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# *In Vitro* Destruction of Tumor Cells by Human Monocytes[1] (38363)

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The role of large mononuclear leukocytes in host defense against neoplastic cells has recently attracted increased attention. It has been suggested that such leukocytes are effectors in a primitive surveillance mechanism against aberrant cells (1). A phenomenon possibly related to a local amplification of this primitive surveillance mechanism in man is regression of cutaneous neoplasms after recruitment of mononuclear inflammatory infiltrates at the tumor sites by induction of delayed type hypersensitivity reactions to chemical haptens (2,3), microbial antigens, or lymphokines (4,5). Increased resistance to carcinogens and transplanted tumors after administration of various agents with a stimulatory effect on the reticuloendothelial system (6,7) may be a reflection of a systemic activation of the same mechanism. This implies that large mononuclear cells are selectively cytotoxic for neoplastic cells. In support of this view are the findings of a striking cytotoxicity of activated peritoneal leukocytes (about 70% large mononuclear cells) of rats (8–10) and mice (11,12) towards neoplastic cells with minor or no effect on cells of normal tissue origin.

Since the large mononuclear cells of the peritoneum and other tissues, at least in part, are derived from the monocytes of the blood (18) one might anticipate that these latter cells also exhibit cytotoxic properties. Here we report preliminary observations of tumor cell destruction by human blood monocytes *in vitro*.

*Materials and Methods.* Cells of a human osteosarcoma and a reticulum cell sarcoma were established *in vitro*. When used for experimental purposes the cells were in the eighth to tenth passage. Human monocytes of healthy donors were isolated by passage of whole heparinized blood through "LP- Leuko-Pak" leukocyte filters (Fenwall Laboratories, Morton Grove, Illinois). The adherent cells were eluted by flushing the filters with ABO compatible plasma acidified by addition of citrate and lactate (21). Separation of the eluted cells was achieved by centrifugation onto a Ficoll-Isopaque cushion (13). The resulting preparations consisted of about 90% or more large mononuclear cells. The remaining cells were lymphocytes and granulocytes.

Purified lymphocytes were prepared from human blood buffy coat leukocytes by Ficoll-Isopaque sedimentation. The granulocyte contamination of the resulting cell population was less than 2%. Lymphokines were obtained by *in vitro* stimulation of purified lymphocytes by the mitogen concanavalin A (Miles Laboratories, Kankakee, IL, catalogue #79–001). Cultures of lymphocytes maintained in tissue culture medium supplemented with 10% autologous serum and mitogen, 2.5 $\mu$g/ml, were incubated at 37° on a roller drum for 24 hr. The supernatant fluids were collected and tested by intradermal injection of human skin with 0.1 ml quantities of material. Reactions measuring up to 40 mm in diameter of erythema and induration reached

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peak intensity between 6 and 8 hr. Control preparations of mitogen alone or supernatants of nonstimulated lymphocyte cultures induced reactions of erythema and induration measuring 3–4 mm in diameter. Microscopic examination of skin biopsies obtained 24 hr after intradermal injection of supernatant fluid of mitogen stimulated lymphocyte cultures revealed an inflammatory infiltrate of the dermis consisting predominantly of large mononuclear cells with an admixture of lymphocytes and polymorphonucleated granulocytes.

*Experimental.* For experimental use tumor cells were seeded in round, 35 mm plastic dishes using an inoculum of 150,000 cells per dish. After 24 hr, fluids were removed and replaced with either of the following: fresh medium; a 1/20 dilution of supernatant fluid of mitogen stimulated lymphocytes in fresh medium; or monocytes suspended in either of these two vehicles at a ratio of 20 monocytes per target cell. The cultures were incubated in a water saturated atmosphere containing 5% $CO_2$ at 37° for 60 hr at which time they were harvested and the target cells enumerated by the use of a Coulter counter (20,1). The mean numbers of target cells from duplicate cultures of an experiment, at the time of harvest, expressed in terms of percent of the numbers of cells of unchallenged control cultures are presented in Fig. 1. When a 1/20 dilution of supernatant fluid of mitogen stimulated lymphocytes (K) in fresh medium was added to the target cells the final cell counts of the time of harvest were about 80% of the cell counts of the control cultures receiving fresh medium only. After challenge with monocytes (Mo), the final target cell numbers were 45 or 60% of the unchallenged companion cultures. Challenge with monocytes in combination with a 1/20 dilution of supernatant fluids of mitogen stimulated lymphocytes (MoK) resulted in a further reduction of the final numbers of target cells. In these latter cultures the final target cell numbers were below the initial numbers at the time of addition of monocytes to the cultures. Comparable results were obtained in two additional experiments. The effect of monocytes in combination with supernatant fluids of mitogen stimulated lymphocytes on cultures of the reticulum cell sarcoma is illustrated in Fig. 2.

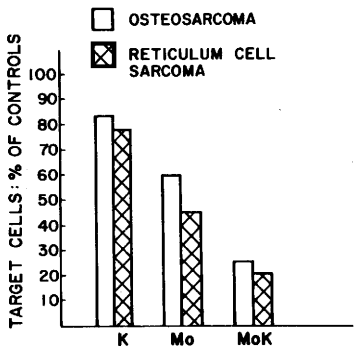


FIG. 1. Cells of a human osteosarcoma and a reticulum cell sarcoma were cocultivated *in vitro* with monocytes (Mo). or monocytes activated with lymphokines (MoK). Control cultures received lymphokines (K) or media in lieu of monocytes. After 60 hr the cultures were harvested and the target cells enumerated. The graph shows the target cell numbers expressed in terms of percent of the cell numbers of the media controls.

*Discussion.* The results of these preliminary experiments indicate that human peripheral blood monocytes are cytotoxic for human tumor cells. The increase in observed cytotoxicity by combining monocytes with supernatant fluids from nonspecifically stimulated lymphocytes can hardly be accounted for by the relatively minor inhibitory effect on the target cells by these supernatant fluids *per se*. A more likely interpretation is an activation of the monocytes by lymphokines present in these supernatant fluids. In favor of this view is stimulation of a number of macrophage functions by mediators produced by lymphocytes (14).

Lymphokines prepared by mitogen stimulation of lymphocytes *in vitro* when injected intradermally induce an inflammatory response similar to that of delayed hypersensitivity reactions (15). Inflammatory reactions induced by lymphokines when elicited at the site of neoplasms involving the skin of man have been found to result in tumor regression (14). A characteristic feature of such inflammatory reactions is accumulation of lymphocytes and large mononuclear cells. We have previously proposed that the large mononuclear cells are part of the effector mechanism in tumor cell destruction at sites of induced delayed type hypersensitivity reactions (1,9,10). Implicit in this proposition is that large mononuclear cells may be equipped with a mechanism for recognition which permit them to differentiate between normal and neoplastic cells. This suggestion is supported by the observation that peritoneal leukocytes of nonspecifically stimulated rats (1,9,10) and mice (11,12) are selectively cytotoxic for isologous as well as

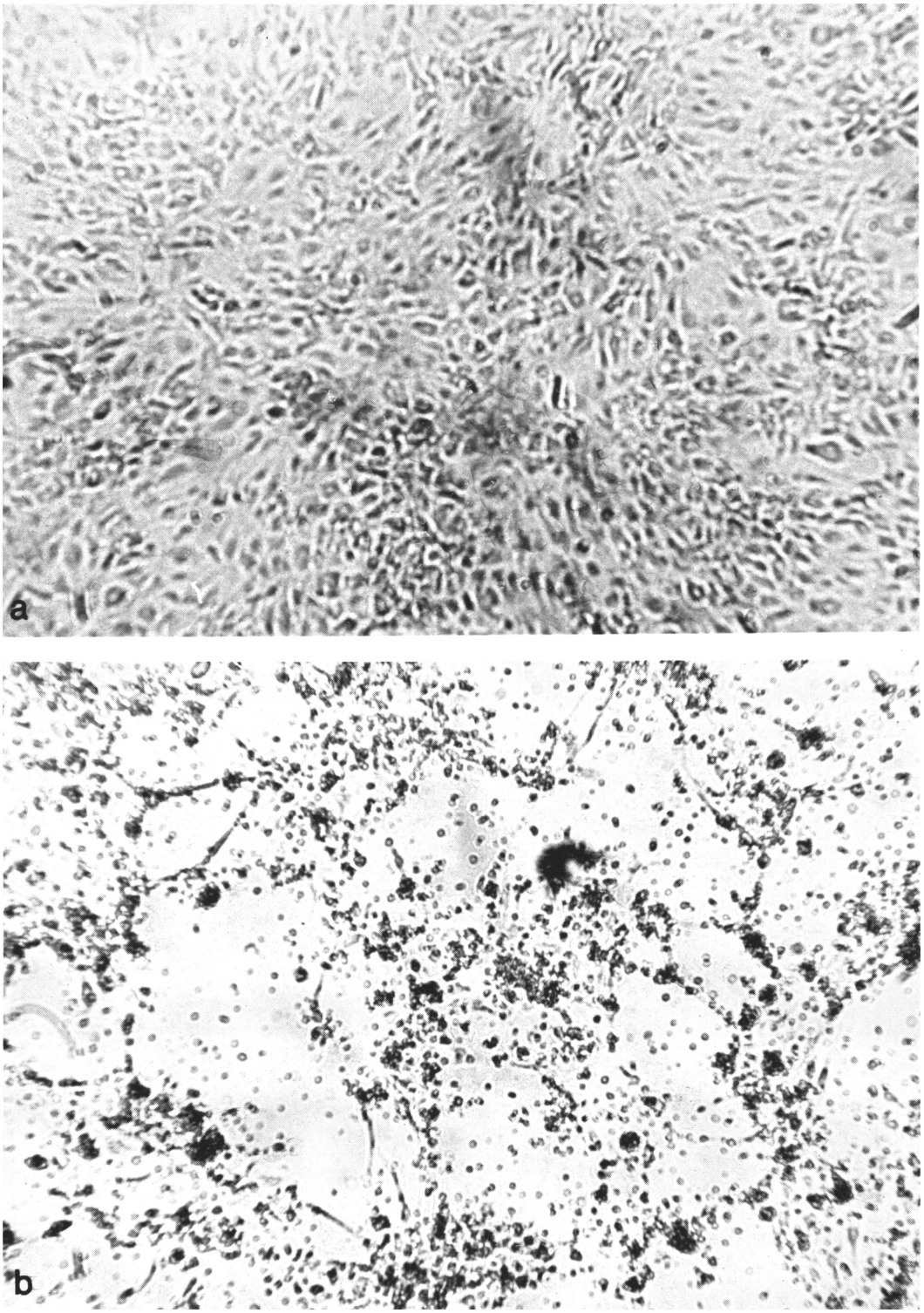


FIG. 2. a. Culture of human reticulum cell sarcoma. b. Comparison culture of the one shown in "a" challenged 60 hr previously with activated monocytes. Note extensive destruction of the sarcoma cells. (Magnification of both microphotographs before printing: 350×).

homologous neoplastic cells, with significantly less or no adverse effect on cells of normal tissue origin. The basis for this selective cytotoxicity of large mononuclear cells does not seem to be antigenic differences between tumor cells and cells or normal tissue origin. Rather, some unique properties of the cytoplasmic membrane of neoplastic cells seem to be involved (1, 9). Whether human blood monocytes exhibit a similar selectivity in their cytotoxicity is currently being investigated.

Serum of rats carrying tumors induced by polyoma virus have in some instances been found to increase cytotoxicity of syngeneic peritoneal leukocytes towards polyoma tumor cells *in vitro* (8), and it is therefore conceivable that in addition to an intrinsic recognition mechanism tumor specific antibody may be involved in imparting specificicity or selectivity on the cytotoxicity of large mononuclear cells. The antibody may be cytophilic for these leukocytes or may alternatively primarily bind to tumor cells and thus earmarking them for destruction by the mononuclear cells. Such mechanisms may be related to the antibody dependent cell mediated cytotoxicity reported by Perlmann and Holm (16) or the "arming" of macrophages described by Evans and Alexander (17).

In terms of phylogenetic development, large mononuclear cells are older than the more specialized small lymphocytes (18). Conceivably, large mononuclear cells are among the effectors in a surveillance mechanism against aberrant cells which is more primitive than that ascribed to T cells. Regression of cutaneous neoplasms at sites of induced delayed hypersensitivity reactions may represent a local recruitment of this primitive surveillance mechanism. Alternative means of activation of this surveillance mechanism may prove to be of value in the management of cancer. The use of local administration of lymphokines has so far yielded promising results in the treatment of superficial basal cell carcinomas, mycosis fungoides (4) and adenocarcinoma of the breast metastatic to the skin (5). Established lines of human lymphoid cells might be a convenient source of lymphokines in the quantities necessary for extension of these initial clinical trials (19).

*Summary.* Human blood monocytes were cytotoxic towards cells of a human osteosarcoma and a reticulum cell sarcoma *in vitro*. Activation of the monocytes by supernatant fluids of mitogen stimulated human lymphocytes markedly increased their cytotoxicity towards the neoplastic cells. Intradermal injection of these supernatant fluids into human skin induced an inflammatory infiltrate consisting predominantly of large mononuclear cells. The role of large mononuclear cells in cancer surveillance is discussed.

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