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Bovine pan T-cell monoclonal antibodies reactive with a molecule similar to CD2

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SUMMARY

Monoclonal antibodies (mAb) CH128A and CH61A react with molecules of 50,000–60,000 MW. They are expressed by all T cells in cattle, comprising 44–69% of peripheral blood mononuclear leucocytes (PBM), the majority of lymphocytes in T-dependent areas of lymph node, and 75–80% of cells derived from the thymus including both cortical and medullary thymocytes. The molecule recognized by these mAbs is not expressed on B lymphocytes, monocytes/macrophages, or granulocytes. Both mAb inhibit spontaneous rosette formation by sheep erythrocytes and bovine lymphocytes. We postulate that these mAb see the bovine homologue of the human sheep red blood cell receptor CD2 and has been named BoT2.

Spontaneous rosette formation with xenogeneic erythrocytes (E rosettes) has been used to identify T lymphocytes of several species (Coombs *et al.*, 1970; Cockerell & Baldwin, 1979; Outteridge, Fahey & Lee, 1981; Renshaw, 1981). In humans, the capacity to form rosettes is associated with a 50,000 MW protein (the sheep red blood cell receptor, SRBCr) that appears early in the ontogeny of T lymphocytes in the thymus (Kamoun *et al.*, 1981). Its presence on both immature and mature T lymphocytes has made it useful as a pan T-cell marker, and recent studies on the human SRBCr, T11 or CD2 with monoclonal antibodies (mAb) have shown this molecule to be functionally important (Meuer *et al.*, 1984; Reinherz, 1985). Antibodies to homologous molecules in other species have not been reported.

We have reported previously the phenotypic and functional characterization of distinct subsets of bovine T lymphocytes (BoT4$^+$ and BoT8$^+$) using mAb (Baldwin *et al.*, 1986; Ellis *et al.*, 1986). In the present report we describe two mAb, CH128A and CH61A, which identify a pan T-cell molecule on thymocytes and peripheral blood lymphocytes, believed to be the bovine homologue of CD2. The hybridomas producing mAb CH61A and CH128A, both of the IgG1 isotype, were derived from the splenocytes of BALB/c mice that had been hyperimmunized as described previously (Davis, McGuire & Perryman, 1983) with peripheral blood mononuclear leucocytes (PBM) from the following species: bovine, caprine, porcine, equine, canine, mink and human.

Abbreviations: mAb, monoclonal antibody; PBM, peripheral blood mononuclear leucocytes; SDS–PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; SRBCr, sheep red blood cell receptors.

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Clinically normal female and castrated male *Bos taurus* and *B. indicus* cattle, 6–36 months of age, were used as a source of leucocytes, thymocytes and tissues. These were prepared as described previously (Baldwin *et al.*, 1986; Ellis *et al.*, 1986). Cells were stained by indirect immunofluorescence and analysed on a fluorescence-activated cell sorter (FACS II, Becton-Dickinson, Sunnyvale, CA) as described by Lalor *et al.* (1986). CH128A reacted with between 44·0% and 68·9% (mean 55·9%) of PBM from 24 cattle, while CH61A reacted with between 45·7% and 65·8% (mean 57·6%). When PBM were reacted with mAb CH128A and CH61A together, the percentage of positive cells did not increase above the values obtained when samples of the same populations were stained with the mAb individually. In populations of cells enriched for monocytes (approximately 75–90%), both mAb reacted only with a few of the small scatter cells, consistent with the level of lymphocyte contamination. Neither mAb reacted with purified neutrophils. Dual fluorescence analysis, carried out by labelling PBM directly with goat anti-bovine IgM conjugated with tetramethyl-rhodamine-isothiocyanate and indirectly with mAb and fluoresceinated sheep anti-mouse Ig (Lalor *et al.*, 1986), indicated that neither CH128A nor CH61A reacted with B cells. Both mAb reacted with 75–80% of cells in suspensions derived from thymus.

The distribution of cells reacting with CH128A and CH61A in thymus and lymph node were determined using an indirect immunoperoxidase staining method on frozen sections of tissue as previously described (Ellis *et al.*, 1986). In cryostat sections of thymus, both mAb reacted with the majority of thymocytes in both cortex and medulla (Fig. 1). However, in the outer cortex

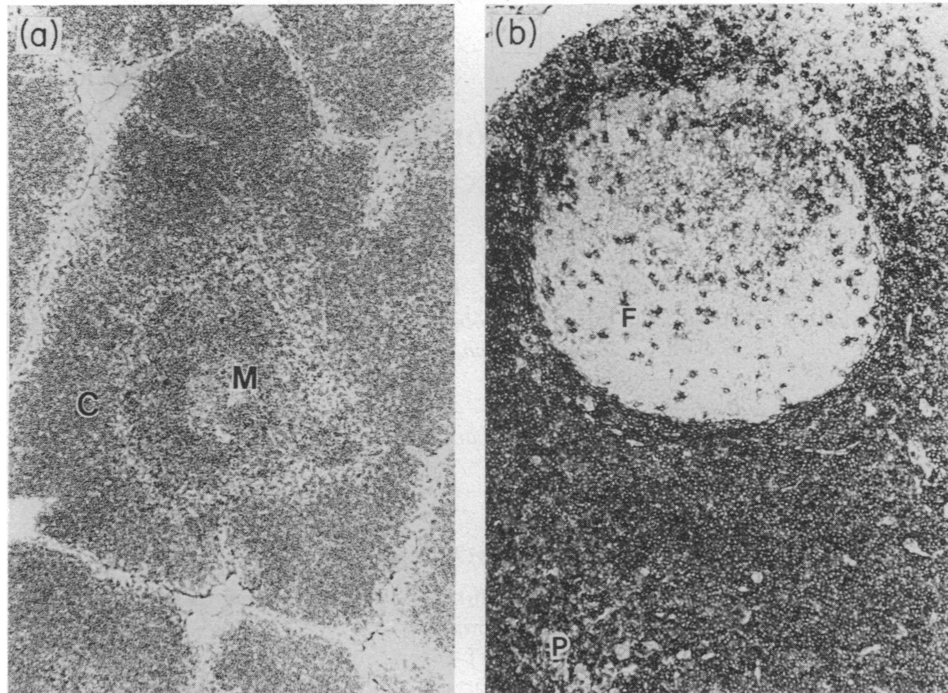

Figure 1. Cryostat section of normal bovine lymphoid tissue stained by the indirect immunoperoxidase method with CH128A. In the thymus (a), the cortex (C) and medulla (M) are indicated; in the lymph node (b), the paracortex (P) and the follicles (F) are indicated. Magnifications 80 × for thymus and 100 × for lymph node.

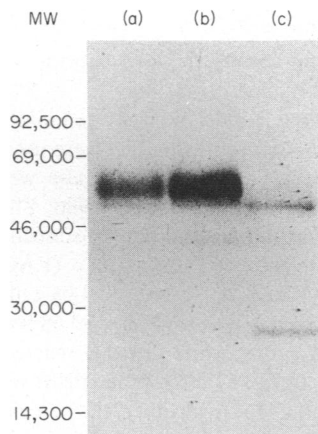

Figure 2. Analysis by SDS–PAGE, using reducing conditions, of the molecules precipitated from surface-biotinylated lymphoblasts with CH61A (a), CH128A (b), and an irrelevant IgG1 mAb (c). Molecular weights are indicated.

some of the lymphocytes lying immediately beneath the thymic capsule were not stained. Virtually all lymphocytes in the T-dependent paracortex of lymph nodes were reactive with CH128A and CH61A. In addition, small numbers of positive cells were present in the B-dependent follicular areas (Fig. 1).

In order to test reactivity with the bovine SRBCr, the mAb were tested for their ability to inhibit E-rosette formation. Sheep erythrocytes were prepared for use in the rosetting assay by treatment with 2-aminoethylisothiouronium (Paul *et al.*, 1979). PBM (50 μl at 10^6 cells/ml) were preincubated with 50 μl of a saturating concentration of the mAb for 30 min at 4°, washed twice with cold rosetting medium (RPMI-1640 with 10% fetal bovine serum that had been absorbed overnight with sheep erythrocytes) and then mixed with 50 ml of a 2% solution of sheep erythrocytes in rosetting medium, centrifuged at 200 ***g*** and incubated overnight at 4°. Before examination, the pellets were fixed in 0·8% formaldehyde for 2 hr, and stained with 0·5% methylene blue. Two-hundred PBM in each sample were examined for rosette formation. Preincubation of PBM with either CH128A or CH61A markedly inhibited E-rosette formation, from 91% to 99% with PBM from five cattle including both *B. taurus* and *B. indicus*. In contrast, there was no inhibition of rosette formation when PBM were preincubated with mAb of the same isotype which react with other bovine T-lymphocyte determinants.

In order to determine the molecular mass of target molecules, cell surface proteins were biotinylated according to the methods of Hurley, Finkelstein & Holst (1985). The molecules recognized by the mAb were immunoprecipitated, electrophoresed, blotted as described elsewhere (Ellis *et al.*, 1986), and reacted with (^{125}I)-streptavidin (Amersham International, Amersham, Bucks, U.K.) according to the manufacturer's specifications, prior to autoradiography. Both mAb precipitated molecules of approximately 50,000–60,000 MW from a cloned population of bovine lymphoblasts (Fig. 2) and a molecule of identical molecular mass from biotinylated thymocytes (data not shown).

The patterns of reactivity of CH128A and CH61A in cell suspensions and in tissues were very similar to those reported for mAb detecting the SRBCr in humans (Kamoun *et al.*, 1981; Verbi *et al.*, 1982). The mAb were reactive with both immature and mature thymocytes, and indeed in man T11 (CD2) is one of the first known T-lineage specific markers to appear during the ontogeny of T lymphocytes (Kamoun *et al.*, 1981). The data are

also consistent with the results of previous work in cattle which demonstrated that the majority of bovine fetal thymocytes form E rosettes (Renshaw, 1981). With respect to molecular masses of target molecules, previous studies have demonstrated that bovine T-lymphocyte differentiation antigens have biochemical characteristics similar, but not identical, to homologous antigens of other species (Baldwin *et al.*, 1986; Ellis *et al.*, 1986). In comparisons of potential analogues in man, it should be noted that the use of surface biotinylation may add 10% to the apparent molecular weight of labelled molecules (Neumaier, Fenger & Wagener, 1986). This, at least in part, may account for the higher molecular weight obtained for the bovine SRBCr than for the human CD2 molecule (50,000 MW; Kamoun *et al.*, 1981).

In humans, mAb raised against the T-lineage-specific 50,000 MW CD2 protein define three functionally distinct epitopes (Meuer *et al.*, 1984). These include mAb to T11.1, which are capable of blocking rosette formation by sheep red blood cells and human T lymphocytes. By analogy, we would conclude that mAb CH128A and CH61A recognize a T11.1-like epitope in cattle, and that these data provide biochemical characterization of the bovine homologue of the CD2 molecule (Meuer *et al.*, 1984), designated BoT2 (Baldwin *et al.* 1987). The mAb CH128A and CH61A will be useful in further definition of the functional properties of bovine T lymphocytes and contrast with the expression of some other bovine pan T-cell markers described elsewhere (Baldwin *et al.* 1987; Rabinovsky & Yang, 1986) which arise later in thymic development, as evidenced by their expression primarily on medullary thymocytes.

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REFERENCES

Baldwin C.L., MacHugh N.D., Ellis J.A., Naessens J., Newson J. & Morrison W.I. (1987) Monoclonal antibodies which react with bovine T-lymphocyte antigens and induce blastogenesis: tissue distribution and functional characteristics. *Immunology* (in press).

Baldwin C.L., Teale A.J., Naessens J., Goddeeris B.M., MacHugh N.D. & Morrison W.I. (1986) Characterization of a subset of bovine T lymphocytes that express BoT4 by monoclonal antibodies and function: similarity to lymphocytes defined by human T4 and murine L3T4. *J. Immunol.* **136,** 4385.

Cockerell G.L. & Baldwin C.L. (1979) Increased spontaneous erythrocyte rosette formation of feline lymphocytes preincubated at 37°C. *J. immunol. Meth.* **28,** 369.

Coombs R.R.A., Gurner B.W., Wilson A.B., Holm G. & Lindgren B. (1970) Rosette formation between human lymphocytes and sheep red blood cells not involving immunoglobulin receptors. *Int. Archs Allergy Appl. Immunol.* **39,** 658.

Davis W.C., McGuire T.C. & Perryman L.E. (1983) Biomedical and biological application of monoclonal antibody technology in developing countries. *Periodicum Biologorum,* **85,** 259.

Ellis J.A., Baldwin C.L., MacHugh N.D., Bensaid A., Teale A.J., Goddeeris B.M. & Morrison W.I. (1986) Characterization by a monoclonal antibody and functional analysis of a subset of bovine T lymphocytes that express BoT8, a molecule analogous to human CD8. *Immunology,* **58,** 351.

Hurley W.L., Finkelstein E. & Holst B.D. (1985) Identification of surface proteins on bovine leukocytes by a biotin avidin protein blotting technique. *J. immunol. Meth.* **85,** 195.

Kamoun M., Martin P.J., Hanson J.A., Brown M.A., Siadak A.W. & Nowinski R.C. (1981) Identification of a human T-lymphocyte surface protein associated with the E-rosette receptor. *J. exp. Med.* **153,** 207.

Lalor P.A., Morrison W.I., Goddeeris B.M., Jack R.J. & Black S.J. (1986) Monoclonal antibodies identify phenotypically and functionally distinct cell types in the bovine lymphoid system. *Vet. Immunol. Immunopathol.* **13,** 121.

Meuer S.C., Hussey R.E., Fabbi M., Fox D., Acuto O., Fitzgerald K.A., Hodgdon J.C., Protentis J.P., Schlossman S.F. & Reinherz E.L. (1984) An alternative pathway of T-cell activation: a functional role for the 50 kD T11 sheep erythrocyte receptor protein. *Cell,* **36,** 897.

Neumaier M., Fenger U. & Wagener C. (1986) Transblot studies with biotin-labeled proteins: electrophoretic mobilities and detection limits. *Analyt. Biochem.* **156,** 76.

Outteridge P.M., Fahey K.J. & Lee C.S. (1981) Lymphocyte surface markers in sheep blood and lymph. *Aust. J. exp. Biol. Med. Sci.* **59,** 143.

Paul P.S., Senogles D.R., Muscoplat D.R. & Johnson D.W. (1979) Enumeration of T cells, B cells and monocytes in the peripheral blood of normal and lymphocytotic cattle. *Clin. exp. Immunol.* **35,** 306.

Rabinovsky E.D. & Yang T.J. (1986) Production and characterization of a bovine T cell-specific monoclonal antibody identifying a mature differentiation antigen. *J. Immunol.* **136,** 609.

Reinherz E.L. (1985) A molecular basis for thymic selection: regulation of T11 induced thymocyte expansion by the T3-Ti antigen/MHC receptor pathways. *Immunol. Today,* **6,** 75.

Renshaw H.E. (1981) Non immune erythrocyte rosette formation of bovine peripheral blood and thymus lymphocytes. *Comp. Immunol. Microbiol. infect. Dis.* **4,** 75.

Verbi W., Greaves M.F., Scheider C., Koubek K., Janossy G., Stein H., Kung P. & Goldstein G. (1982) Monoclonal antibodies OKT11 and OKT11A have pan T-cell reactivity and block sheep erythrocyte receptor. *Eur. J. Immunol,* **12,** 81.