A MACROPHAGE-MONOCYTE CELL LINE FROM A DOG WITH MALIGNANT HISTIOCYTOSIS

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SUMMARY

The DH82 cell line was established from the neoplastic progenitor cells of canine MH and was characterized as histiocytic in origin based on light microscopic and ultrastructural morphology, positive staining reactions for alpha naphthyl acetate esterase and acid phosphatase, presence of Fc receptors, phagocytosis of latex beads, and plastic adherence in culture.

Key words: malignant histiocytosis; cell line; canine.

INTRODUCTION

Malignant histiocytosis (MH) is an uncommon, systemic, neoplastic proliferation of atypical histiocytes. Recent ultrastructural (6,20,25), cytochemical (1,3,6,10,26), immunological (7,8,20,26), and functional (1,20) findings support the concept that the neoplastic cells of human MH belong to the mononuclear-phagocyte system. Twenty cases of MH have been reported in dogs (5,15,22,23,27). The clinical and pathologic features of MH in the dog resemble those of the human disease (15,26). The objective of this study was to establish a continuous cell line from the neoplastic progenitor cells of canine MH and to document the histiocytic origin of this tumor using light microscopic and ultrastructural morphology, cytochemical stains, immunologic markers, and functional characteristics. This established cell line has been designated DH82.

MATERIALS AND METHODS

Patient. A 10-yr-old intact male Golden Retriever was presented to The Ohio State University Small Animal Clinic for lethargy, anorexia, and weight loss of 3 mos. duration. The dog had peripheral lymphadenopathy and hepatosplenomegaly. Initial laboratory data revealed a mildly regenerative anemia (hematocrit = 27%; normal = 35 to 54%) and thrombocytopenia (47 x 10^9/liter; normal = 150 to 400 x 10^9/liter). The leukogram showed a normal white blood cell count (6 x 10^9/liter; normal = 6 to 18 x 10^9/liter) and lymphopenia. No atypical cells were seen on the peripheral blood film. A direct Coomb's test and an antinuclear antibody test were negative. A bone marrow aspirate was characterized by a decreased myeloid:erythroid ratio, plasma cell hyperplasia, and frequent large, atypical histiocytes with erythropagocytosis. The anemia and thrombocytopenia were unresponsive to therapy with prednisone, vincristine (On-
intervals thereafter. Cultured cells periodically were frozen in supplemented medium with 10% dimethylsulfoxide, and stored in liquid nitrogen.

After several months in culture, the cells were transferred to 75-cm² flasks (Corning Glass Works, Corning, NY) and adapted to growth by weekly passage in Eagle's minimal essential medium containing 10.0% FBS, 1.0% glutamine, and 1.0% antibiotics. The cultures have been maintained for 1.5 yr through at least 75 passages.

Cell morphology. Cytocentrifuge preparations of trypsinized cells were stained with Wright-Giemsa for light microscopic morphologic evaluation. For ultrastructural examination, trypsinized cells were fixed in 3.0% glutaraldehyde, postfixed in osmium tetroxide, dehydrated in graded alcohols, and embedded in epoxy resin (Epon). Ultrathin sections were stained with lead citrate and uranyl acetate, then examined with a transmission electron microscope.

In vitro growth characteristics. In vitro growth characteristics were determined by sequential 48-hr cell counts from separate 25-cm² flasks plated identically with 1 X 10⁶ cells/flask at time 0. Trypsinized cells stained with trypan blue were counted with a hemacytometer to establish a growth curve.

Cytochemical stains. Cytocentrifuge preparations of trypsinized cells were stained with several enzyme-specific stains for cytochemical analysis. Cytochemical staining kits (Sigma Chemical Co., St. Louis, MO) were used for alpha naphthyl acetate esterase (ANAEE), acid phosphatase (ACP), Sudan black B (SBB), naphthol-AS-D chloracetate esterase (CAE), alkaline phosphatase (ALP), and myeloperoxidase (PX). The cells also were stained for alpha naphthyl butyrate esterase (ANBE) (28). Normal canine bone marrow samples were used as controls for each of these cytochemical stains. Terminal deoxynucleotidyl transferase (TdT) was determined by indirect immunofluorescence with an antibody against TdT (Bethesda Research Laboratories, Bethesda, MD). Feline thymocytes were used as a positive control and feline spleen cells were used as a negative for control TdT (12).

Surface markers. Trypsinized cultured cells were examined for the presence of surface receptors for the Fc portion of IgG or IgM or both (Fcγ and Fcα, respectively) and the 3b component of complement (C3b) using ovine erythrocyte rosette assays as described elsewhere (11). The presence of surface immunoglobulin (sIgG or sIgM) was evaluated by immunofluorescence with a fluorescein-conjugated polyvalent anticanine immunoglobulin as previously described (11). Thy-1 (theta) antigen was detected using a murine anticanine Thy-1 monoclonal antibody (18).

Functional characteristics. Phagocytic capability was determined by incubating cells in culture with 0.8 μm latex beads (Sigma) or washed canine red blood cells at 37°C overnight. The presence of phagocytized material was evaluated both in culture flasks with phase contrast microscopy and in Wright-Giemsa stained cytocentrifuge preparations of trypsinized cells using light microscopy.

Natural killer (NK) cell activity of the DH82 cells was assessed using a ⁵¹chromium release assay and NK

Fig. 1. Light microscopy of cytocentrifuge preparation of cultured DH82 cells. Arrow indicates large nucleolus in phagocytized cell. Wright-Giemsa stain. ×1726.