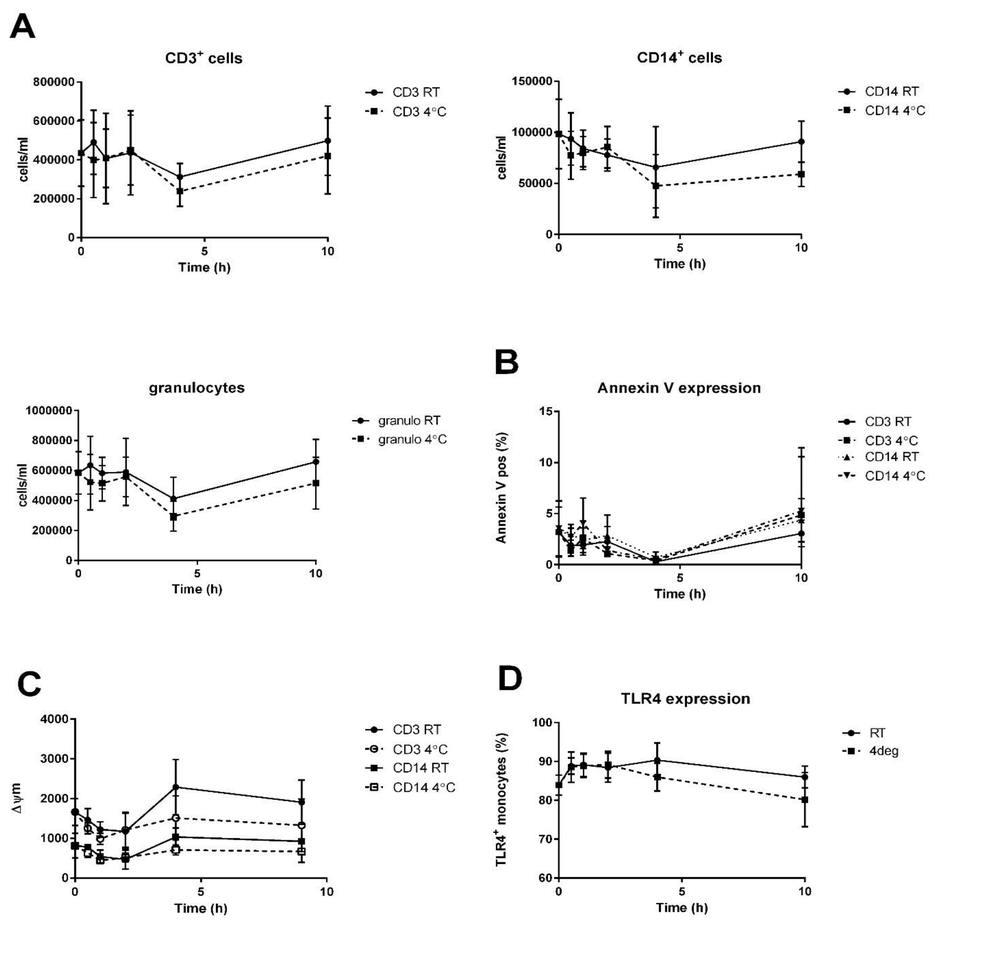

Figure 2: cell viability (cell counts (A), annexin V (B), MMP (C)) and TLR4 expression (D) in whole blood cultures (n=6, average + SD).

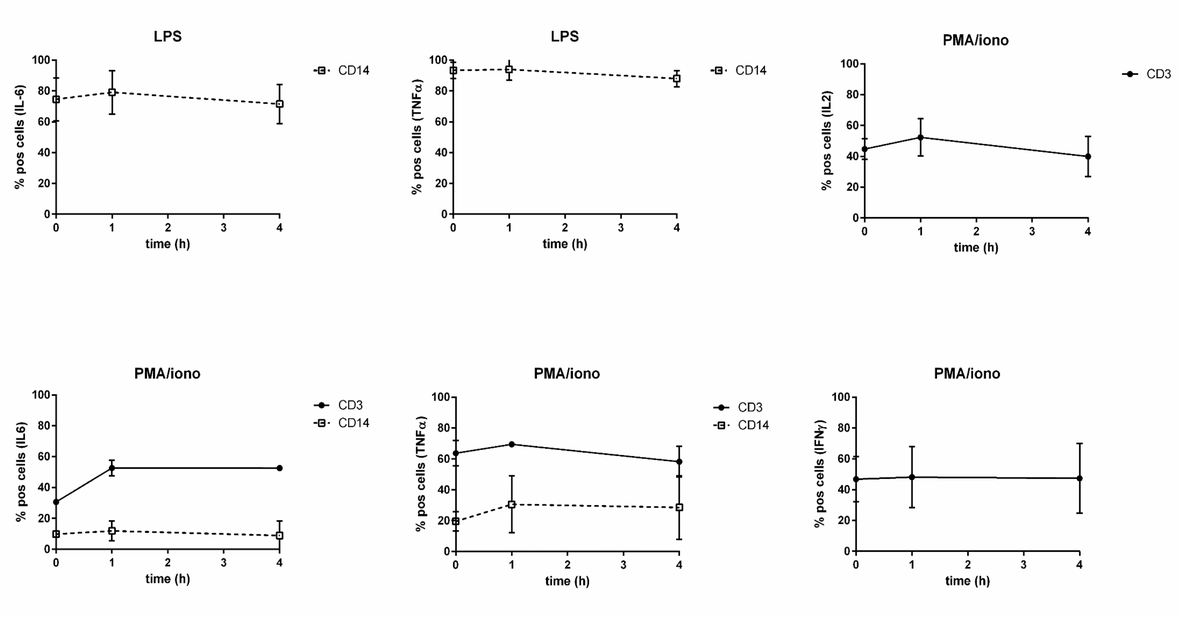

Figure 3: cytokine-producing cells in whole blood cultures (n=5, average + SD)

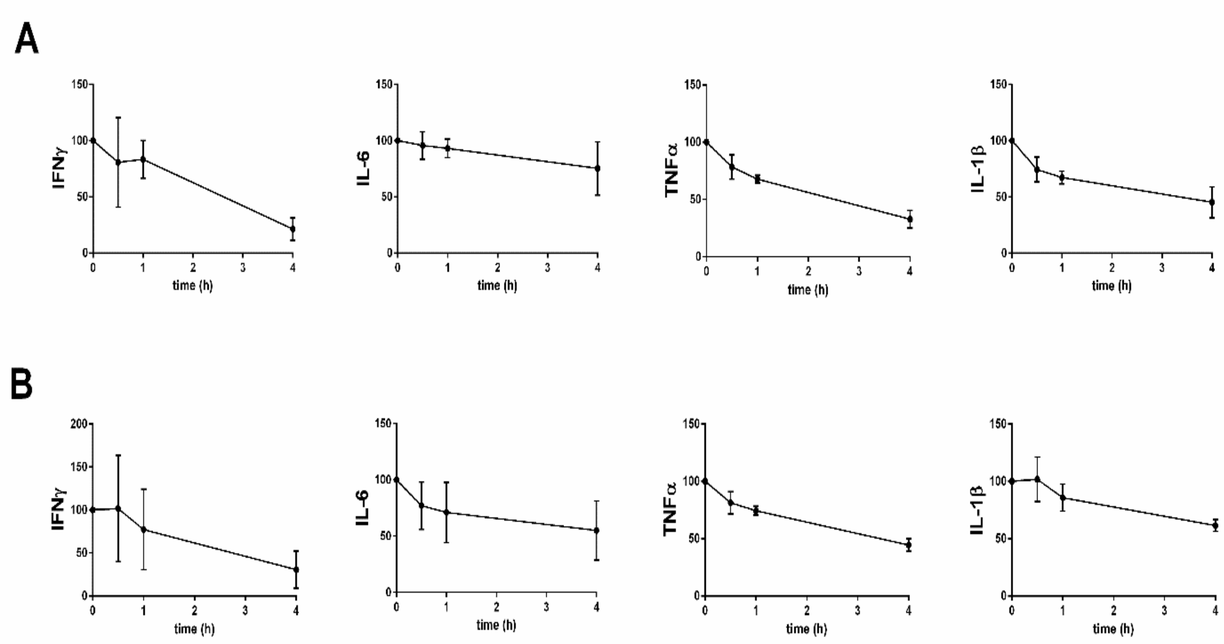

Figure 4: cytokine-producing cells in whole blood cultures (n=3, average + SD), in absence of RPMI (A) and diluted 1:1 with RPMI during storage (B)

CONCLUSIONS

The responsiveness of monocytes to LPS decreases drastically during sample aging, while the responsiveness of T-cells remains more stable (figure 1). Cell viability and stress markers show that the cells do not undergo apoptosis during a maximum of 10 hours of aging. Also TLR4 expression on monocytes remains stable (figure 2). Intracellular cytokine staining showed that the amount of cytokine producing cells remains the same, indicating that the same amount of cells respond, but the amount of cytokine secreted decreases (figure 3). Addition of culture medium (RPMI 1640) during storage does not prevent loss of monocyte responsiveness to LPS (figure 4).

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