Surface Membrane Antigens of PY-3T3 Mouse Fibroblasts

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Summary

NIH Swiss mice produce a strong cellular response to polyoma-transformed mouse 3T3 cells (PY-3T3) in contrast to that of BALB/c mice. The present study sought to determine if plasma membranes isolated from PY-3T3 cells could induce similar delayed-type reactions. Surface membranes were prepared from intact cells using Dounce homogenization and density gradient centrifugation. Mice were immunized with emulsions of membranes in complete adjuvant, and foot pad tested 7 days post-immunization with membranes to detect delayed immune reactions. Measurement of the pad swelling 24 hours after challenge demonstrated that 33/40 NIH Swiss mice yielded positive results in contrast to 0/19 similarly treated BALB/c mice. Histologic examination of challenged and control foot pads substantiated the above interpretations. NIH Swiss and BALB/c mice immunized and challenged with normal 3T3 membranes failed to elicit a significant response at 24 hours. The findings suggest that this surface membrane fraction contained the tumor-specific transplantation antigens of the PY-3T3 cells.

1. Introduction

The induction of delayed-type immunity has been used to detect the antigenicity of oncogenic virus-transformed cells in mice (4, 11, 14, 19). In addition to the appearance of a new nuclear T antigen within a few hours after polyoma (7) or SV40 infection (2) in vitro, the presence of tumor-specific transplantation antigens (TSTA) on the cell surface are characteristic of these oncogenic infections. One of the procedures used with mice to demonstrate the development of cell-mediated immunity against these antigens is the foot-pad-swelling assay (4, 14). A challenge injection of tumor cells into the hind foot pad of immunized mice resulted in the production of delayed reactions specific for the tumor antigens. Tumor homogenates have also been shown to elicit cellular immune responses which are specific for the tumor (1, 3, 10).
The major emphasis in the present investigation was to determine if a tumor cell membrane fraction could induce an antigenic stimulus similar to that of the intact tumor cells. The plasma membranes from polyoma-transformed mouse fibroblasts were isolated, purified and the immunogenicity of the preparations tested in mice.

2. Materials and Methods

2.1. Animals

Female NIH Swiss and BALB/c mice 8—10 weeks old prior to immunization were used in this study. Both strains of mice were fed pelleted Purina mouse diet and water ad libitum.

2.2. Cells

Two lines of fibroblasts were employed in the study. These were the 3T3, an established cell line of BALB/c mouse fibroblasts, and PY-3T3, an established line of polyoma virus-transformed 3T3 fibroblasts. Normal 3T3 cells were purchased from Flow Laboratories (Rockville, Maryland). The initial culture of PY-3T3 cells was kindly provided by Dr. Howard Green (Massachusetts Institute of Technology). All cultures were grown in Dulbecco's modified Eagles medium (Grand Island Biological Company), supplemented with 10 per cent calf serum and 0.9 per cent antibiotic solution (10,000 units penicillin and 10,000 mcg streptomycin/ml antibiotic solution). The PY-3T3 and non-transformed 3T3 cell lines showed the typical appearance of mouse fibroblasts, and were free from mycoplasma.

2.3. Preparation of Surface Membranes

Fibroblasts were harvested when confluent multilayers (PY-3T3) or monolayers (3T3) of cells had formed. The cells were collected by scraping them off the glass with a rubber policeman and pipeting the suspensions into sterile screw-cap tubes. These were centrifuged at 800 × g for 10 minutes and the cell pellet washed 3 times with 0.15 M phosphate-buffered saline (PBS), pH 7.0. After the final wash viable cell counts were determined by trypan blue dye exclusion and resuspended in PBS to a concentration of 2 × 10⁶ viable cells per ml. The surface membranes were prepared from these suspensions by the method of Bosmann, Hagojian and Eylar (5) utilizing Dounce homogenization and density gradient centrifugation. The protein concentration of the membrane preparations was determined by the method of Lowry, et al. (12), with bovine serum (Pentex) as a standard.

2.4. Electron Microscopy

Suspensions of cell membranes were diluted in equal volumes of 1 per cent phosphotungstic acid, mounted on 300 mesh copper grids, and coated with 1 per cent collodion. Following subsequent carbon-coating the prepared grids were examined with a Hitachi #57 electron microscope.

2.5. Immunization with Surface Membranes

Suspensions of PY-3T3 and 3T3 plasma cell membranes (4.0 mg protein/ml) were prepared in 0.05 M Tris, pH 7.0, and diluted 1:2 in complete Freund's adjuvant (CFA, Difco Laboratories) to form stable emulsions. All experimental animals were immunized subcutaneously in the nape of the neck with 0.2 ml of the final preparations.

2.6. Foot Pad Assay for Cellular Immune Responses

At 7 days post-immunization, experimental NIH Swiss and BALB/c mice were foot pad tested to detect delayed hypersensitive reactions. Preliminary experiments had determined the 7 day rest interval as the optimum time for elicitation of the response. Cellular immune reactions were demonstrated by measuring the extent of pad swelling following challenge with 0.05 ml of a suspension of either PY-3T3 or 3T3 membranes (2.0 mg protein/ml) in one hind foot pad, as compared to a control