ERYTHROCYTE GHOSTS' INFLUENCE ON THE PHENOTYPIC PLASTICITY OF MACROPHAGE

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Correction of phenotypic plasticity of macrophages for reprogramming inflammatory response is of great interest to modern medicine. To date, there are lots of factors, which can alter macrophages' phenotype to M1 or M2. The introduction of drug containing autologous erythrocyte ghosts is one of such factors.

We have developed a method for the targeted delivery of autologous erythrocyte ghosts loaded with antibiotic (pharmacocytes) directly into the wound tissues. Introduction of pharmacocytes to the purulent wound ensures high therapeutic concentrations of the antibiotic, which leads to rapid arresting of the phase of the inflammatory process and thus results in early healing.

In order to explain this phenomenon, we investigated the effect of the pharmacocytes on the phenotypic polarization of macrophages.

Material and methods: Human monocytes, erythrocytes and plasma were prepared from the peripheral blood of healthy volunteers. Encapsulation of rifampicin into the erythrocyte ghosts was performed by the method of hypotonic preswelling. Suspension of monocytes was isolated by centrifugation on Histopaque gradient density 1.077 g/cm3. Cell population that contains more than 90% monocytes was obtained by the magnetic cell sorting using a monocyte isolation kit Dynabeads® (Invitrogen). For the purpose of activation of pro-inflammatory phenotype in the incubation medium of monocytes, 100 ng/ml gamma interferon (INF γ) was added, and for stimulation of anti-inflammatory phenotype, 10 ng / ml interleukin 4 (IL4) was added. Incubation was carried out for 3 and 5 days at 370C in water vapor saturated atmosphere with 5% carbon dioxide. The concentration of the cytokines in the culture medium was determined using the ELISA kits (Sigma-Aldrich).

Results: Addition of pharmacocytes to the culture of monocytes regardless of their phenotypes leads to reduced levels (non-significant) of secretion of pro-inflammatory cytokines on the 3rd day of incubation: IL-1 β and TNFα and a substantial (about 6 folds), significant increase in the production of anti-inflammatory cytokines IL1ra (up to (0.584±0.15, $p<0.01$) and PARC / CCL18 (up to 646.49±35.76, $p<0.001$). After 5 days of incubation, with rifampicin pharmacocytes, concentration level of PARC/CCL18 was high (585.65 pg/ml) in the population of monocytes, and without pharmacocytes, concentration level of PARC/CCL18 was low (6.082 pg/ml). This suggests that the production of PARC/CCL18 is stimulated in the presence of pharmacocytes. The concentration of cytokine IL1ra was stably elevated in all groups for 3 and 5 days, but the secretion of pro-inflammatory cytokines TNFα was increased by 40% on the 5th day in the population of macrophages incubated with pharmacocytes.

Conclusions: Co-culture of monocytes with pharmacocytes leads to an increased production of anti-inflammatory cytokine PARC/CCL18. PARC/CCL18 is a well-known marker for M2 macrophage phenotype, and formation of an anti-inflammatory phenotype is possible, and thus ultimately exerts positive influence on the acceleration of inflammatory process. Therefore increased effectiveness of the pharmacocytes in the treatment of purulent wounds could be explained by the change of the polarization of macrophages in the direction of M2 at the site of infection.