

In Vitro Culture of a Primary Plasmacytoma that has Retained Its Dependence on Pristane Conditioned Microenvironment for Growth

A. Degrassi, D. M. Hilbert, ²A. O. Anderson, M. Potter, H. G. Coon

Laboratory of Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD, 20892 USA; ²US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD, 21701 USA

INTRODUCTION

In BALB/cAn mice, intraperitoneal implantation of plastics (Merwin and Redmon 1963), paraffin oils or the pure alkane pristane (2,6,10,14 tetramethylpentadecane), induces the formation of plasmacytomas (PCT) that arise in the peritoneal inflammatory tissues evoked by these agents (Potter and MacCardle 1964). The paraffin oils and pristane are known to induce the formation of a chronic oil granulomatous (OG) tissue on peritoneal surfaces (Potter and MacCardle 1964, Anderson et al 1985). This tissue contains predominantly macrophages and neutrophils that have ingested or surrounded oil droplets and become fixed to peritoneal surfaces (Anderson et al 1985). The OG tissue is vascularized by angiogenesis from mesenteric blood vessels and covered by mesothelium. In addition, the OG tissue contains a rich supportive stroma of fibroblasts and reticular fibroblasts.

One of the hallmarks of primary plasmacytomas is the fact that free ascitic tumor cells in doses of 10^6 cannot be successfully transplanted when introduced into the normal peritoneal cavities of syngeneic BALB/cAn mice (Potter et al 1972). Such tumors can only be transplanted into pristane primed animals. However, the dependence of primary plasmacytomas on the oil-induced microenvironment is usually lost after several transplant generations. These findings suggest the growth requirements of primary plasmacytomas may differ from those of long term transplanted plasmacytomas. Consistent with this view has been the inability to grow primary plasmacytomas in vitro. In contrast, many serially transplanted plasmacytomas have been adapted to tissue culture and have proved invaluable in defining their growth requirements (Nordan and Potter 1986). In an effort to understand the growth requirements of granuloma-dependent tumors we have established granuloma-derived adherent stromal cell lines. We report here the in vitro growth of a primary plasmacytoma (5-25) on such lines. The resultant PCT cell line has retained its dependence on the oil-induced microenvironment for in vivo growth in spite of 8 months of in vitro passage.

RESULTS AND DISCUSSION

The in vivo dependence of primary plasmacytoma growth on

granulomatous tissue suggests that successful in vitro growth of primary plasmacytomas may require the presence of stromal/adherent cells from the primary site of plasmacytomagenesis, the granuloma. Accordingly, a primary plasmacytoma tumor (5-25) was removed from the peritoneal cavity of a BALB/cAn previously injected with pristane (0.5ml ip.) 150 days prior to sacrifice. The granulomatous tissue and plasmacytoma cells were disassociated for one hour at 37°C in isotonic buffer containing collagenase (60U/ml) trypsin (0.1%) and chicken serum (2%) with mild agitation. Cells were washed twice in medium (RPMI 1640 containing 10% fetal calf serum (FCS), 2mM glutamine, 20mM HEPES, non-essential amino acids, and 50µg/ml gentamycin) and placed in 60mm dishes at a density of 10⁶ cells/ml. Cultures were maintained at 37°C in a humidified atmosphere of 5% CO₂. The medium was changed every two days and when necessary the cells were passed to new dishes following treatment with collagenase and trypsin as described above. EM analyses of the resultant cultures revealed an adherent stromal layer (Figure 1, left) on which foci of semi-adherent plasmacytoma cells grew (Figure 1b). Fluorescent Activated Cell Sorter (FACS) analyses of the plasmacytoma cells indicated they were CD-45⁺, FcR⁺, IgA⁺, ThB⁺, CD-45R⁺, Thy-1⁺, and Mac-1⁺, a phenotype typical of many plasmacytomas.

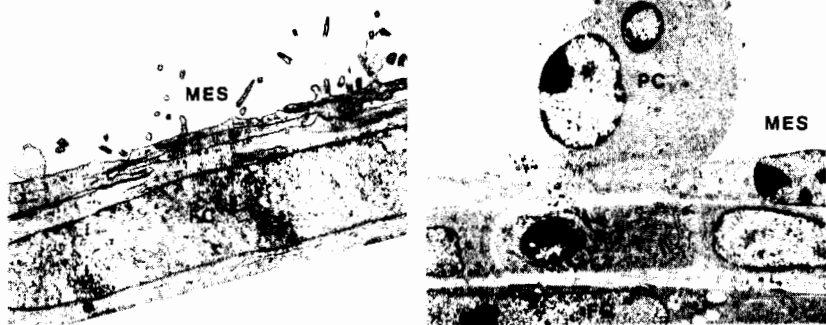


Fig. 1: Electronmicrographs of in vitro cultured granuloma-derived adherent cell layer (left) depicting mesothelial cell (MES) and fibroblastic cell (FC) types. The right micrograph contains 5-25 plasmacytoma cells (PC) which preferentially grow either on the microvillous surface of the mesothelial cells or in the intercellular space between the fibroblastic and mesothelial cells.

To date, two novel growth characteristics of in vitro cultured 5-25 PCT cells have been observed. First, establishment of the 5-25 PCT in vitro required an autologous, granuloma-derived adherent cell layer consisting of mesothelial, and fibroblastic cells. This requirement could not be replaced by rIL-6 or by conditioned medium from autologous feeder cultures. Second, EM analyses suggest the spatial relationship of the PCT and its stromal layer is critical since PCT growth is restricted to either the microvillous surface of the mesothelial cells or the intercellular space between the mesothelial and fibroblastic cells (Figure 1). It remains unclear whether the oriented growth of 5-25 PCT is dictated by local concentrations of diffusible factors or by the interactions of PCT- and stromal-specific adhesion molecules. These interactions are currently under investigation.

To determine if in vivo growth of the 5-25 cell line was characteristic of a primary plasmacytoma (i.e. granuloma dependent), 5-25 PCT cells were injected into a total of 17 BALB/cAn mice, 8 of which received 0.5ml of pristane ip. 3 days prior to tumor transplant. Four weeks post injection PCT growth was observed in 6/8 of the pristane primed mice. In contrast, 17 weeks post injection no tumor growth was observed in the 9 untreated mice indicating the in vitro passaged 5-25 PCT had retained its granuloma-dependence despite more than seven months of in vitro passage.

These results provide evidence for the critical role of adherent cell layers in the growth of early stage plasmacytomas and suggests the possibility that any such tumor can be grown on appropriate feeder layers. Furthermore, the ability to maintain such tumors in the "primary" state in vitro should provide a system for studying early stages of plasmacytoma development not previously accessible.

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