Cytochemical and Immunological Properties of Leukemic Cells from a Cat with Reticuloendotheliosis

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Feline reticuloendotheliosis (RE) was classified as a very primitive type of feline myeloproliferative diseases and believed to be derived from a leukemic transformation of undifferentiated stem cells, based on the findings of their cytological features and clinical conversion into granulocytic leukemias and/or erythremic myelosis [1, 2, 4, 7]. The leukemic cells characteristic of RE were described to be large ovoid in shape, and to contain a eccentrically placed round nucleus with one or two prominent nucleoli. However, the precise classification of RE cells has not yet been done. In the present study, we describe several cytochemical and immunological properties of RE cells from a naturally occurring case.

A female cat weighing 2 kg was referred to the Veterinary Hospital, Faculty of Agriculture, The University of Tokyo, because of sudden anorexia. Physical examinations revealed pale mucus membranes and the slight enlargement of the submandibular lymph nodes. The thoracic and abdominal radiography showed normal appearance. In the laboratory examinations, severe non-regenerative anemia (1530000/µl), accompanied by remarkably decreased number of white blood cells (2600/µl) and platelets (130000/µl) was observed. The enzyme-

Fig. 1. Morphological appearance of leukemic cells. a, a leukemic cell in the peripheral blood stained with Giemsa. ×1250. b, transmission electron microscopy. ×25000. c, α-naphthyl butyrate esterase staining of cells from the bone marrow. ×1250. d, β-glucuronidase staining of cells from the bone marrow. ×1250.
linked immunosorbent assay for feline leukemia virus was strongly positive. Other than the elevated level of serum lactate dehydrogenase (367 IU/l), no abnormal serum chemistry profiles were noted. Sixteen percent of cells in the peripheral blood were round or oval in shape, and contained a large nucleus which was eccentrically situated in the one side of the cell (Fig. 1a). The nucleus had one or two prominent nucleoli, being poor in chromatin, and the cytoplasm stained slightly greyish blue in Giemsa staining. Cytological studies of bone marrow aspirate revealed the accumulation of leukemic cells morphologically similar to the cells in the peripheral blood and severe loss of granulocytic and erythrocytic precursors. Electron microscopy disclosed that the cytoplasm was poorly differentiated and contained numerous free ribosomes (Fig. 1b). The nucleus had fine stippled chromatin with one definite nucleolus. We diagnosed this case as RE on the basis of these clinical and cytological findings, and the cytochemical and immunological properties of the RE cells in this case were further examined. In cytochemical stainings [9], these cells were negative for peroxidase and Sudan black B, and faintly positive for acid phosphatase. A diffuse staining pattern for α-naphthyl butyrate esterase was noted in some but not all leukemic cells (Fig. 1c). These cells were also positive for β-glucuronidase in a stippled staining pattern (Fig. 1d). These cytochemical features suggested that the RE cells had staining properties of monocytic/lymphocytic lineages. Cell surface and cytoplasmic immunoglobulins (Ig) were examined by a direct immunofluorescence using fluorescein isothiocyanate (FITC)-labeled anti-cat Ig (Cappel Laboratories, Cochranville, PA). These cells lacked surface and cytoplasmic Ig. The surface phenotype of these RE cells was also examined by using a monoclonal antibody (9F23) which is directed to feline interleukin 2 receptor α-subunit (IL-2Rα) [3, 6]. Cells obtained from the peripheral blood and bone marrow by Ficoll-Hypaque gradient centrifugation were incubated with a saturating amount of 9F23

**Fig. 2.** Expression of IL-2Rα on leukemic cells from the peripheral blood and bone marrow. Shaded area represents the background fluorescence of cells stained with an irrelevant antibody instead of 9F23.

**Fig. 3.** Histopathological appearance of leukemic cells infiltrated into the spleen (a; ×65) and the liver (b; ×250). HE stain.
antibody at 4°C for 30 min, washed twice with sorting
buffer containing 1% bovine serum albumin and 0.1%
sodium azide, and then incubated with FITC-labeled
rabbit anti-mouse IgG (Cappel) at 4°C for 30 min. Washed
cells were examined under a CYTOACE cytofluorometer
(Japan Spectroscopic Co., Tokyo, Japan). As shown in
Fig. 2, RE cells from the peripheral blood and bone
marrow were weakly, but significantly stained with 9F23,
indicating the presence of IL-2Rα on the cell surface of
RE cells. In addition, the cells expressing IL-2Rα might
not be contaminating activated T cells, because these cells
failed to react with the monoclonal antibody which
recognizes the antigen on feline activated lymphocytes
(data not shown). Initially, the expression of IL-2Rα was
considered to be essentially restricted to activated mature
T cells, B cells and NK cells. Recently, the presence of
IL-2Rα was reported on interleukin 3 (IL-3)-dependent
pro-T and pro-B cell lines [8]. Furthermore, IL-2Rα has
also been detected on some myeloid leukemia cells [11].
From these findings, the possible roles of IL-2Rα have
been postulated not only in the activation of lymphoid
cells but also in the oncogeny of lymphoid and myeloid
cells. Although we could not determine the function and
affinity of IL-2R on these RE cells, the expression of
IL-2Rα might be associated with the abnormal prolifera-
tion of RE cells, as described in human adult T cell
leukemia [10].

Because of life-threatening anemia, several blood trans-
fusions were carried out, and subsequently chemotherapy
was initiated. The total counts of red blood cells and
platelets were recovered by the therapy with moderate
improvement of general condition, but leukemic cells did
not disappear from the peripheral blood. The total cell
counts were suddenly decreased on 7 days after the
initiation of the therapy, and the cat became soon
deteriorated.

At necropsy, the spleen was remarkably enlarged with
indistinct follicles. The systemic lymph nodes including
submandibular, mesenteric, and hilar lymph nodes were
also slightly enlarged. Multiple white nodules were distrib-
uted throughout the liver with numerous small hemmor-
rhage foci. Spotted hemorrhage was seen on the surface of
the lungs. The femoral bone marrow was discolored and
parenchymatous. Histopathologically, the severe diffuse
infiltration of RE cells as seen in the peripheral blood
smears with frequent mitosis was observed in many organs
including the spleen, lymph nodes, liver, and lungs. The
red pulp of the spleen was atrophied with the invasion of
RE cells (Fig. 3a). The original architecture of the
submandibular lymph nodes was disappeared and re-
placed by proliferating RE cells. The RE cells also
infiltrated into the sinusoids and interlobular connective
tissues of the liver (Fig. 3b).

Although the definitive classification of RE cells could
not be done, RE cells in this case appeared to be
committed to monocytic/lymphocytic lineage. In contrast,
it has been reported that RE cells originated from
granulocytic and/or erythroidic stem cells [1, 2, 4, 7]. This
difference regarding the origin or RE cells might be
attributed to the heterogeneity of leukemic cell popula-
tions in RE. Furthermore, the immature progenitor cell
line which has the capacity to differentiate into myeloid as
well as lymphoid cells has been established [5]. Thus, the
RE cells might be a immature stem cell with a multilineage
differentiation potential. Therefore, whenever RE was
experienced, the careful cytochemical and immunological
analyses of RE cells should be carried out to clarify the
origin of RE cells and to recategorize this obscure disease
entity.

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