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Original article

Mononuclear cells phagocytic activity affects the crosstalk between immune and cancer cells

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ABSTRACT

The "professional phagocytes", i.e. monocytes and macrophages, play an important role as eliminators of pathogens and as essential components of the immune system. It is well established that monocytes induced for phagocytosis by various stimulators, produce cytokines that are closely related to inflammation. Considering the role of inflammation in carcinogenesis and the existence of an immune dialog between mononuclear and cancer cells, the aim of the present work was to examine cytokine production by immune cells stimulated for phagocytosis by latex particles and incubated with cells from HT-29 and RKO human cancer lines. Human peripheral blood mononuclear cells (PBMC) were incubated with various numbers of polysterene latex beads, 0.8 μm in diameter and the secretion of the following cytokines: TNF-α, IL-1β and IL-6, IL-10 and IL-1ra was examined before and after further incubation with cells of the both cancer lines. Phagocytosis of latex beads by PBMC caused an increased production of TNF-α, IL-1β and IL-10, whereas that of IL-6 declined. PBMC activated by latex beads and co-cultured with cancer cells generated lesser amount of the three pro-inflammatory cytokines TNF-α, IL-1β and IL-6, while that of the anti-inflammatory IL-10 and IL-1ra remained unchanged. The results indicate that phagocytosis of polystyrene latex beads by human PBMC alters the dialogue between immune and cells of human colon carcinoma lines, an observation that may clarify the role of the immune cells in attenuating inflammation and restraining carcinogenesis.

1. Introduction

The phagocytic capacity of the peripheral blood mononuclear cells is an important component of the innate immune system. From morphological point of view, it depends in part on the cell membrane fluidity that permits a rapid change from a spherical to irregular shape, pursued by membrane ruffling, pseudopodia-like formations and finally engulfment of target particles. The ultrastructural alterations accompanying phagocytosis have been reviewed by Djaldetti et al. [1]. The engulfing activity of monocytes assigned as "professional phagocytes" may be activated by a large number of factors, acting as phagocytic stimulators, such as pathogens, latex beads [2], migration inhibitory factor (MIF) [3], zymosan particles [4], antibiotics [5], carbon nanotubes [6], intralipid [7,8], as well as lipopolysaccharide (LPS) and phorbol myristate acetase (PMA) [9,10]. Considering the role of phagocytes in the function of the immune system, the essential point to be stressed is that these cells are engaged not only in clearance and destroying pathogens by a number of molecular mechanisms, but they are also capable to produce cytokines linked with inflammatory processes throughout the phagocytic process. Thus, human mononuclear cells incubated with zymozane or latex particles produced one- or two-fold amounts of IL-8 [4]. Rat peritoneal macrophages expressed a marked increase in IL-1β secretion following phagocytosis of latex beads comparable to cells stimulated with LPS [11]. Monocytes activated for phagocytosis by antibiotics, such as erythromycin, mixifloxacin and doxycyclin showed a significantly increased production of IL-1β and IL-6 [5]. On the other hand, cytokines added to phagocytic cells may affect their engulfing ability. Thus, while TNF-α and granulocyte/macrophage colony stimulating factor enhanced microglial cells phagocytosis, IL-4 and transforming growth factor-beta exerted an inhibitory effect on that cell function [12]. In a previous study [13], we have reported that human peripheral blood mononuclear cells (PBMC) are vividly activated for cytokine production following incubation with cancer cells, creating an immune dialogue between these two types of cells. The released inflammatory cytokines play an important role in initiation, development and maintenance of chronic inflammation linked closely with carcinogenesis. Moreover,

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we have shown that the crosstalk between immune and cancer cells is affected by a number of drugs, nutrients and chemicals [14]. Following these observations, the question has been posed if phagocytosis of latex particles will affect the immune crosstalk between human PBMC and cells of two human colon cancer lines, expressed by a modulation of cytokine production. The aim of the study therefore, was to examine the production of the pro-inflammatory cytokines TNF-α, IL-1β, and IL-6, as well as the anti-inflammatory cytokines IL-10 and IL-1ra by PBMC incubated with latex beads and to assess potential modulation of the immune crosstalk between phagocytes and cells of HT-29 and RKO human cancer lines.

2. Materials and methods

2.1. Latex particles

Polysterene latex beads, 0.8 μm in diameter, supplied as an aqueous suspension were used (Sigma, Israel). The number of latex particle/mL (n) was calculated using equation provided by the manufacturer. Latex beads' suspension was diluted in 0.9% sodium chloride solution and 0.01 mL of various amounts of latex beads was added to 1.0 mL of cell cultures to get a final number of 10^8, 5×10^8, 10^9, and 5×10^9 particles/mL.

2.2. Cell preparation and culture conditions

PBMC were separated from venous blood obtained from adult blood bank donors by gradient centrifugation using Lymphoprep-1077 (Axis-Shield PoC AS, Oslo, Norway). The cells were washed twice in phosphate buffered saline (PBS) and suspended in RPMI-1640 medium (Biological Industries, Beith Haemek, Israel) containing 1% penicillin, streptomycin and nystatin and supplemented with 10% fetal calf serum (designated as complete medium CM).

2.3. Effect of latex phagocytosis on cytokine production

An amount of 2×10^6/ml of PBMC suspended in CM were incubated for 24 h without or with latex particles, at numbers as described above. The plates were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. At the end of the incubation period, the culture media were collected, the cells were removed by centrifugation and the supernatants were kept at –70 °C until assayed for cytokine content.

2.4. Cell lines

HT-29 and RKO human colon cancer cell lines were obtained from the American Type Cultural Collection, Rockville, MD. The cells were maintained in CM containing MacCoy's 5A (Sigma, Israel) and modified eagle medium (MEM-Biological Industries Co, Beth-Haemek, Israel) respectively, supplemented with 10% FCS, 2 mM L-glutamine and antibiotics (penicillin, streptomycin and nystatin-Biological Industries Co, Beth-Haemek, Israel). The cells were grown in T-75 culture flasks (Nunc, Roskidle, Denmark) at 37 °C in a humidified atmosphere containing 5% CO_2.

2.5. Effect of phagocytosis on cytokine production by PBMC activated by cancer cells

A 0.5 mL of PBMC (4×10^6/mL of CM) was incubated with 0.5 mL of CM or 0.5 mL of each type of cancer cells (4×10^5/mL suspended in the appropriate CM). Then, 0.01 mL of various latex particles suspensions was added at the onset of cultures to reach the final numbers indicated above. Cultures without latex served as controls. The cultures were maintained for 24 h at 37 °C in a humidified atmosphere containing 5% CO_2. At the end of the incubation period, the cells and latex particles were removed by centrifugation at 12,000 rpm for 10 min, the supernatants were collected and kept at –70 °C until assayed for cytokine content.

2.6. Effect of phagocytosis on cytokine production by colon cancer cells

An amount of 2×10^5 of HT-29 or RKO cells suspended in 1 mL of appropriate CM were incubated for 24 h at 37 °C in a humidified atmosphere containing 5% CO_2 without or with latex particles at numbers as indicated above. At the end of the incubation period, the supernatants were collected and kept at –70 °C until assayed for cytokine content.

2.7. Cytokine content in the supernatants

The concentration of cytokines in the supernatants was tested using ELISA kits specific for human cytokines (Biosource International, Camarillo, CA) as detailed in the guidelines provided by the manufacturer. Each kit is specific for one individual cytokine. The detection level of all cytokines was 30 pg/mL. The percentage of the CV of the ELISA assay for the cytokines examined was 2% to the mean or less.

2.8. Statistical analysis

Data was analyzed using one way ANOVA with repeated measures to evaluate the effect of phagocytosis of latex particle on each cytokine production and two-tailed paired Student's t-test to compare between the effect of the various numbers of latex particles and the control (incubated without latex). The results are expressed as mean ± SEM. P value of < 0.05 was considered as statistically significant.

3. Results

3.1. Effect of phagocytosis on cytokine production by colon cancer cells

Supernatants obtained from HT-29 or RKO cells incubated for 24 h without or with latex particles did not contain detectable amounts of any of the cytokines tested in the current study.

3.2. Effect of phagocytosis on IL-1β production

A dose-dependent stimulation of IL-1β secretion was observed when non-stimulated PBMC were incubated with increased number of latex beads ($P = 0.0021$) (Fig. 1). At doses of 5×10^8, 10^9 and 5×10^9 particles/mL, IL-1β secretion was enhanced by 24%, 43% and 90% respectively ($P = 0.5$, $P = 0.027$, and $P = 0.0003$, respectively). PBMC incubated with either HT-29 or RKO colon cancer cells produced IL-1β at amounts up to 5.5- and 3.3-fold higher, respectively. A dose-dependent inhibition of HT-29-induced IL-1β production was found when increased doses of latex particles were added ($P = 0.013$). At 10^9 and 5×10^9 particles/mL, IL-1β secretion by HT-29-stimulated PBMC was reduced by 17% and 48%, respectively ($P = 0.069$ and $P = 0.0008$, respectively). At lower numbers of latex particle (below 5×10^8/mL), the secretion of IL-1β by either non-stimulated or HT-29 stimulated PBMC was not affected. The addition of latex particles at any of the doses tested had no effect on RKO-induced IL-1β secretion by PBMC ($P = 0.971$).