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THE INCUBATION PERIOD AND REACTION OF ONE TYPE OF ANTIBODY TO CD3 ANTIGEN IN LYMPHOCYTE SMEARS OF DIFFERENT ANIMALS

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Immunocytochemical detection of CD3 cells in lymphocyte smears of several animals species (dog, pig, sheep, and cattle) is described. Different incubation times with polyclonal antibodies were used (30 min, 90 min, 6 h, and 18 h). CD3 lymphocytes showed an intense immune reaction in the cytoplasmic membrane of these animal cells. An incubation time of 90 min is suitable for using that antiserum in lymphocyte smears.

Key work: animals, lymphocite smears, CD3 antigen, antibody

INTRODUCTION

The identification of animal T lymphocytes with the same antibody in lymphocyte smears is very useful for routine diagnostic haematology and immunology. In human medicine the use of monoclonal antibodies (MoAbs) which serve to identify different lymphocyte populations has become widespread.

However, only a few antibodies prepared against lymphocytes of one animal species cross-react with cells of another animal species. Storing antibodies against cells from different kinds of animals is expensive for small clinical laboratories.

Detection of T lymphocytes in paraffin wax-embedded tissues by means of polyclonal antibodies (PoAbs) to CD3 antigen has been described in dogs (Ledecky et al., 1995), swine (Levkut et al., 1995), cattle (Kolodzieyski and Revajova,1996) and poultry (Ševčikova in press).

The aim of this work was to determine the reaction to lymphocyte smears in different animals. Similarly, we tried to find a suitable incubation time for PoAbs without interference after the incubation period.

MATERIAL AND METHODS

Cell suspension. Venous blood from four species (dogs, pigs, sheep and cattle) of animals was collected into heparin. Mononuclear cells were separated from erythrocytes and polymorphonuclear cells by sedimentation on a Ficoll-Hypaque gradient (Boyum, 1974).

Polyclonal antibodies. A rabbit anti-human T cell CD3 serum (Dakopatts, Glostrup, Denmark) raised against synthetic human CD3 epsilon chain peptide was used.

Immunocytochemistry. A drop of re-suspended mononuclear cells was applied to degreased slides and smeared. The slides were air dried overnight at room temperature. The dry slides were fixed in acetone for 10 min and stored in sheets of aluminium at 20^{0}C.

CD3 antigen was detected immunohistochemically by a biotin-streptavidin amplified peroxidase detection (B-SA) system (Biogenex, San Ramon, CA, USA). Briefly, slides were left for 10 min at room temperature. After rinsing, they were incubated with the CD3 polyclonal antibodies at a dilution of 1:300 for 30 min, 90 min or 6 h at room temperature or 18 h at 4^{0}C respectively.They were then incubated with biotinylated anti immunoglobulins and finally with peroxidase-labelled streptavidin in phosphate-byffered saline (PBS). The immunological reaction was identified by amino-ethyl-carbazole (ACT). Cells were counter-stained with Mayer's haematoxylin. Preparations were viewed under a Nikon microscope at a total magnification x 1000. Approximately 200 lymphocytes were counted in individual samples, with a determination of percentage of positive cells for CD3 antigen.

RESULTS AND DISCUSSION

The percentage of CD3 positive cells in the peripheral blood monouclear cells incubated with the primary antibodies may be seen in Table 1. The results showed, that anti CD3 antiserum can be used in lymphocyte smears of dogs, swine, sheep, and cattle. The reaction of CD3 antigen of cells to the polyclonal antibodies after different incubation periods can also be seen in Table 1.

Table 1. The percentage of CD3 positive cells in lymphocyte smears from different animals incubated with rabbit anti-human T cell CD 3 antibodies.

Animal (No.)	Incubation time of lymphocyte with anti-CD3 PoAb			
	30 min	90 min	6 h	18 h
Dog (7)	54.4 ± 4	55.1 ± 3	52.9 ± 4	62.2 ± 2
Pig (5)	55.3 ± 3	56.4 ± 2	51.1 ± 2	53.2 ± 2
Sheep (6)	53.0 ± 5	51.8 ± 5	50.6 ± 6	52.3 ± 6
Cattle (7)	49.2 ± 4	49.3 ± 6	51.2 ± 6	53.8 ± 5

Cells stained with CD3 antiserum had a red reaction in the cell membrane and with increased incubation time the cytoplasm was also stained (Figure 1). Similarly we showed, that a relatively short incubation period (30 min) is needed for labelling CD3 cells with the polyclonal antibodies studied. Lymphocyte smears have an advantage compared with incubation in tissue where the time taken is 18 h (Ferrer et al., 1992). Lymphocyte smears using the shortest incubation time (30 min) had the lowest percentage of CD3 labelled cells. It is suggested that not all CD3 cells react with the antibodies used during that time.

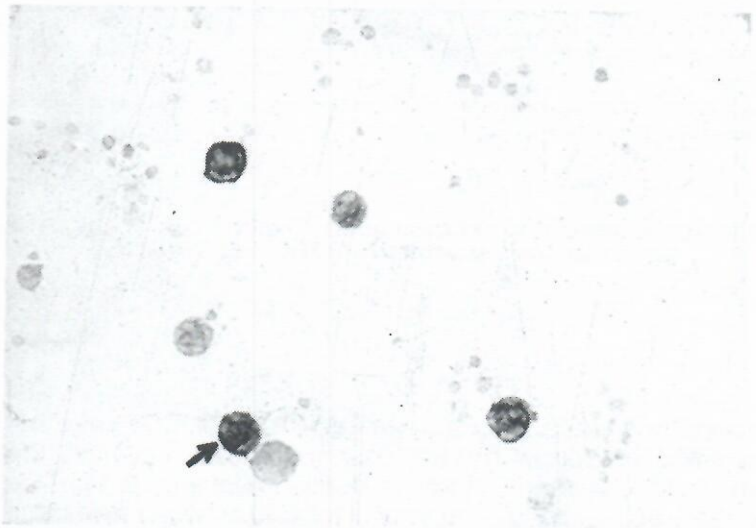

Figure 1. CD3 cells (arrow) intensively stained in lymphocyte smears of the peripheral blood of a dog. Immunocytochemical staining with a rabbit anti-human T cell CD3 antibody. Biotin-streptavidin amplified peroxidase system. x200.

In our opinion an incubation time of 90 min is suitable for using that antiserum in lymphocyte smears. Tissue cells of some animal species have reduced activity to this polyclonal antiserum (Ramos-Vara et al., 1994) and it is necessary to digest the tissues. Lymphocyte smears of these animal species do not need to be digested.It is believed, that polyclonal CD3 antibodies can be used as a suitable marker for the routine determination of T lymphocytes in veterinary haematological laboratories. Thus, techniques based on erythrocyte rosetting can be avoided (Levkutova et al., 1993) and FACS cytometry temporarily replaced in the laboratories of small veterinary clinics.

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INKUBACIONI PERIOD I REAKCIJA JEDNOG TIPA ANTITELA NA CD3 ANTIGEN U LIMFOCITNIM MRLJAMA RAZLIČITIH ŽIVOTINJA

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SADRŽAJ

U radu je opisana imunocitohemijska detekcija CD3 ćelija u limfocitnim mrljama nekoliko životinjskih vrsta (pas, svinja, ovca i goveče). U ogledu je korišćeno različito vreme inkubacije sa poliklonalnim antitelima. Pokazano je da CD3 limfociti ispoljavaju snažnu imunu reakciju u svojoj membrani kod svih ispitivanih životinjskih vrsta, pošto su nosioci CD3 antigena.