Assay development for phagocytosis activity evaluation

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Introduction

The efficiency of phagocytic activity is a significant organism's indicator which decreases with the aging of the immune system. The medications are able to influence phagocytosis, having a blocking or activating effect, and therefore are important modulators of immune function. We are developing an ex vivo assay indispensable for medication screening in human and Macaca fascicularis whole blood. For assay verification several published control drugs were successfully used for blocking of phagocytosis.

Materials and methods

The object for phagocytosis was *E.coli* stained in house with FITC (Lumiprobe) for 3 h, 37˚C. Phagocytosis was carried out in whole blood without adhesion under near physiological conditions (T=37˚C, 5% CO_2). After 1 h incubation with *E.coli*, cells were treated with Protein transport inhibitor, containing monensin (BD GolgiStop), according to the instruction, or placed on ice, or treated with combination of lidocaine (0.1-3 mg/ml) – epinephrine (0.34-10.2 μM) for 5 min. Then the erythrocytes were lysed with BD Pharm Lyse according to the instruction. Besides, cells were pre-treated with these agents for 5 min before adding bacteria. Sample analysis was performed on a BD LSRFortessa flow cytometer. To correctly identify leucocytes, CD45 APC-eFluor780 (HI30, eBioscience; 1:100) was used.

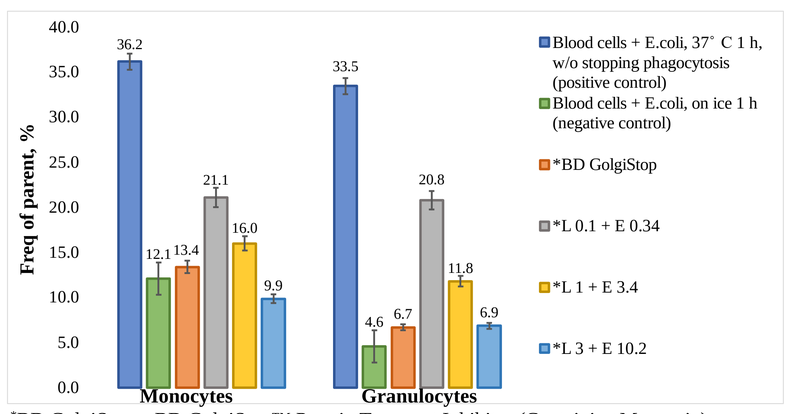
Objective

Quantification of phagocytised cells, which were treated with published inhibition agents, interfering with phagocytic activity, for pharmaceutical, research applications, and for further method development.

Results

Pre-treated with different agents blood cells were incubated with *E.coli* as described; after that percentage of phagocytosed cells (PC; activated monocytes or granulocytes, as the most prominent phagocytosed populations) was evaluated (Fig.1). In comparison to non-treated cells (PC ~ 33%), treated cells' phagocytic activity plummeted (PC 4.6-20.8%), and lidocaine-epinephrine treatment showed dose-dependent decrease, effectively blocked phagocytosis in the same manner as commercial GolgiStop in optimal concentration. This trend was linear for both granulocytes and monocytes (R^2 = 0.972 and R^2 = 0.997, respectively, Fig.2).

Moreover, treatment after 1 h incubation with *E.coli*, was indistinguishable from control sample without inhibitors of phagocytosis (Fig.3). Despite there was no difference, it can be used in modifications of phagocytic dynamic test where both engulfed and adhered bacteria are detected in defined timelapse. In case of phagocytosis was stopped on ice, percentage of PC was lower in comparison with treated and non-treated samples, and it can be supposed that cells undergo degranulation and cell death on ice. Further analysis confirmed that degranulation and death of phagocytosed cells on ice occurs, and that additionally validates usage of chemical agents to block phagocytosis.



*BD GolgiStop = BD GolgiStop™ Protein Transport Inhibitor (Containing Monensin)
L = Lidocaine concentration (mg/ml)
E = Epinephrine concentration (μM)

Fig.1. Evaluation of activated cells percentage, while treated for 5 min with phagocytic activity inhibitors before phagocytosis modulation.

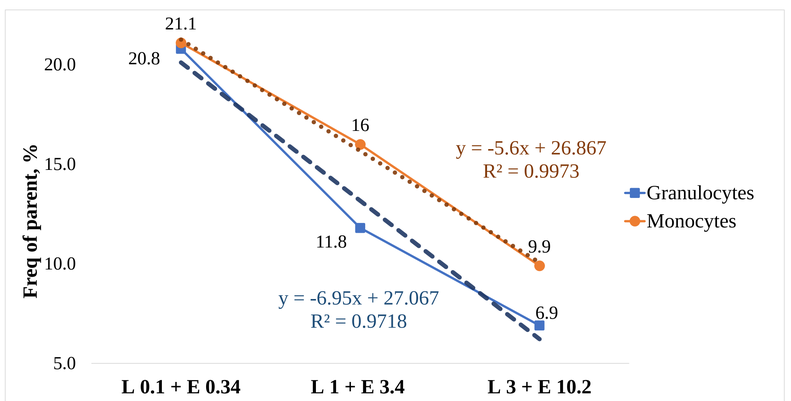


Fig.2. Linear dependence of activated cells percentage on lidocaine-epinephrine concentrations.

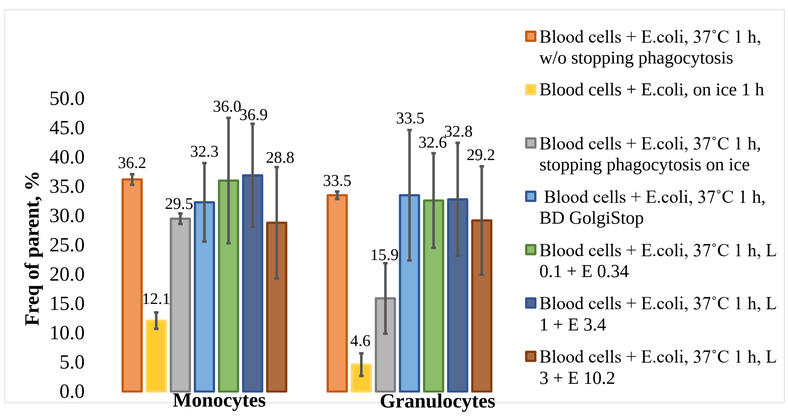


Fig.3. Evaluation of activated cells percentage, when treated for 5 min with phagocytic activity inhibitors after phagocytosis modulation.

Conclusion

Lidocaine-epinephrine mixture inhibits phagocytosis and, if necessary, can be used as a stop-reagent as a cheaper and effective analogue to commercial reagents and ice. Phagocytic activity evaluation by FITC-conjugated bacteria and developed protocol in whole blood suggested to use as an essential part for medication screening. Validation of perspective medications is recommended for elderly people whose phagocytosis is impaired. Moreover, phagocytosis testing in whole blood under near physiological conditions offers useful and feasible approach for a personalized medicine.

Acknowledgments

The work was carried out with the support of the Russian Science Foundation, project No. 23-15-00443 (https://rscf.ru/project/23-15-00443).

References

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