A simple procedure for removal of intra- and extra-tumoral macrophages from primary tumor cultures[1]

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Summary. Improved growth of tumor cells in primary cell suspensions isolated from solid tumors can be achieved by addition of an antifungal agent, mycostatin, to the culture medium. Mycostatin inhibits the cytotoxicity of macrophages and also quickly detaches phagocytes without any apparent retardation of the growth of tumor cells. This method is particularly useful for obtaining cultures of lung tumor cells or cells from lung metastases of other tumors.

Complex nutritional requirements and hormonal influences contribute to the difficulty of establishing tumor cell cultures in vitro, and the outgrowth of fibroblasts can cause additional technical problems. Another difficulty is the presence of in situ immunological effector cells within solid tumors, as described for both human and animal tumors[3-8]. Since some in situ effector cells have been shown by conventional microcytotoxicity tests to be directly cytotoxic or cytostatic to tumor cells in vitro, the growth of tumor cells can be significantly affected when mixed cell populations are cultured.

Furthermore, the proliferative capacity of some intratumoral macrophages may contribute to this problem. In tumor specimens obtained from the lung, the presence of highly cytotoxic alveolar macrophages in addition to intratumoral macrophages also adds to the difficulty of obtaining viable tumor cell cultures.

Methods currently used for removal of macrophages from mixed cell suspensions or from cultures are complex and usually ineffective. We have found that the inclusion of mycostatin, a common antifungal antibiotic, in the culture medium provides a simple and effective alternative.

Materials and methods. An uncharacterized, highly metastatic tumor, PR-IV, which was isolated in our laboratory from a spontaneous mammary tumor of a DBA/2J retired female breeder mouse (Jackson Laboratory, Bar Harbor, ME) was maintained as a monolayer culture using alpha medium (Gibco) plus 10% fetal calf serum. Suspensions of cultured tumor cells were prepared by trypsinization, and 10^5 viable cells were injected s.c. into syngeneic (DBA/2J) hosts. The s.c. tumors grew slowly, reaching approximately 10–15 mm diameter by day 45 after injection, when the animals were killed. All the hosts developed extensive lung metastases, which were surgically removed and transferred into tissue culture medium. The tissue was minced and trypsinized to obtain single-cell suspensions. The cells were grown in multiwell tissue culture plates (Falcon 3008) in alpha medium plus 10% fetal calf serum, with or without mycostatin (50–100 units/ml). The medium was changed every 3 days. At the end of the culture period, the cells were fixed in 5% phosphate-buffered glutaraldehyde and stained with Giemsa.

Results and discussion. Neoplastic growths are usually associated with local inflammatory responses, which may either inhibit or enhance tumor growth. On one hand, some macrophages isolated from either in situ or distant peripheral sources have been shown to promote tumor cell proliferation in vitro[9,10]. On the other hand, the presence of specific anti-tumor effector cell populations within solid tumors[3-8] not only supports the association of 'round cell' infiltration with prognosis of human tumors[11-13] but also may explain in part the difficulty of establishing tumor cell cultures in vitro. Effector populations found in situ include both cytotoxic T lymphocytes, as described in human breast carcinomas[5], and macrophages. The former are nonadherent and can be washed out of cell cultures after the tumor cells have attached to the plate; however, inactivation and/or removal of macrophages is more difficult. Most anti-macrophage sera are ineffective for producing long-lasting macrophage inactivation, and they are not suitable

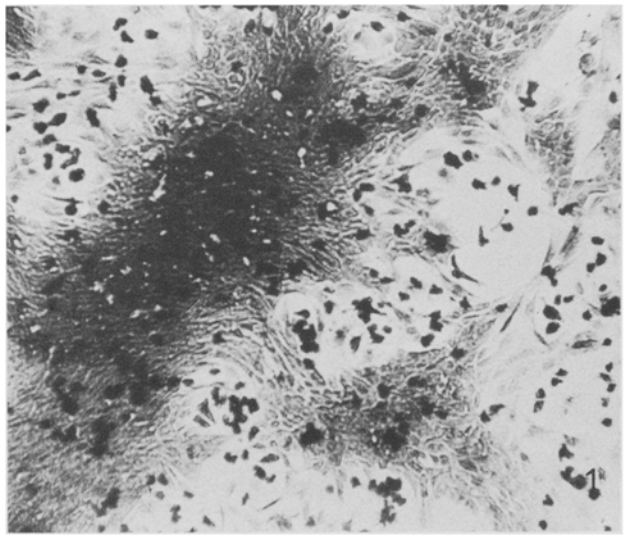

Fig. 1. 1-week-old culture of tumor cells from a lung metastasis of a murine tumor (PR-IV) grown without mycostatin. Numerous macrophages (very dark stain) are evident, as well as many holes in the monolayer where tumor cells have been destroyed by macrophages.

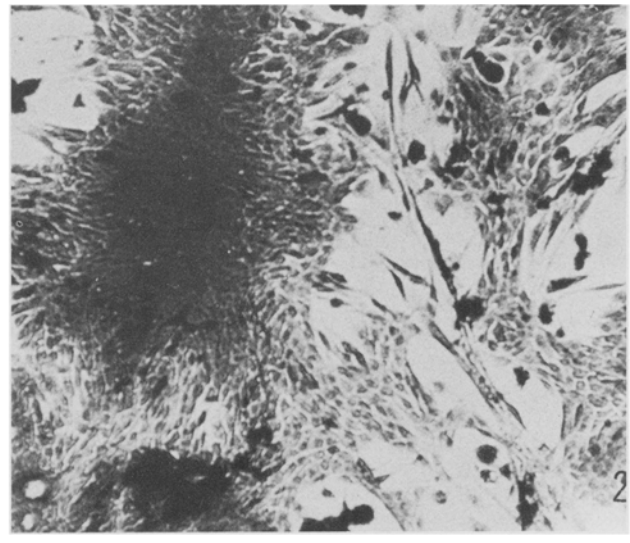

Fig. 2. 10-day-old culture of PR-IV cells with mycostatin (added after 5 days). In comparison with figure 1 there is a significant decrease in the number of macrophages and an increase in the uniformity of the tumor cell monolayer.

for treating large numbers of cells. Attempts to remove macrophages by density gradient sedimentation or by formation of rosettes with antibody-coated sheep erythrocytes (EA rosettes) have had only limited success. Although macrophages free of tumor cells can be obtained by culturing mixed cell populations in medium containing low concentrations of trypsin[14], this method is not useful for isolating tumor cells since the 'nonadherent' population from such cultures contains large numbers of less adherent macrophages/monocytes. Moreover, proliferation of intratumoral macrophages has been observed with various tumors[3], resulting in significant inhibition of tumor cell growth in vitro. In the present study we have examined the capacity of intratumoral and alveolar macrophages to inhibit the growth of tumor cells from metastatic murine lung tumors in vitro and the use of mycostatin to suppress the activity of macrophages in vitro.

Mycostatin is a common antifungal antibiotic that has long been used as an antifungal agent in tissue culture, with well-established safety and effectiveness. It is suspended in the medium and, due to its low solubility, is quickly phagocytosed by macrophages, which apparently are unable to digest it. In cultures without mycostatin, tumor cell monolayers were irregular in shape and contained numerous macrophages (figure 1). After prolonged culture periods, the tumor cells were often completely eliminated. Addition of mycostatin early in the culture period quickly and effectively eliminated macrophages and restored the growth of tumor cells (figure 2). Mycostatin-saturated macrophages were apparently unable to affect tumor cells, which began to regrow in the areas where they had been eradicated.

In conclusion, we have found that the addition of mycostatin to tissue culture medium not only prevents fungal contamination but also improves tumor cell growth by inhibiting the cytotoxicity of intra- and extra-tumoral macrophages. This is especially useful for establishing primary cultures of cells from lung tumors or lung metastases.

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Studies on the mechanisms of neurulation in the chick: The intracellular distribution of Ca^{++}[1]

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Summary. Coated vesicles were found to accumulate Ca^{++} in neuroepithelial cells and may play a role in regulating the contractile activities of apical microfilament bundles during uplifting of neural folds in the chick.

During neurulation in the chick the neural plate folds at its midline and eventually rolls itself into a neural tube. The origin of the motive forces for these movements has been the subject of numerous studies over the past decade[3,4]. Microfilament bundles, which encircle the cell apex, are generally thought to play an active role in uplifting of neural folds. Microfilaments forming these bundles are capable of binding heavy meromyosin and therefore contain actin[5]. Indirect immunofluorescence has revealed that myosin is also present in neuroepithelial cells and is most concentrated in the cell apex where the actin-like microfilaments are organized into discrete bundles[6]. These findings along with our previous observation that apical microfilament bundles exhibit substructural features resembling myofibrils[5] strongly suggest that apical microfilament bundles generate the motive forces for uplifting of neural folds by a sliding filament mechanism similar to that of skeletal muscle. This is in line with Burnside's[7] observation of a progressive increase in the thickness of microfilament bundles during apical constriction of amphibian neuroepithelial cells. It is well known that muscle contraction is regulated by the availability of intracellular free Ca^{++}. This raises the possibility that Ca^{++} also serves as a regulator of microfilament contraction in the developing neuroepithelium. This view is supported by the recent findings that chemical agents (e.g., papaverine and ionophore A23187) known to alter intracellular free Ca^{++} levels disrupt the uplifting and alignment of neural folds through their action on microfilaments[8–11]. For Ca^{++} to serve as a regulatory ion, neuroepithelial cells must have a means of controlling local intracellular Ca^{++} levels. Thus, knowledge of the distribution of Ca^{++} in neuroepithelial cells could augment our understanding of the mechanisms which control uplifting of neural folds. As a first part of our interest in this problem, the present study was carried out to localize Ca^{++} in neuroepithelial cells by cation precipitation with pyroantimonate[12,13]. An attempt was also made to identify those structures that have the capacity to serve as intracellular reservoirs from which Ca^{++} may be mobilized to regulate microfilament contraction.

Materials and methods. Fertile White Leghorn eggs were incubated at 37.5 °C to obtain embryos at stage 8 or 8+ of development[14]. These stages were chosen because the forming neural tube exhibits a gradual variation in the degree of