Cytotoxic Effects of Macrophages and Asbestos on Transformed Rat Mesothelial Cells

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Asbestos produced a cytotoxic effect on transformed cells of rat pleural mesothelium and on IAR2 epithelial cells and Rat1 fibroblasts transformed by *ras* oncogene, but not on normal cells of these strains under conditions of coculturing with peritoneal macrophages. Contact of mesothelioma cells, but not macrophages with asbestos was necessary and sufficient for attaining the cytotoxic effect. Macrophage-conditioned medium potentiated asbestos cytotoxicity for transformed mesothelial cells, but not for IARS-*ras* and Rat1-*ras*.

Key Words: *mesothelium; macrophages; asbestos*

Like pulmonary epithelium, mesothelium is a target for biological effect of asbestos and some other mineral fibers. The mechanism of transforming effect of asbestos and the role of other cells, in particular macrophages, in this type of carcinogenesis remain little studied. It was shown that macrophages play an important role in the development of asbestosis [7], another disease caused by mineral fibers. *In vitro* experiments demonstrated that macrophages inhibit asbestos-induced transformation of rat pleural mesothelial cells and this inhibition is associated with selective elimination of transformed cells [10]. However, factors responsible for selective cytotoxicity and cell specificity of this phenomenon are little studied.

The purpose of this study was to elucidate whether factors secreted by macrophages exert a direct tumoricidal effect or they increase the sensitivity of transformed mesothelial cells to cytotoxic effect of asbestos and to study the specificity of this effect.

MATERIALS AND METHODS

Pleural mesothelial cells of Wistar rats and mesotheliomas [4,9], fibroblasts Rat1 and Rat1-*ras*, and epithelial cells IAR2 and IAR2-*ras* were cultured in 25 cm^2 flasks (Costar) at 37°C and 6% CO_2 in medium-1 containing F12 (Sigma), 10% ETC (Gibco), and 2 mM glutamine (Gibco). The cells were subcultured using 0.25% trypsin and 0.025% EDTA (both from Gibco).

Peritoneal macrophages of Wistar rats were obtained by washing the abdominal cavity with Hanks' solution and subsequent adhesion on plastic [1]. Granulocytes, lymphocytes, and monocytes were isolated from human peripheral blood on a Ficoll-Hypaque gradient (Pharmacia) [5].

Conditioned media were obtained 24 h after culturing of 3×10^5 cells in 2 ml of medium-1 in 35-mm Petri dishes (Costar).

For evaluation of the cytotoxic effect of macrophages, 3×10^4 cells were seeded on 35-mm Petri dishes and after 24 h macrophages in different doses (1.3×10^6, 2.8×10^5, 5.5×10^4, 1.1×10^4, 2.2×10^3, and 4.4×10^2) were added to the cultures. On the next day textile Bazhenov chrysotile asbestos (50% fibers thinner than 1 μ, 70% fibers 10-50 μ long) was added to a final concentration of 5 μg/cm^2, and after 48 h the number of live cells was determined using trypan blue staining. For studies of the cytotoxic effect of conditioned media, 3×10^4 studied cells were seeded on 35-mm Petri dishes; conditioned media (final concentration 10%) and asbestos (final concentration 5 μg/cm^2) were added after 24 h, and

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after 48 h live cells were counted using trypan blue exclusion test.

For identification of cell targets of asbestos toxicity the cells were cultured in Petri dishes with inserts allowing culturing of two cell cultures on different surfaces in the same volume of medium. Mesothelioma cells (3×10^4) were seeded in 35-mm Petri dishes, after 24 h 25-mm inserts with Anopore membrane (0.2-μ pores; Nunc) were inserted into dishes and 2.2×10^3 macrophages were seeded on these inserts. After the next 24 h asbestos (final concentration 5 μg/cm^2) was added on one or both surfaces, and after 48 h live mesothelioma cells were counted using trypan blue staining.

Statistical analysis was carried out using Student's *t* test.

RESULTS

Malignant mesothelioma cells obtained *in vivo* were most sensitive to the cytotoxic effect of macrophages and asbestos (Fig. 1); *in vitro* transformed cells (spontaneously transformed mesothelium of late passages, *ras*-transformed IAR2 epithelial cells and *ras*-transformed Rat1 fibroblasts) were less sensitive. Mesothelial cells of early passages and nontransformed IAR2 and Rat1 cells exhibited low sensitivity to the toxic effect.

The highest toxicity was observed in macrophage-mesothelioma coculture on the same surface (Fig. 2). When two cell populations were grown on different surfaces, the cytotoxic effect was observed only after asbestos contact with mesothelioma cells, while macrophage contact with mineral fibers was of no importance. Hence, macrophages secrete two classes of factors inducing death of transformed mesothelial cells: some factors are characterized by a very short life span insufficient for diffusion from the surface of the insert to the bottom of Petri dish, while others are stable. These stable factors possess no intrinsic cytotoxic effect, but potentiate asbestos cytotoxicity.

Only transformed mesothelioma cells were sensitive to combined effect of asbestos and macrophage-conditioned medium (Fig. 3). Epithelial IAR2 cells, Rat1 fibroblasts (both normal and transformed) and normal mesothelial cells were resistant to these effects. Macrophage-conditioned medium alone produced no cytotoxic effects, while the effect of asbestos alone was negligible (death of about 5% cells; data not presented).

Only media conditioned by rat peritoneal macrophages and human peripheral blood monocytes potentiated the cytotoxic effect of asbestos, while media conditioned by IAR2 and Rat1 cells (both normal and *ras*-transformed), rat mesothelial and mesothelioma cells, and human lymphocytes and granulocytes were inactive (data not presented).

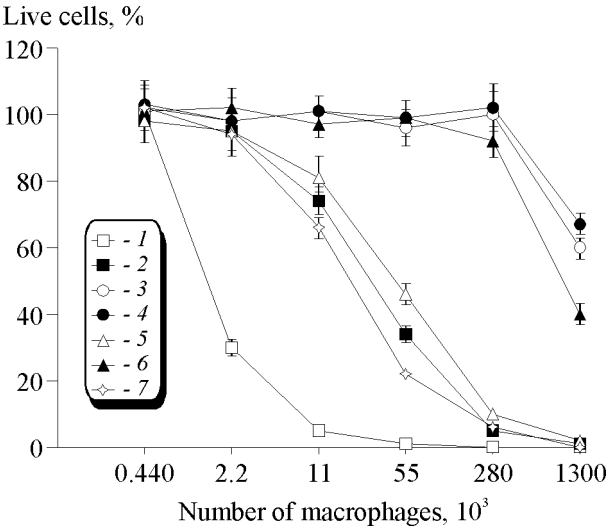

Fig. 1. Survival of different cell lines in coculture with macrophage and asbestos. *1*) mesothelioma; *2*, *3*) mesothelium; passages 30 and 8, respectively; *4*) IAR2; *5*) IAR2-*ras*; *6*) Rat1; *7*) Rat1-*ras*.

It can be hypothesized that the short-lived cytotoxic factors secreted by macrophages after contact with asbestos are highly active oxygen [2,8] or NO [11] derivatives. But of particular importance was to identify the macrophage-derived factors potentiating the cytotoxic effect of asbestos on transformed mesothelial cells.

The mechanisms of cytotoxic effect of asbestos are still unknown. It was demonstrated that endocytosis of mineral fibers is essential for induction of apoptosis in target cells [13], but intracellular mechan-

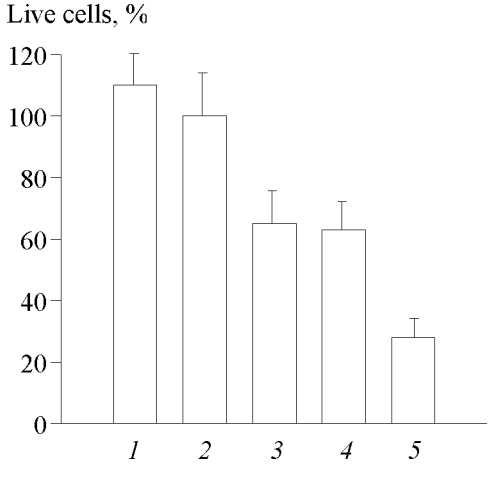

Fig. 2. Survival of mesothelioma cells in coculture with macrophages on different surfaces in the same medium. *1*) macrophages/mesothelioma; *2*) macrophages+asbestos/mesothelioma; *3*) macrophages/mesothelioma+asbestos; *4*) macrophages+asbestos/mesothelioma+asbestos; *5*) mesothelioma+macrophages+asbestos (on the same surface).

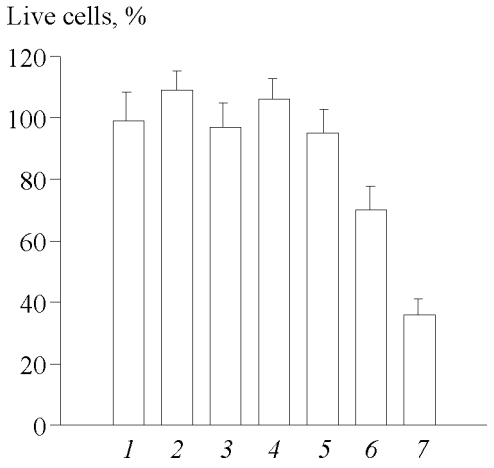

Fig. 3. Survival of different cell strains during combined exposure to asbestos and medium macrophage conditioned. *1*) Rat1; *2*) Rat1-*ras*; *3*) IAR2; *4*) IAR2-*ras*; *5,6*) mesothelium, passages 8 and 30, respectively; *7*) mesothelioma.

isms are little known. An important role in this process is played by free radicals [3,6] and activation of the epidermal growth factor and ERK kinases [14]. The factor detected by us can act via two mechanisms: it can be adsorbed on asbestos fibers and stimulate its endocytosis by mesothelial cells (vitronectin potentiates asbestos toxicity by this mechanism [13]), or it can activate intracellular apoptotic cascades increasing cell sensitivity to asbestos effect (for example, γ-interferon treatment of mouse carcinoma C-26 cells did not induce apoptosis, but sharply potentiated the apoptotic effect of tumor necrosis factor [12]). These mechanisms should be further investigated.

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