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Proceeding Paper

Assay Development for Phagocytosis Activity Evaluation †

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Abstract: The efficiency of phagocytic activity is a significant organism indicator which decreases with the aging of the immune system. 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. This assay assists with finding medications for enhancing the immune response in elderly people and providing a deeper comprehension of the fundamental process of immune system aging.

Keywords: phagocytosis; senescence; immune system; immune response; monkey; drugs

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1. Introduction

The increased risk of developing age-related diseases in elderly people is associated with an imbalance and deficiency in immune response; therefore, specific treatment and vaccination approaches should be developed. The efficiency of phagocytic activity is a significant organism indicator which decreases with the aging of the immune system. Medications are able to influence phagocytosis, having a blocking or activating effect, and are important modulators of immune function. We are developing an ex vivo assay indispensable for medication screening in human and Macaca fascicularis whole blood. M.fascicularis is a well-recognized preclinical model with human-like immune responses. For assay verification, several published control drugs [1] were successfully used for the blocking and acceleration of phagocytosis. Experiments evaluating human and animal phagocytosis indices at different ages will provide statistical standards to be used for medication preclinical and clinical trials. This assay assists with finding medications for enhancing the immune response and antigen presentation in elderly people. Moreover, the screening of medications with an impact on phagocytosis and further studies of their mechanisms will assist in providing a deeper comprehension of the fundamental process of immune system aging and a perspective for immune response correction.

2. Materials and Methods

The object for phagocytosis was *E. coli* stained in-house with FITC (Lumiprobe) for 3 h at 37 °C. Phagocytosis was carried out in whole blood without adhesion [2] under near physiological conditions (T = 37 °C, 5% CO_2). After 1 h of incubation with *E. coli*, cells were treated with Protein transport inhibitor, containing monensin (BD GolgiStop), according to the instructions, placed on ice, or treated with a combination of lidocaine (0.1–3 mg/mL) and epinephrine (0.34–10.2 µM) [1] for 5 min. Then, the erythrocytes were lysed with BD Pharm Lyse according to the instructions. Furthermore, cells were pre-treated with these agents for 5 min before adding bacteria. A sample analysis was performed on a BD LSRFortessa flow cytometer. To correctly identify leucocytes, CD45 APC-eFluor780 (HI30, eBioscience; 1:100) was used.

3. Results and Discussion

Pre-treated with different agents, blood cells were incubated with *E. coli* as described; after that, the percentage of phagocytosed cells (PC; activated monocytes or granulocytes, as the most prominent phagocytosed populations) was evaluated (Figure 1).

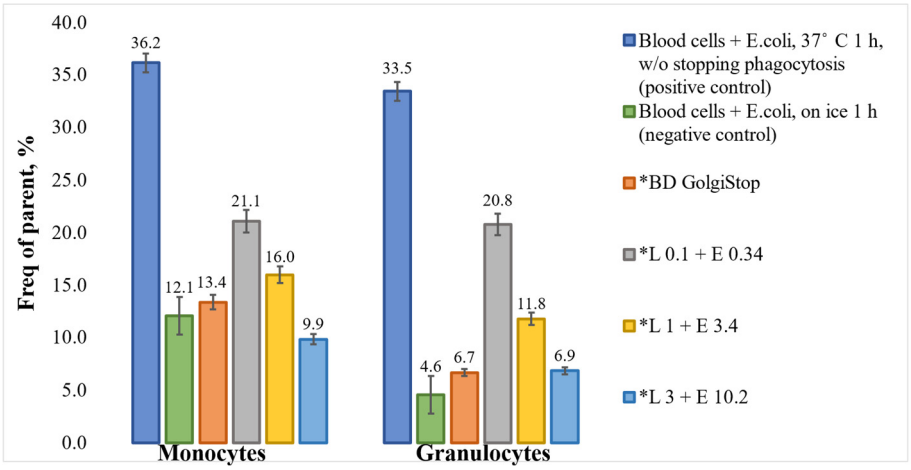

Figure 1. Evaluation of activated cells percentage, while treated for 5 min with phagocytic activity inhibitors before phagocytosis modulation. * BD GolgiStop = BD GolgiStop™ Protein Transport Inhibitor (Containing Monensin). L = Lidocaine concentration (mg/mL). E = Epinephrine concentration (μM).

In comparison to the non-treated cells (PC ~ 33%), the treated cells' phagocytic activity plummeted (PC 4.6–20.8%) and the lidocaine-epinephrine treatment showed a dose-dependent decrease, effectively blocking phagocytosis in the same manner as commercial GolgiStop in its optimal concentration. This trend was linear for both granulocytes and monocytes (R^2 = 0.972 and R^2 = 0.997, respectively, Figure 2).

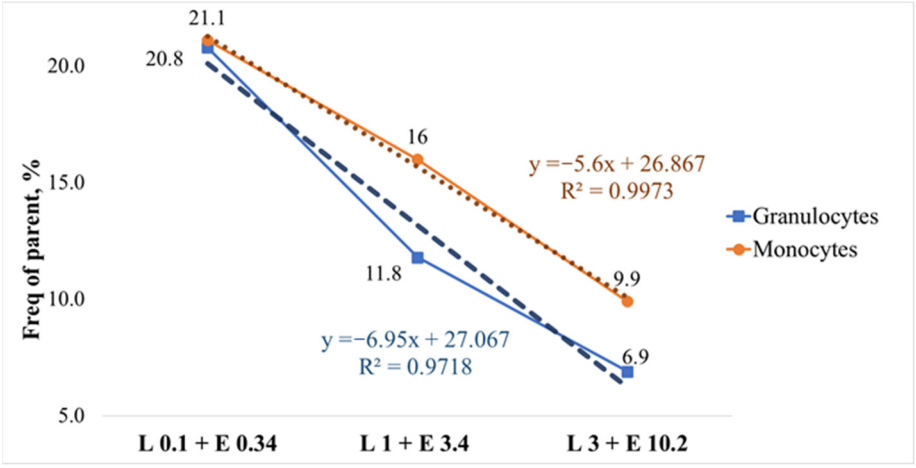

Figure 2. Linear dependence of activated cells percentage on lidocaine-epinephrine concentrations. L = Lidocaine concentration (mg/mL). E = Epinephrine concentration (μM).

Moreover, treatment after 1 h of incubation with *E. coli* was indistinguishable from the control sample without inhibitors of phagocytosis (Figure 3).

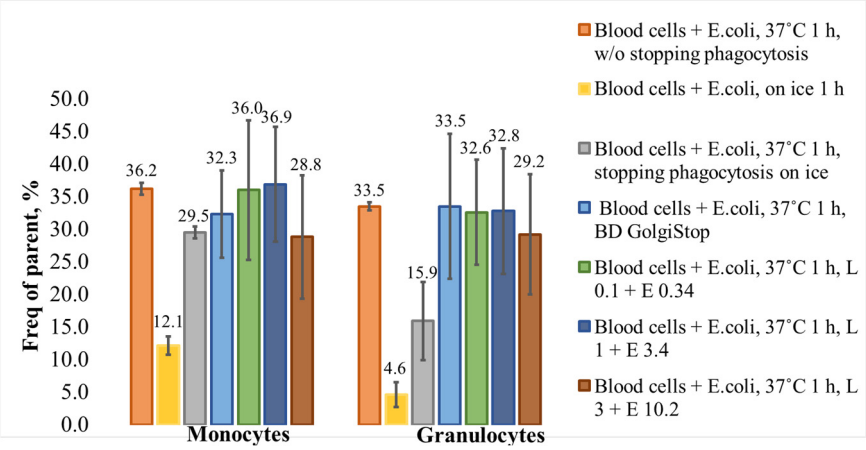

Figure 3. Linear dependence of activated cells percentage on lidocaine-epinephrine concentrations. * BD GolgiStop = BD GolgiStop™ Protein Transport Inhibitor (Containing Monensin). L = Lidocaine concentration (mg/mL). E = Epinephrine concentration (μM).

Despite there being no difference, this can be used in modifications of phagocytic dynamic tests where both engulfed and adhered bacteria are detected in a defined time-lapse. In the case of phagocytosis being stopped on ice, the percentage of PC was lower in comparison with the treated and non-treated samples, and it can be supposed that cells undergo degranulation and cell death on ice. Further analysis confirmed that the degranulation and death of phagocytosed cells on ice occurs, which additionally validates the usage of chemical agents to block phagocytosis.

4. Conclusions

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. A phagocytic activity evaluation with FITC-conjugated bacteria and a developed protocol in whole blood suggested its use as an essential part of medication screening. The validation of perspective medications is recommended for elderly people whose phagocytosis is impaired. Moreover, phagocytosis testing in whole blood under near physiological conditions offers a useful and feasible approach for personalized medicine.

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Institutional Review Board Statement: All procedures performed in the study involving human subjects complied with national ethical standards, the 1964 Declaration of Helsinki and its subsequent amendments and was approved by the Bioethics Committee of Sirius University (protocol dated 3 June 2023).

Informed Consent Statement: Informed voluntary consent was obtained from all individuals participating in the study to use the obtained data for publication before taking blood samples. The data was used and processed on the basis of anonymity and protection of personal data in accordance with national legislation.

Data Availability Statement: All data presented in this manuscript, such as flow cytometry and haemoanalyser files or other manual entries in workbooks, are available on request and in accordance with the Federal Law (Russian Federation of 27 July 2006 N 152-FZ).

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