The Antibody against Human CD25, ACT-1, Recognizes Canine T-lymphocytes in the G2/M and G0/G1 Phases of the Cell Cycle during Proliferation

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Abstract. Because of the lack of an appropriate antibody against the canine CD25 molecule, we investigated whether anti-human CD25 antibody, ACT-1, could be useful in detecting canine T-lymphocyte proliferation. Peripheral mononuclear cells from a dog were cultured for 4 days with or without concanavalin A stimulation. In the last 24 hr, bromodeoxyuridine (BrdU) and human recombinant IL-2 were added. While the cell cycle was detected using anti-BrdU antibody and 7-amino-actinomycin (7-AAD), the cultured cells were stained with anti-canine CD4 antibody and ACT-1. The results showed that T-lymphocytes reactive to ACT-1 were present in the G2/M and G0/G1 phases in 94.4% and 70.0% of CD4-positive T-lymphocytes, respectively, suggesting that flow cytometry with ACT-1 might be useful in detecting canine T-lymphocytes during and after activation.

Key words: CD25, dogs, lymphocytes.

Interleukin-2 (IL-2) receptor (CD25) is one of the major and important markers on the cell surface of T-lymphocytes during activation and proliferation in humans [2, 9] as well as dogs [3, 4, 6]. When stimulated with mitogen, canine peripheral blood lymphocytes are known to proliferate and express CD25 [4, 6]. Because of the lack of an appropriate antibody against canine CD25, anti-human CD25 antibody (ACT-1) has been examined and reported to be cross-reactive to activated canine T-lymphocytes in immunohistochemistry [3] and flow cytometry [8]. However, it is still unclear whether this antibody can recognize proliferating lymphocytes in dogs. Therefore, in this study, canine lymphocytes stimulated with a T-lymphocyte mitogen, concanavalin A (Con A), were investigated during the cell cycle in terms of reactivity to ACT-1.

Purification of peripheral blood mononuclear cells (PBMCs) from whole blood and flow cytometry were performed as reported previously with slight modifications [8]. Briefly, whole blood samples were obtained from a healthy beagle using EDTA-treated collection tubes. PBMCs were purified from the whole blood using Ficoll-Hypaque (Nycomed Pharma AS, Oslo, Norway) and cultured in RPMI 1640 medium containing 10% fetal bovine serum and antibiotics (100 µg/ml of streptomycin and 100 U/ml of penicillin) with or without 1 µg/ml of Con A (Sigma) at 37°C under 5% CO2 in air for a total of 4 days. In the last 24 hr, bromodeoxyuridine (BrdU) was added into the culture at a concentration of 10 µM together with human recombinant IL-2 (Strathmann Biotech AG, Hamburg, Germany) at a concentration of 100 U/ml. The cultured cells were then stained with Alexa 647-labeled anti-canine CD4 antibody (Serotec Ltd, Oxford, UK) and PE-labeled anti-human CD25 antibody (clone, ACT-1; DAKO A/S, Denmark). As isotype controls, Alexa 647-labeled purified rat IgG2a (Serotec Ltd) and PE-labeled mouse IgG1 (Serotec Ltd) were also used to determine threshold lines for the fluorescent intensities of both Alexa 647 and PE in flow cytometry (Fig. 1). After washing with PBS, the cells were fixed, permeabilized and stained with anti-BrdU antibody labeled with FITC according to the instructions in a FITC BrdU Flow Kit (BD Pharmingen, San Diego, CA, U.S.A.). After staining with 7-amino-actinomycin (7-AAD) to detect total DNA content, the percentages of cells reactive to anti-canine CD4 antibody and ACT-1 were examined with a FACScanII flow cytometer using the FACSdiva software (BD Biosciences, San Jose, CA, U.S.A.).

The cell cycle phases of lymphocytes stimulated with Con A were detected with FITC labeled anti-BrdU antibody and 7-AAD, showing a typical horseshoe pattern similar to the data in other studies (Fig. 1) [1, 7]. There were 4 different phases of cell cycle in the typical lymphocyte gate: the S, G2/M, G0/G1 and apoptotic phases in the cells stimulated with Con A. The percentages of cells in the S, G2/M, G0/G1 and apoptotic phases with Con A stimulation were 7.6, 2.9, 74.8 and 14.7, respectively. Without stimulation, cells in the S and apoptotic phases were rarely detected, while cells in the G0/G1 and G2/M phases were more frequently identified (G0/G1, 98.1%; G2/M, 1.8%). This indicated that Con A stimulation could activate the cell cycle of PBMCs during culture. In the S, G2/M and G0/G1 phases of the cells stimulated with Con A, the percentages of T-lymphocytes reactive to ACT-1 in CD4-positive lymphocytes were 30.2%, 94.4%, and 70.0%, respectively. In contrast, although CD4-positive lymphocytes were detected in the G0/G1 and G2/M phases in the cells cultured without Con A, few CD4-positive lymphocytes were reactive to ACT-1 in those gates (G0/G1, 0.0%; G2/M, 1.2%; Fig. 1). Thus, most of the CD4-positive T-lymphocytes during and after activation were reactive to ACT-1.
The ability of human CD25 antibody, ACT-1, to recognize proliferating canine T-lymphocytes has been questioned. It has been reported that ACT-1 is reactive to canine lymphocytes stimulated with PHA [3]. In another study, CD4-positive lymphocytes reactive to ACT-1 appeared when PBMCs from a dog colony spontaneously sensitive to corn antigen were cultured with the antigen [8]. The present study further indicated that canine CD4-positive T-lymphocytes reactive to ACT-1 are mostly present during and after mitosis (G0/G1 and G2/M phases). Taken together, the present study confirmed the ability of ACT-1 to recognize proliferating T-lymphocytes in dogs, suggesting that flow cytometry using ACT-1 could be an alternative to the assay with 3H-thymidine incorporation for detection of canine lymphocyte proliferation. For instance, antigen-reactive lymphocytes are known to involve in the pathogenesis of canine food allergies, which has been examined with the 3H-thymidine incorporation assay [5]; however, proliferation of T-lymphocytes against food antigens can be examined by assay using flow cytometry with ACT-1. Thus, the assay system comprised of flow cytometry with ACT-1 would be useful in investigation of CD4-positive T-lymphocyte-related allergic diseases in dogs, although further investigation is necessary to determine whether the antibody can recognize the canine CD25 molecule itself using its expressing cells or recombinant protein.

In summary, the human CD25 antibody, ACT-1, was shown to recognize proliferation of canine T-lymphocytes. The assay system comprised of flow cytometry with this antibody can be a novel tool for investigation of diseases related to T-lymphocyte proliferation in dogs, such as allergies, autoimmune diseases, and cancer.

REFERENCES


