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Influence of Colchicine and Cytochalasin B on Pinocytosis, Phagocytosis, and Antibody-Dependent Cell-Mediated Cytotoxicity

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A. Introduction

The cytoskeleton is an organelle of eukaryotic cells engaged in organization and external and internal cell movements, e.g., endocytosis. Our previous experiments proved that colchicine (an antitubular drug) and cytochalasin B (cyt B, an antimicrofilament agent) have significant influence on the development of the process [8, 9]. Reports on the inhibiting effect of the development of antibody-dependent cell-mediated cytotoxicity (ADCC) are inconsistent. The aim of the present work was to examine the influence of colchicine and cyt B on the development and mutual dependence of pinocytosis, phagocytosis, and cytotoxic effect.

Peritoneal resident cells (PRC) isolated from Swiss mice were used as effector cells in our experiments. Macrophages constituted 60%–80% of this cell population. PRC at a concentration of 4×10^6/ml were incubated 2 h in MEM in 37 °C, 5% CO_2 with addition of: (a) colchicine 10^{-6} *M*; (b) cyt B 4 μg/ml; and (c) pure medium (control).

B. Pinocytosis

After 2 h of incubation, 0.5 ml MEM containing horseradish peroxidase (HRP) at a concentration of 3 mg/ml was added to the culture. After 90 min incubation, the cells were centrifuged, washed five times and after sonication in the presence of 0.05% Triton X-100, HRP was determined according to Karnowsky's method [4].

C. Phagocytosis

After 2 h of incubation as described, opsonized sheep red blood cells (SRBC) were added to the effector cell culture. The cultures were incubated "under" standard conditions for 90 min. The cells were centrifuged and supernatants were collected for determination of released hemoglobin (Hb). Nonphagocytized erythrocytes were removed by means of double lysis with 0.5 *M* NH_4Cl solution. After additional washing with PBS, the cells were sonicated in the presence of 0.05% Triton X-100. The Hb content in homogenates was determined as a measure of the effectiveness of phagocytosis. Phagocytosis was also examined using ^{51}Cr-labeled SRBC and measuring the radioactivity of the effector cells at the end of the experiment. Phagocytosis was also confirmed by morphological observations.

D. ADCC

In supernatants collected from PRC cultures with opsonized SRBC, Hb was determined according to a modified Karnowsky's method. When ^{51}Cr-labeled SRBC were used as the target cells, the

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radioactivity of the ^{51}Cr released was determined in a gamma scintillation counter. Full details of this assay procedure are described elsewhere [11, 12].

E. Results and Discussion

We have observed that cyt B and colchicine decreased pinocytosis of HRP by PRC by more than 30% (Table 1). In the case of colchicine treatment, phagocytosis was inhibited similarly to pinocytosis, but the process was completely stopped by cyt B (Table 2).

The results of endocytized Hb determination were identical with the determination by the isotope method. Analogously, ADCC activity was found to be identical with both methods (Hb or ^{51}Cr). PRC colchicine treatment had no influence on the development of ADCC, but cyt B treatment almost completely stopped the reaction (Table 3). The literature shows that a cytotoxic effect may be caused by enzymes [1, 2, 5–7]. This role is ascribed to lysosomal hydrolases released to the environment or to the enzymes producing oxygen radicals (superoxide) and hydrogen peroxide. It is considered that endo- and exocytosis are in constant dynamic balance. In both processes, the same cellular organelles: actin microfilaments and microtubules, take part. It was suggested in this and other work that the strong inhibition of pinocytosis and phagocytosis after microtubule destruction with colchicine might be connected with the strong decrease of enzyme release.

The effect of this should be a decrease in ADCC activity. In our work, PRC colchicine treatment had no influence on the reaction. The second possibility concerning the role of oxygen radicals seems to be inadequate to explain the ADCC mechanism. There are reports on monocytes unable to produce hydrogen peroxide and oxygen radicals that show strong activity in ADCC [3]. Cyt B in the concentrations examined causes disorganization of movement in the cell membrane. The effect of this is capping inhibition on the lymphocyte surface [10]. The destruction of actin microfilaments caused almost complete inhibition of ADCC by PRC.

It is known that the cell movements and the movements of the cell membrane elements are connected with actin microfilaments. So it is possible that the specific configurations of these elements in the cell membranes of the PRC, together with hydrophobic domains that lyse the membranes of the target cells, are responsible for the PRC cytotoxic effect in ADCC. This mechanism would be similar to that of complement action during formation of the membrane attack complex.

Table 1. Influence of colchicine and cyt B on pinocytosis

Medium	Number of endocytized HRP (ng/mg protein)	Percentage of control ratio
MEM	3 300	100%
MEM + colchicine	2 125	64%
MEM + cyt B	2 046	62%

Table 2. Influence of colchicine and cyt B on phagocytosis

Medium	Number of phagocytized SRBC/ 10^6 PRC	Percentage of control ratio
MEM	2 112 000	100%
MEM + colchicine	1 221 000	58%
MEM + cyt B	Undetermined	–

Table 3. Influence of colchicine and cyt B on ADCC

Medium	Number of SRBC destroyed by 1×10^6 PRC	Percentage of control ratio
MEM	4 212 000	100%
MEM + colchicine	4 085 000	97%
MEM + cyt B	385 000	9%

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