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# An established feline monocytic cell line

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**SUMMARY**

A feline monocytic cell line was established from a venous blood sample obtained from a healthy male donor cat. The cloned cells, temporarily named FLMo/K02, were successively passaged in vitro with cell growth medium consisting of RPMI-1640 supplemented with 10% heat-inactivated fetal calf serum. Non-specific esterase and acid phosphatase, as marker enzymes, were clearly demonstrated by cytochemical examinations. The cells treated with allogeneic serum for two hours in advance showed enhanced reactivity to monoclonal antibodies, feline CD1a, canine CD11b, feline CD11c, canine CD11d, and feline MHC-II.

*KEY WORDS:* feline, monocyte, cell line

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## INTRODUCTION

Among the immunocompetent cells, the monocyte-macrophage lineage are known to be key cells for antigen trapping, processing, and presentation functions.

These cells are important tools for immunological studies, but few feline monocytic cell lines have been reported.

Encouraged by our former work on the establishment of canine (Kadoi, 2000) and swine moncytic cell lines (Kadoi *et al.,* 2001) further trials were performed with a blood sample collected from a young healthy cat and a cell line was established.

The fundamental cytological property of the cell line is reported.

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## MATERIALS AND METHODS

*Primary monocyte culture*: Primary culture was made on the monocytes separated from venous blood of a healthy male cat as mentioned previously (Kadoi 2000; Kadoi *et al.*, 2001) Briefly, the cellular fraction mainly consisting of lymphocytes and monocytes was obtained by a gradient centrifugation in the use of Lymphoprep ($\rho$ = 1.077, AXIS-SHIELD PoC AS, Norway). The washed cells were suspended in cell growth medium (GM) consisting of RPMI-1640 (Sigma-Aldrich, USA) containing glucose at 3 grams per liter (RPMI), supplemented with heat-inactivated fetal calf serum (FCS) (JRH Bioscience, USA) at 20%. Antibiotics, like ampicillin sodium, kanamycin sulfate, and streptomycin sulfate, were included in GM at 200 mcg per ml in final concentrations. Since adhesive monocytes were stable to grow in flask culture (Nunclon, Delta Surface, Denmark) after successful passages, cellular cloning was made by a limited dilution method in seeding cell suspension to microplates. One of clones, named FL/K02, was successively passaged in flasks with GM containing 10% FCS. The cells at the passage level 12th to 14th were used for karyotyping, and cyto-

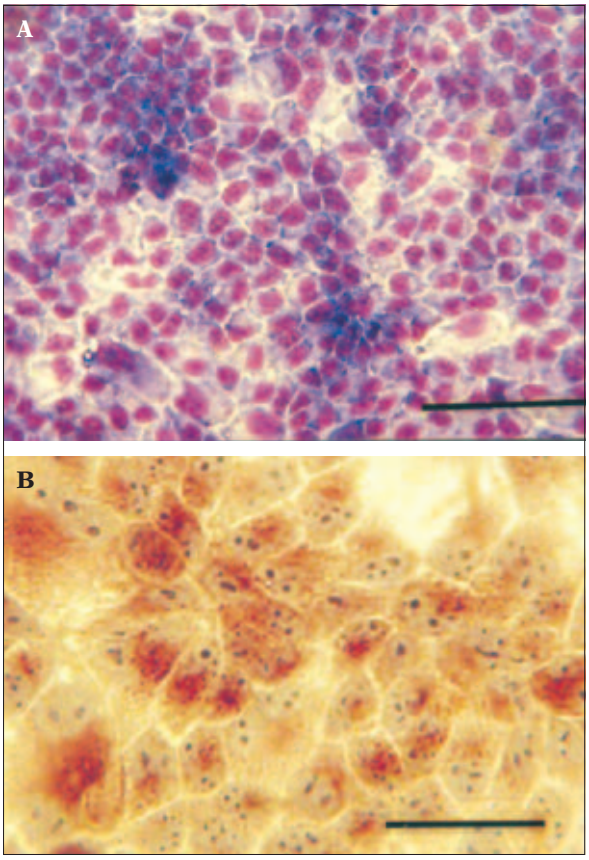


FIGURE 1. **A** *Monolayer of feline monocytic cells (FLMo/K02), showing epithelioid morphology. Methanol fixed and Giemsa stained. (Bar = 100 µm);* **B** *Non-specific esterase reaction demonstrated on the cells by cytochemistry. (Bar = 50 µm).*

chemistry. Cells at 24th and 25th passage levels were examined for the cell surface antigen expression by flow cytometry with monoclonal antibodies.

*Karyotyping:* Karyotyping of cells was made by adding a small amount of cholchicine to growing cells and was examined as usual.

Morphology and cytochemistry: Morphological and cytochemical studies were made on the slip cultures in Leighton tubes. Two to three days after seeding cells, slip cultures were dried and fixed with cold methanol. Giemsa staining was mainly made for morphological studies. Both non-specific esterase (Li *et al.,* 1973) and acid phosphatase were examined by commercially available kits (Muto Chem. Japan).

*Monoclonal antibodies:* Five monoclonal antibodies (mAb) mainly related to monocyte-macrophage lineage (supplied from Prof. Moore, University of California, Davis, USA) were used. They were feline CD1a (FE1.5F4), canine CD11b (CA16.3E10), feline 11c (FE5.5C1), canine CD11d (CA16.3D3), and feline MHC-II (42.3). Both canine CD11b and canine CD11d are known to cross-react between canine and feline (personal communication with Prof. Moore). Secondary antibody used was anti-mouse IgG (L+H) goat IgG FITC-conjugated (Jackson Immuno Research, USA).

*Flow cytometry:* FL/K02 cells grown in flasks were dispersed by EDTA-trypsin mixture solution and washed with GM, and then Dulbecco's phosphate buffered saline without Ca++ and Mg++ (PBS) containing 0.2% bovine serum albumin (Sigma-Aldrich, USA) (PBS/BSA). The cell suspension was divided into two groups. One of them was supplemented with normal feline serum at 20% and kept at room temperature for two hours. Afterwards cells were washed 3 times with PBS/BSA. Both groups of cell suspensions were further divided into 6 groups, each containing ca. 100,000 viable cells in 0.5 ml PBS/BSA. For labeling, cells first received the mAb for 30 minutes at 4°C, then secondary antibody for 20 minutes at 4°C. Each cell suspension was analyzed using FACScan (Nippon Becton Dickinson) as usual.

## RESULTS AND DISCUSSION

The modal chromosome number detected in 50 metaphase cells was 38 (2n) and the major population was a quasi-diploid. Seeding with ca. two million cells suspended in 20 ml of GM in the flask (culture dimension: 80 $cm^2$), three to four times growth were regularly obtained after three to four days' incubation at 36.5-37.0°C. The plating efficiency was more than 95%. A single cell had a triangular shape, but densely grown cells showed an epithelioid morphology. Both non-specific esterase and acid phosphatase were clearly demonstrated in the cytoplasmic location. The cells, pretreated with allogeneic serum, were more reactive to all five monoclonal antibodies. Results mentioned above indicate that the cells showed monocytic properties. It was an imagination that feline monocytic cells have particular susceptibility to feline coronaviruses.

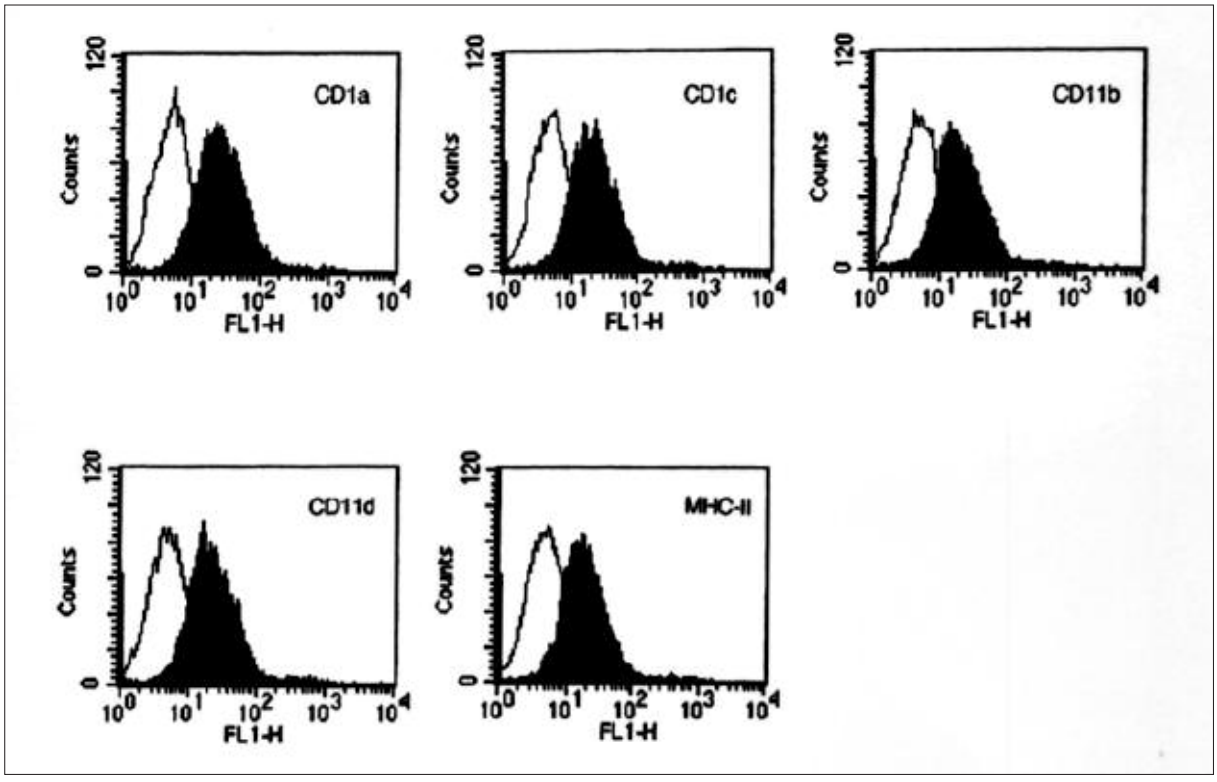


FIGURE 2 - *Reactivity difference of cells to five monoclonal antibodies distinguished allogeneic serum-treated cells (filled histogram) from non-treated cells (empty histogram).*

Therefore, two strains of feline infectious peritonitis viruses (WSU 79-1146 and UCD-2) adapted to grow in CRFK and fcwf-4 cells were inoculated to FL/K02 cells. However, infectious progenies were not clearly produced. The cellular homogenates prepared from more than five million cells were inoculated to CRFK and fcwf-4 cells. None of cytopathic viruses were isolated. Similar homogenates were examined for feline leukemia virus (FeLV) antigen by a kit (Witness FeLV, Agen Biomedical Ltd, Australia). The frozen stock of the donor serum was tested for specific antibody against feline immunodeficiency virus by a kit (Witness FIV, Agen Biomedical Ltd, Australia). In both tests the results were negative. Latent viral infections caused by the member of Coronaviridae, Retroviridae, and Parvoviridae are common in cats. Such diseases must be deeply related to feline immunological dynamics. In these circumstances this cell line can be applied as a tool for further feline immunological studies. It is generally known that mammalian monocytes differentiate to dendritic cells by the addition of cytokines such as GM-CSF and IL-4, and GM-CSF and TN (Pickl *et al.,* 1996; Romani *et al.,* 1994; Sallusto *et al.,* 1994; Zhou *et al.,* 1996). Since dendritic cells are major antigen presentation cells, many works have focused on these cells. Such differential pathway has ready been indicated (Caux *et al.,* 2001). There might be a possibility that the cell line established in this report has progenitor cells for feline dendritic cells. It was proved in the present experiment that the allogeneic serum treatment for monocytic cells induced an enhanced reactivity to monoclonal antibodies employed. A similar reactive property was also observed in our work on canine and porcine monocytic cells (Kadoi's unpublished data). The mechanism of this phenomenon remains to be studied.

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