IMMUNOLOGY AND MICROBIOLOGY

Cytokine Secreted by Rat Macrophages and Inhibiting Proliferation of Mesothelial Cells

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Rat peritoneal macrophages and human peripheral blood monocytes secrete a protein with a molecular weight of 450 kDa, which specifically inhibits proliferation of cultured rat pleural mesothelial cells, but not fibroblasts and epitheliocytes. Protein secretion does not depend on the activation of macrophages. This cytokine is not a cobalamin-binding protein and has no arginase activity.

Key Words: *mesothelium; macrophages; cytokines*

The mesothelium lining secondary body cavities in humans and animals and lung epithelium are the main targets for carcinogens, including asbestos and other mineral fibers [8]. The mechanism of asbestos-induced cell transformation and the role of other cells (e.g., macrophages involved in the pathogenesis of asbestosis caused by mineral fibers [7]) in carcinogenesis remain unknown.

Macrophages secrete various cytokines, including tumor necrosis factor-α, interleukin-1, and colony-stimulating factor-1 regulating proliferation of fibroblasts, smooth muscle cells, T and B lymphocytes, and other cells [2,10,11]. The effect of macrophages on proliferation of mesothelial cell is poorly understood. The interaction between co-cultured human macrophages and peritoneal mesothelial cells was previously studied [6]. However, biologically active compounds were not isolated and assayed.

Here we studied the effect of substances produced by macrophages on proliferation of mesothelial cells, evaluated the specificity of this influence for mesothelial cells, and characterized cytokines.

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MATERIALS AND METHODS

Cultures pleural mesothelial cells from Wistar rats were obtained as described elsewhere [3,9]. 3T3 and NRK fibroblasts, P388 D1 myeloid cells (Flow), and IAR2 epithelial cells gifted by E. M. Levina were also used. The cells were cultured in 25-cm^2 flasks (Costar) with medium 1 containing F12 medium (Flow), 10% fetal bovine serum (FBS, Gibco) and 2 mM glutamine (Flow) at 37°C and 6% CO_2. These cells were subcultured using 0.25% trypsin and 0.025% ethylenediaminetetraacetate (Serva).

Peritoneal macrophages were obtained from peritoneal lavage fluid followed by adhesion on plastic plates [1]. The cells (3×10^5) were placed in 35-mm Petri dishes with 2 ml medium 1, and conditioned medium was collected after 24 h. DQ12 silica, chrysotile-asbestos, or *E. coli* lipopolysaccharide (LPS, Sigma) in concentrations of 1 μg/cm^2, 5 μg/cm^2, and 50 μg/ml, respectively, were added 3 h after cell inoculation to evaluate the effect of activated macrophages on cytokine secretion.

Granulocytes and monocytes were isolated from human peripheral blood [4]. Erythrocytes were lysed for 5 min in 0.83% NH_4Cl, and the ghosts were removed. The cell suspension was centrifuged in a Ficoll-Hypaque gradient (Pharmacia) at 1500*g* for 45

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min. Precipitated granulocytes (5×10^7 cells) were placed in 35-mm Petri dishes with 2 ml medium 1, and after 24 h conditioned medium was collected. Mononuclear cells collected from the surface layer of Ficoll were inoculated into plastic Petri dishes and washed from nonadherent cells 24 h later. Monocyte-conditioned medium was obtained similarly to the medium conditioned by rat macrophages.

NRK and P388 D1 cells (3×10^5 and 3×10^6, respectively) were placed in 35-mm Petri dishes with 2 ml medium 1, and conditioned medium was collected 24 h later.

Arginine concentration in 24-well plates with passage 6 mesothelial cells was measured 48 h after the addition of macrophage-conditioned medium by the method of Sakagushi [5].

Molecular weights of secreted cytokines were estimated by gel filtration of a 10-fold concentrated macrophage-conditioned medium (Ultracent-10 filter, Bio-Rad) on a 0.8×52-cm column (Whatman) packed with Sephacryl S300 (Pharmacia) and balanced with Hank's solution at a flow rate of 4 ml/h. After chromatography 0.6 ml fractions were sterilized by filtration, and inhibition of mesothelial cell proliferation was assayed.

Cell proliferation was studied by ^{3}H-thymidine incorporation. Passage 6 mesothelial or NRK/3T3/IAR2 cells were grown in 24-well plates (Costar, 5×10^4 cells/well) to monolayers and then reinoculated into F12 medium with 0.2% FBS for 48 h. FBS alone (10%) or in combination with test samples was added to control or experimental wells, respectively. ^{3}H-Thymidine (0.5 μCi/well) was added 24 h later. After 24 h, the cells were washed 3 times with physiological saline and 5% trichloroacetic acid. ^{3}H-Thymidine incorporation into acid-insoluble fraction was measured using a ZhS-8 scintillation cocktail.

The results were analyzed by Student's *t* test.

RESULTS

Macrophage-conditioned medium dose-dependently inhibited ^{3}H-thymidine incorporation into DNA of normal mesothelial cells (Fig. 1). Human monocyte-conditioned medium also suppressed proliferation of mesothelial cells, while media conditioned by granulocytes, NRK fibroblasts, and P388 D1 myeloid cells produced no significant changes. These results indicate that secretion of this inhibitor is specific for macrophages and monocytes, but it is not species-specific.

Activation of macrophages with DQ12 silica, chrysotile-asbestos, or *E. coli* LPS did not modulate secretion of this cytokine. It was reported that secretion of various proteins sharply increases after activation of macrophages. However, there are data that lysozyme production remains unchanged under these conditions.

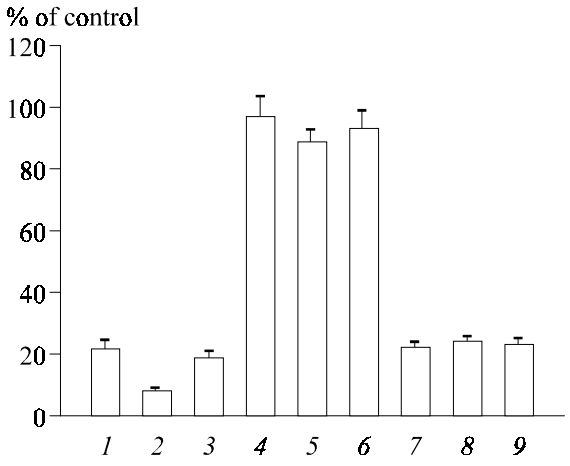

Fig. 1. Effects of conditioned media on ^{3}H-thymidine incorporation into mesothelial cell DNA: *1*) 10% macrophage-conditioned medium, *2*) 40% macrophage-conditioned medium, *3*) 10% monocyte-conditioned medium, *4*) 10% granulocyte-conditioned medium, *5*) 10% NRK cell-conditioned medium, *6*) 10% P388 D1 cell-conditioned medium, *7*) 10% medium conditioned by macrophages treated with DQ 12 (1 μg/cm^2), *8*) 10% medium conditioned by macrophages treated with asbestos (5 μg/cm^2), and *9*) 10% medium conditioned by macrophages treated with *E. coli* LPS (50 μg/ml).

The addition of macrophage-derived factor had no effect on ^{3}H-thymidine incorporation into DNA of 3T3 and NRK fibroblasts and IAR2 epithelial cells, which indicated its specific influence on mesothelial cells (Fig. 2).

Cobalamin (1.5 and 3 μg/ml) added to conditioned medium did not abolish its inhibitory effect on mesothelial cells. Therefore, inhibition of mesothelial cell proliferation was not related to secretion of vitamin B_{12}-binding proteins by macrophages.

Incubation of mesothelial cells in macrophage-conditioned medium did not decrease arginine concen-

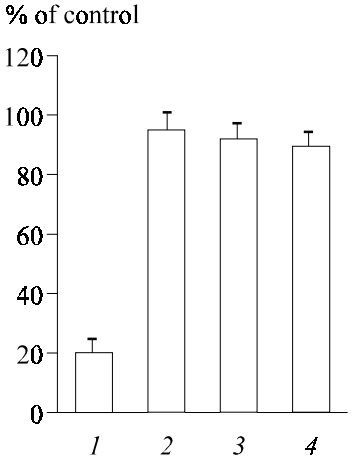

Fig. 2. Effects of macrophage-conditioned medium on ^{3}H-thymidine incorporation into DNA of mesothelial cells (*1*), 3T3 fibroblasts (*2*), NRK fibroblasts (*3*), and IAR2 epithelium (*4*).

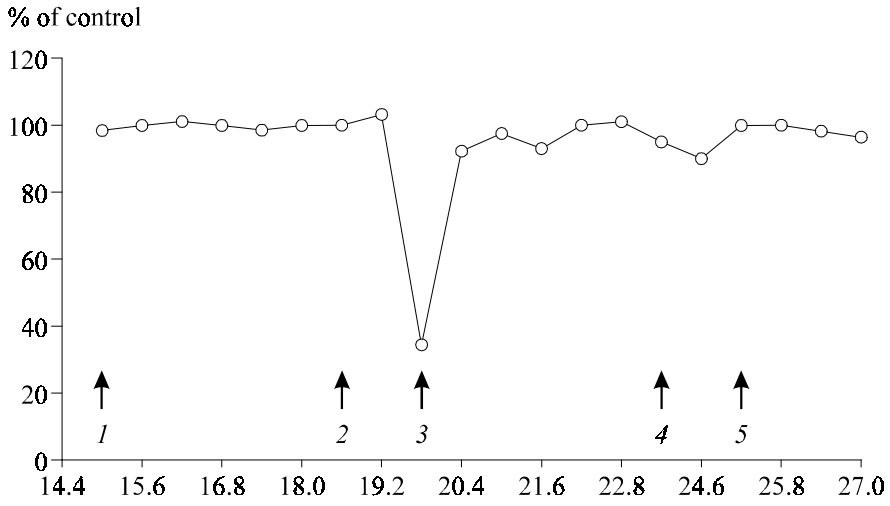

Fig. 3. Effects of protein fractions obtained by chromatography of macrophage-conditioned medium on Sephacryl S300 on ^{3}H-thymidine incorporation into mesothelial cell DNA: free volume (*1*), proteins with molecular weights of 669 (*2*), 440 (*3*), 160 (*4*), and 80 kDa (*5*).

tration compared to the control, which indicated that the inhibitory effect was not associated with arginase.

Measurements of the molecular weight of macrophage-derived cytokine inhibiting proliferation of mesothelial cells showed that this substance did not pass through a dialysis bag and Amicon PM 10, PM 30, XM 100 and XM 300 filters. Gel filtration revealed that this cytokine had a molecular weight of 450 kDa (Fig. 3).

Thus, macrophages secrete a protein that specifically inhibits proliferation of mesothelial cells. The molecular weight of this protein differs from that of interleukin-1, interleukin-3, interleukin-6, interleukin-8, interleukin-10, interleukin-12, interleukin-15, tumor necrosis factor-α, granulocyte and granulocyte/macrophage colony-simulating factors, and tumor growth factor-β. Under certain conditions, macrophages and monocytes synthesize cobalamin-binding proteins and arginase. Both proteins suppress cell division, but in our experiments they were not involved in the inhibition of cell proliferation.

The test cytokine can play a role in tumor promotion during transformation of mesothelial cells, if partially transformed cells are less susceptible to the inhibition of proliferation than normal cells. This problem requires further detailed investigations.

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