Monoclonal Antibody S37 Induces Apoptosis in Bovine T Lymphoma Cells

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(Received 1 February 1994/Accepted 6 April 1994)

ABSTRACT. Influences of 38 monoclonal antibodies (mAbs) on the growth of bovine T lymphoma cells (BTL-27) were examined. One of them, S37, inhibited the cell growth strikingly when it was added to the cultures. S37-treated cells showed DNA fragmentation containing ~200-base pair multiple patterns and chromatin condensation. This evidence suggested that mAb S37 induced apoptosis in BTL-27 cells.—**KEY WORDS:** apoptosis, bovine T lymphoma, mAb.

J. Vet. Med. Sci. 56(5): 957–959, 1994

Growth of lymphocytes is influenced by their environment mainly through cell surface molecules. These molecules include receptors for growth-stimulating cytokines [1, 2, 9] and those for transmitting signals essential for cell death [8, 11, 13]. It has been thought that suppression of death pathways leads to an altered increase in cell number and often contributes to tumor development. Recently, we raised 38 mouse monoclonal antibodies (mAbs) directed against cell surface molecules of three different types (calf, skin, and thymic) of sporadic bovine leukosis (SBL) cell lines [10]. To investigate their biological activities, the 38 mAbs were examined to determine whether they had any influence on the growth of tumor cells. We found that one of them (mAb S37) induced apoptotic cell death in T lymphoma cells. We report here the characteristic features of mAb S37-induced apoptosis in bovine lymphoma cells.

In this study, the 38 mAbs directed against three different types of SBL cell lines were used [10]. Immunoglobulin-rich fractions containing mAbs were prepared from heat-inactivated ascites by precipitation with 50% saturated ammonium sulfate solution and subsequently filtrated through a Sephadex G25 column. These immunoglobulin-rich fractions were used as sources of mAbs. The T lymphoma cell line BTL-27 (BoCD3⁺, BoCD4⁺, BoCD8⁺, TCR1⁺), a clone of BTL-PC3 derived from the calf type of SBL [6], and bovine leukemia virus (BLV)-transformed B cell line BL312 [7] were examined for growth influence by mAbs. These cells were cultured in 96-well flat-bottomed plates with 1.0–200 µg/ml of mAbs for 6 days. As a control, the immunoglobulin fraction from normal BALB/c mouse serum was used. Cell viability was assessed by trypan blue exclusion and MTT assay as described by Green *et al.* [4].

Among the 38 mAbs tested, two mAbs, S37 and T60, inhibited proliferation of BTL-27 cells, whereas one mAb, C183, promoted it. In the presence of 100 µg/ml of C183, the cell numbers of BTL-27 increased to twice those with control immunoglobulin, while with 100 µg/ml of T60, cell growth was retarded by 50% for 6 days incubation compared with the control culture (data not shown). At 100 µg/ml of S37 directed against skin type of SBL cell line BLS1, the number of viable cells appeared to decrease to about 80% after 2 days of incubation by trypan blue exclusion test (Fig. 1, A). On the other hand, the MTT assay showed that the mitochondrial function in the cells appeared to decrease to about 50% after 1 day of culture,

although the number of viable cells counted by trypan blue exclusion was nearly the same as in the control culture (Fig. 1, A). This suggests that the cells treated with mAb S37 were going to die at 1 day of culture irrespective of having the ability to exclude trypan blue. Cell growth inhibition of BTL-27 by mAb S37 was observed in a dose-dependent manner (Fig. 1, B). Apoptotic cell death was not observed, however, in cells cultured with the other 37 mAbs, including mAb T60.

A B lymphoma cell line, BL312, expressed antigen recognized by S37 in approximately the same amount as BTL-27. When BL312 (1×10^5 cells) were used as target cells, growth inhibition was observed at a higher concentration of S37 than of BTL-27, but the cells did not die even at 200 µg/ml of S37. On the contrary, growth of BL312 seemed to be enhanced at low concentrations (Fig. 1, C).

It has been reported that one of the mechanisms possibly responsible for tumor regression is apoptosis characterized by membrane blebbing, chromatin condensation and DNA fragmentation [12]. To investigate the mechanism of growth inhibition of BTL-27 by mAb S37, DNA fragmentation was analyzed using agarose gel electrophoresis as described by Ishigami *et al.* [5]. A striking fragmentation of the DNA into multiple units of approximately 180 base pairs was clearly observed in BTL-27 treated with S37 at 24 hr (Fig. 2, lane 3). No significant DNA fragmentation was observed in the control cells, which were not treated with S37, even after cultivation for 48 hr (Fig. 2, lane 2). We also observed the morphological changes of dying cells simultaneously. The cells were fixed with ethanol, washed with PBS, and their nuclei were stained with propidium iodide and observed by fluorescent microscopy. Many cells shrunk, and the chromatin was condensed and/or fragmented at 24 hr after addition of S37 (Fig. 3). This evidence suggests that mAb S37 leads to growth inhibition of BTL-27 by inducing apoptosis.

The antigens recognized by S37 had molecular weights of 90 and 35 kilodaltons and were expressed on some bovine T and B lymphomas and on a part of resting and mitogen-stimulated peripheral blood lymphocytes [10]. It is notable that S37 not only retarded the proliferation of BTL-27 but also caused cell death in the absence of complement. Furthermore, by agarose gel electrophoresis, we found low-molecular-weight ladder bands, the most characteristic feature for apoptosis [3, 12], in the

Fig. 1. Effect of mAb S37 on the growth of tumor cells. T lymphoma cell line BTL-27 (A and B) and B lymphoma cell line BL312 (C) were incubated with mAb S37 (—, □), or with normal mouse immunoglobulin (----, ▨) for 1 to 5 days (A) or 6 days (B and C) and examined for mitochondrial function and viable cell numbers. Each upper figure indicates the MTT assay, while the lower ones show the viable cell counts.

Fig. 2. Agarose gel electrophoresis of DNA from BTL-27 cells. DNA of BTL-27 cells incubated in the presence of mAb S37 at the final concentration of 100 μg/ml for 24 hr (lane 3) and for 48 hr (lane 4), or in the absence of mAb S37 for 48 hr (lane 2) was examined by 1.5% agarose gel electrophoresis. Lane 1 contains pBR322 *Hind*III fragments.

Fig. 3. Photographs of fluorescent microscopy. BTL-27 cells were treated with 100 μg/ml of mAb S37 for 24 hr. The nuclei were stained with propidium iodide. A normal nucleus is indicated by an arrow head, and chromatin condensation and fragmentation are indicated by arrows.

DNA extracted from cells treated with S37 (Fig. 2). The cell treated with S37 retained ability for dye exclusion over 1 day, implying that S37-induced apoptosis differ from that induced by anti-T cell receptor or anti-Fas antibodies [3, 13]. Cell death in BL312 expressing the same amounts of antigen as BTL-27 was not observed even at high concentrations of mAb S37 (Fig. 3, C). This indicates that the susceptibility to S37-induced apoptosis may be different in T-cell- and B-cell-lineage tumors, or dependent on the cell differentiation stage. These results suggest that exposure of lymphoid cells to mAb S37 triggers a cascade to apoptosis, but that some mechanisms to transduce the signal or to induce gene expression may be required for the achievement of apoptosis in target cells. Therefore, S37 is considered to be a useful tool for analyzing signal transduction for apoptosis and the mechanisms of malignant processes in tumor growth.

ACKNOWLEDGEMENTS. The authors gratefully acknowledge Dr. Y. Aida for providing bovine leukemia virus-infected cell line, BL312. This work has been supported in part by a Grant-in-Aid (Bio Media Program) from the Ministry of Agriculture, Forestry and Fisheries (BOM 91-IV-1-C).

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