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Monoclonal antibodies which react with bovine T-lymphocyte antigens and induce blastogenesis: tissue distribution and functional characteristics of the target antigens

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SUMMARY

In this study we report on the tissue distribution and functional characteristics of bovine T-cell differentiation antigens recognized by the monoclonal antibodies (mAbs) IL-A26, IL-A27, and IL-A28. All three mAbs are able to stimulate proliferation of peripheral blood mononuclear cells (PBM) and inhibit proliferation of responder cells in mixed leucocyte cultures (MLC). MAbs IL-A27 and IL-A28 are believed to react with the same molecule, which is different to that recognized by IL-A26 as determined by a number of criteria. MAbs IL-A27 and IL-A28 inhibit binding of one another, but not of IL-A26. MAbs IL-A27 and IL-A28 react with 25% of thymocytes confined to the medulla, whereas IL-A26 reacts with approximately 80% of thymocytes, including medullary and cortical populations. MAbs IL-A27 and IL-A28 react with thymocytes which express BoT4 or BoT8 singularly, whereas IL-A26 reacts with all cells which express BoT4 or BoT8, either singularly or dually, in addition to all thymocytes which react with IL-A27/28. Only IL-A26 inhibits spontaneous sheep erythrocyte (E)-rosette formation by bovine T cells. Based on tissue distribution and functional characteristics, IL-A26 is believed to recognize the bovine homologue of CD2, designated BoT2, whereas IL-A27/28 reacts with a mature T-cell antigen. Cells reactive with the mAbs constitute approximately 60% of bovine PBM. Using these mAbs in dual immunofluorescence analyses, at least three populations of bovine T cells are demonstrable in PBM. The majority of T cells are $BoT4^+$ or $BoT8^+$ and also react with IL-A26/27/28. A second small population of PBM is negative for BoT4 and BoT8 but is IL-A26/27/28$^+$. A third population (less than 5%) is $BoT4^-/BoT8^-$/ILA27/28$^-$ but reacts with IL-A26.

INTRODUCTION

In man, monoclonal antibodies (mAbs) have been produced that identify T-lymphocyte cell-surface molecules. The antigens identified include CD2, the sheep erythrocyte (E)-rosetting receptor (Howard *et al.*, 1981; Kamoun *et al.*, 1981; Verbi *et al.*, 1982), CD3, the complex of polypeptides associated with the idiotypic receptor of T cells (Chang *et al.*, 1981; Meuer *et al.*, 1983), CD5, the human homologue of Lyt-1 (Cueppens & Baraja, 1986; Ledbetter *et al.*, 1985), and CD6 (Rieber *et al.*, 1986). All of these molecules are considered to be involved in transduction of activation signals across the T-cell membrane, since some mAb reactive with these antigens are capable of inducing proliferation of resting T cells in the absence of antigenic stimulation (Burns, Boyd & Beverley, 1982; Meuer *et al.*, 1984; Reinherz, 1985; Van Wauwe, DeMey & Goossens, 1980; Rieber *et al.*, 1986). Although all four antigens are exclusively or largely expressed by T cells, the later three CD3, CD5 and CD6, can be distinguished from CD2 by their high level of expression on mature but not immature T cells (Reinherz *et al.*, 1982).

In cattle, as in man, formation of rosettes with sheep erythrocytes has been used as a means of identifying T cells (Paul *et al.*, 1979). In addition, Rabinovsky & Yang (1986) have reported on a mAb which reacts with a molecule on bovine T cells, with cellular distribution and molecular weight similar to CD3 in man, and we have reported on mAbs which block E-rosette formation and precipitate molecules with molecular weights similar to CD2 (Davis *et al.*, 1987). None of those mAbs have demonstrable effects on functional activities of T cells. Herein, we describe the characterization of three mAbs which identify two bovine pan T-cell surface molecules, stimulate

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Abbreviations: E, erythrocyte; FITC, fluorescein isothiocyanate; mAb, monoclonal antibody; MLC, mixed leucocyte cultures; PBM, peripheral blood mononuclear cells

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proliferative responses in the absence of antigen and inhibit proliferation to alloantigens. One of the molecules is similar to CD2 while the other is expressed only by mature T cells.

MATERIALS AND METHODS

Production of monoclonal antibodies

BALB/c mice were immunized on two occasions with $2–4 \times 10^6$ cells of a BoT8$^+$ allo-specific T-cell clone (Teale *et al.*, 1986) in order to generate mAbs IL-A26 and IL-A27, or a BoT8$^+$ *Theileria*-specific T-cell clone (Goddeeris *et al.*, 1986b) to generate mAb IL-A28. MAbs were produced, screened and recloned, the isotypes of the mAbs determined, and ascites produced as described previously (Baldwin *et al.*, 1986).

Experimental animals

Cattle were clinically normal *Bos indicus* or *B. taurus* females or castrated males, between 2 months and 36 months of age.

Isolation of leucocytes and thymocytes

Bovine peripheral blood mononuclear cells (PBM) were isolated from blood which had been collected either in Alsever's solution or defibrinated as described previously (Lalor *et al.*, 1986). PBM from blood collected in Alsever's solution contained 5–20% monocytes, whereas PBM obtained from defibrinated blood and incubated on polystyrene for 60 min at 37° were depleted of monocytes (less than 0·1%) (Goddeeris *et al.*, 1986a). Monocyte-enriched populations were obtained from PBM by adherence to plasma-coated gelatin surfaces (Goddeeris *et al.*, 1986a). Thymic tissue for cryostat sectioning were obtained surgically from calves 2–6 months of age. Suspensions of thymocytes were prepared and granulocytes were obtained from blood as described elsewhere (Baldwin *et al.*, 1986).

Immunohistochemistry

Frozen sections of bovine thymus and lymph node were prepared and stained by the indirect immunoperoxidase method (Baldwin *et al.*, 1986).

Immunofluorescence

Cells were stained by indirect immunofluorescence (Lalor *et al.*, 1986) and two-colour immunofluorescence (Ellis *et al.*, 1986) and analysed with a fluorescence-activated cell sorter (FACS II, Becton-Dickinson, Sunnyvale, CA). The second-step reagents consisted of either biotinylated anti-mouse isotype-specific reagents followed by phycoerythrin/avidin, or fluorescein-conjugated isotype-specific anti-mouse Ig (Southern Biotech, Birmingham, AL) appropriate for the isotype of the mAb. For staining of B cells, dual fluorescence analyses with rhodamine-conjugated anti-bovine Ig were conducted (Lalor *et al.*, 1986).

Erythrocyte rosetting

Bovine PBM were rosetted with sheep red blood cells (SRBC) and inhibition of rosetting by mAb determined as described elsewhere (Davis *et al.*, 1987).

Other monoclonal antibodies used in functional assays and to assess tissue distribution

The following mAbs were used in dual fluorescence assays or as Ig-isotype controls in functional assays: IL-A11 (IgG2a) and IL-A12 (IgG2a), which react with BoT4 (Baldwin *et al.*, 1986), IL-A17 (IgG1), which reacts with BoT8 (Ellis *et al.*, 1986), B5/4 (IgG1), which reacts with bovine IgM (Pinder *et al.*, 1980), CH128A (IgG1), which reacts with BoT2 (Davis *et al.*, 1987), and R1 (IgM) (Lalor *et al.*, 1986) and IL-A21 (IgG1) (J. A. Ellis, unpublished data) which react with monomorphic determinants on bovine class II molecules.

Purification of Ig from ascitic fluid and conjugation with fluorescein isothiocyanate

For mAbs of the IgG2a and IgG1 isotypes, Ig was purified from ascitic fluid by binding to protein A–Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden) at pH 8·0. Those of the IgM isotype were purified by dialysing the ascitic fluid against 0·01 M phosphate-buffered saline, pH 7·2, and resuspending the precipitate, which contained the IgM, in 0·15 M phosphate-buffered saline. Purity of Ig was assessed by electrophoresis on acetate cellulose strips and staining with Ponceau S, Acid Red 112 (MCR, Norwood, OH). Purified Ig was dialysed against RPMI-1640 medium before use and the protein concentration calculated from spectrophotometric absorbance at 280 nm. For use in competitive binding assays, purified Ig of mAb IL-A28 was directly conjugated with fluorescein isothiocyanate (FITC) (Goding, 1976).

Competitive binding assays

PBM were first reacted in an immunofluorescence assay, as described above, with unconjugated mAb, followed by reactivity with a directly conjugated mAb or with an unconjugated second mAb of a different isotype. In the case of an unconjugated second-layer mAb, isotype-specific conjugates were used to distinguish reactivity from that with the first-layer mAb.

Functional assays

For induction of proliferation by mAb, $2·5 \times 10^5$ PBM per well were cultured for 3–6 days in micro-wells with purified soluble Ig at 30 ng/ml to 300 μg/ml final concentration in the well in RPMI-1640 (Gibco, Uxbridge, Middlesex, U.K.) with 10% heat-inactivated fetal bovine serum (Gibco), 2 mM L-glutamine, 50 μg/ml gentamycin and 5×10^{-5} M 2-mercaptoethanol (2-ME). Proliferation was evaluated by measuring incorporation of S[^{125}I]iododeoxyuridine (Amersham International, Amersham, Bucks, U.K.) (Lalor *et al.*, 1986) and results expressed as the mean and SD of c.p.m. of radioactivity of replicate wells.

Allogeneic mixed leucocyte cultures (MLC) were conducted essentially as described previously (Goddeeris *et al.*, 1987). The ability of purified Ig from the mAb to block proliferative responses was evaluated according to the method described elsewhere (Baldwin *et al.*, 1986).

RESULTS

Characteristics of mAb selection

The selection of three mAbs, IL-A26 (IgM), IL-A27 (IgG1) and IL-A28 (IgM), was based on reactivity with T cells. This was determined by reactivity with the majority of cells in paracortical regions of lymph nodes but not cells in lymphoid follicles, reactivity with cells in thymic sections (Fig. 1), and lack of reactivity with peripheral blood granulocytes, B cells or monocytes. With PBM from 10 cattle, IL-A26 reacted with a mean of 62%, ranging from 46–75%, while IL-A27/28 reacted with a

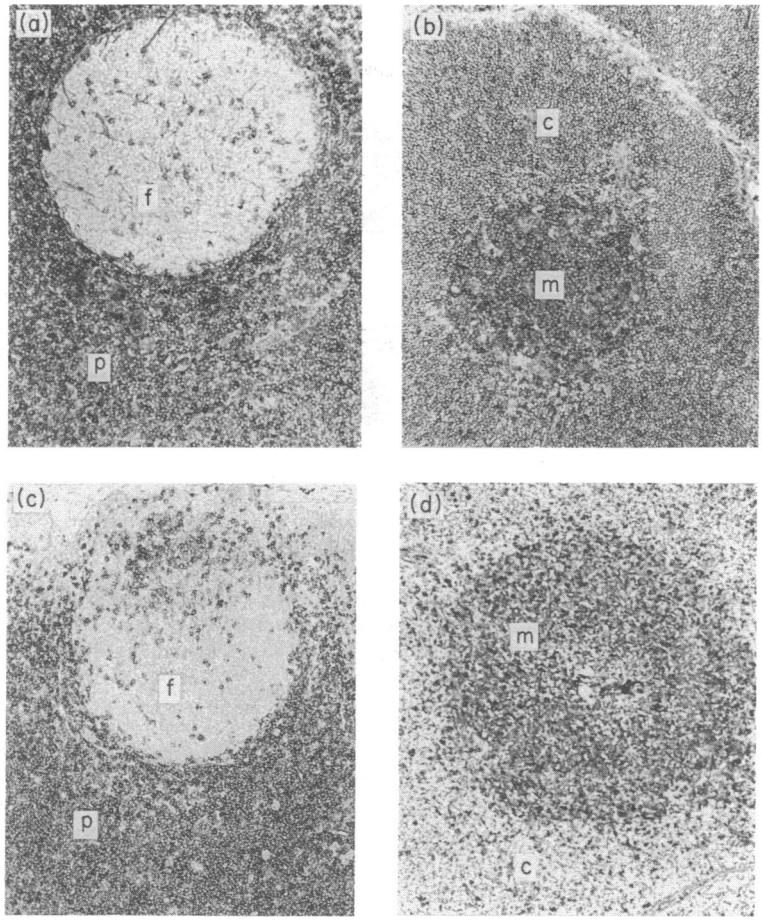

Figure 1. Tissue sections of bovine (a) lymph node or (b) thymus stained with mAb IL-A26; and (c) lymph node or (d) thymus stained with mAb IL-A27, by the indirect immunoperoxidase technique. Magnifications (a) ×81·25, (b) ×55, (c) ×74·75 and (d) ×65. Designated areas are: p, lymph node paracortex (T-dependent area); f, lymph node follicle; c, thymic cortex; and m, thymic medulla.

mean of 62%, ranging from 51% to 70%. The mAbs IL-A27 and IL-A28 differed from IL-A26 in reactivity with thymocytes. In thymic cell suspensions from five cattle, IL-A26 reacted with a mean of 77% (range 75–80%) of the cells whereas IL-A27/28 reacted with a mean of 29·8% (range 21–44%). Furthermore, in sections of bovine thymus IL-A26 reacted with the majority of thymocytes in both the medulla and cortex (Fig. 1b) whereas IL-A27 and IL-A28 reacted mainly with medullary thymocytes (Fig. 1d).

MAb IL-A26 but not IL-A27/28 inhibited spontaneous E-rosette formation, from 95% to 100%, with PBM from 10 cattle. Furthermore, only binding of IL-A26 to PBM was inhibited by mAb CH128A, previously shown to recognize the bovine homologue of CD2 (Davis *et al.*, 1987), whereas IL-A27 but not IL-A26 inhibited binding of FITC-conjugated IL-A28 to PBM.

Distribution of the antigens recognized by mAbs IL-A26, IL-A27 and IL-A28 in relation to the T-cell subset markers BoT4 and BoT8 on lymphocytes and thymocytes

At least five phenotypically distinct populations of cells were discernible in the thymus; these are depicted in Fig. 2. All of the thymocytes which reacted with IL-A27/28 (populations 2–4, Fig. 2) (Fig. 3c), and all the BoT4$^+$ and BoT8$^+$ cells (populations 1, 3 and 4) (Fig. 3d), were within the IL-A26$^+$ population. A small population of IL-A26$^+$ cells that did not express either BoT4 or BoT8 (Fig. 3d) reacted with IL-A27 (population 2, Fig. 2) (compare Fig. 3d and e). Cells which expressed either BoT4 (results not shown) or BoT8 (compare Fig. 3b and f) but not both expressed the molecule detected by IL-A27/28 and accounted for the majority of the IL-A27/28$^+$ thymocytes (populations 3 and 4, Fig. 2).

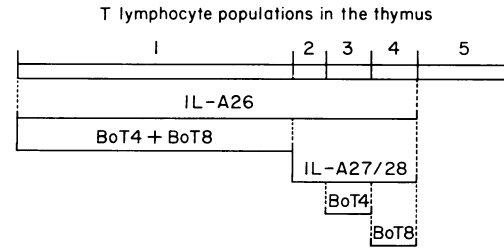

Figure 2. Diagrammatic representation of thymocyte populations (1–5) which react with mAbs IL-A26, IL-A27 or IL-A28, and/or express the differentiation antigens BoT4 and BoT8.

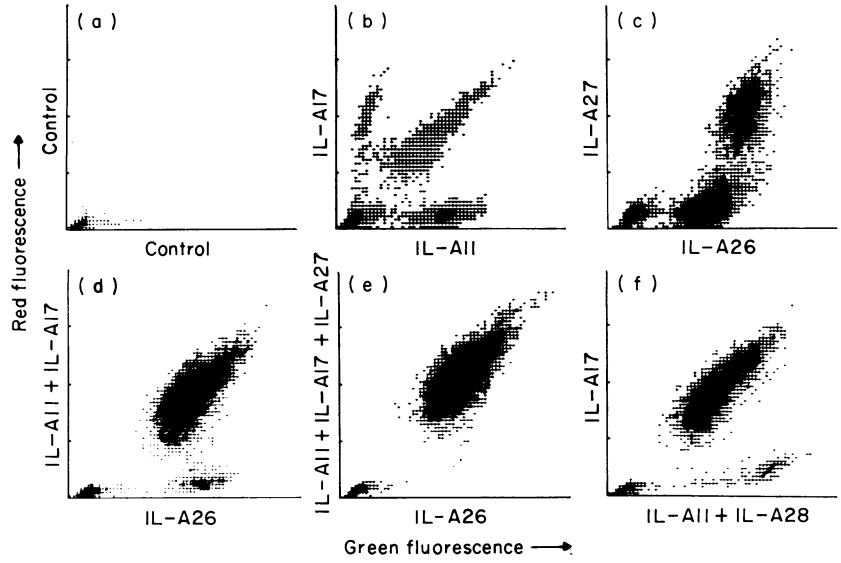

Figure 3. Fluorescence-activated cell sorter analysis of thymocyte suspensions for dual immunofluorescence with mAbs IL-A26, IL-A27 or IL-A28 and mAbs to BoT4 and BoT8, as indicated on the axes. BoT4 was detected by staining with mAb IL-A11, and BoT8 was detected by mAb IL-A17. The ordinate is fluorescence emitted by phycoerythrin (red) and the abscissa by FITC (green).

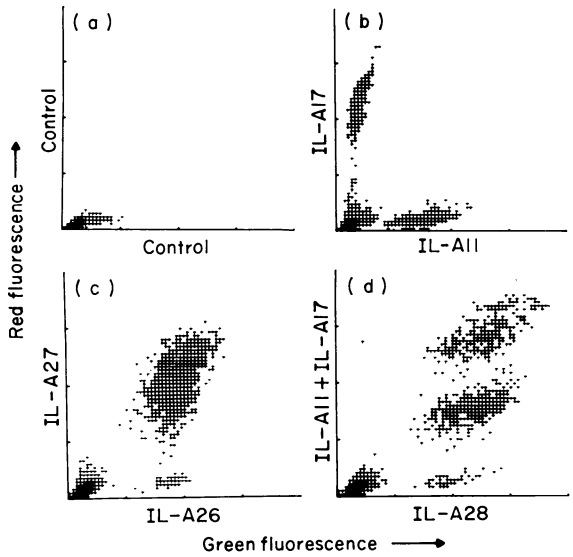

Figure 4. Dual immunofluorescence analysis of staining of PBM for reactivity with mAbs IL-A26, IL-A27, or IL-A28 and expression of differentiation antigens BoT4 (IL-A11) and BoT8 (IL-A17) as indicated in legend of Fig. 3.

The majority of PBM which express BoT4 or BoT8 do so singularly (Ellis *et al.*, 1986) (Fig. 4b), and all of these cells express the molecules recognized by IL-A27/28 (Fig. 4d). Since all PBM which expressed IL-A27/28 also expressed IL-A26 (Fig. 4c), all BoT4/T8$^+$ PBM must also express IL-A26. A small population of PBM (less than 5%) positive for both IL-A26 and IL-A28 was negative for BoT4 and BoT8 (Fig. 4d). In addition, a small but variable population (less than 6%) of PBM was IL-A26$^+$, IL-A27$^-$ (Fig. 4c). Thus, there are two small populations in PBM which lack the T-cell subset markers BoT4 and BoT8 but are IL-A26$^+$, IL-27/28$^+$ (Fig. 4d) or IL-A26$^+$, IL-A27/28$^-$ (Fig. 4c).

Induction of proliferation of PBM by mAbs IL-A26, IL-A27 and IL-A28

Since several mAbs which react with human pan T-cell antigens cause functional effects upon interaction with their target antigens, mAbs IL-A26/27/28 were evaluated for the functional effects they induced when interacting with their target antigens. When PBM were cultured in the presence of 2-mercaptoethanol, all three mAbs stimulated proliferation of PBM from six of 13 cattle tested, whereas control mAbs, IL-A12 and R1, did not. Maximum proliferation was achieved on Day 4–5 (results not shown) with 3–30 μg of soluble Ig/ml or higher. To evaluate further the requirements for induction of proliferation by the mAb, PBM from responder animals were cultured in round-bottomed or flat-bottomed plates either in the presence or absence of monocytes. Examples of results are presented in Table 1. For cultures conducted in flat-bottomed plates, the presence of monocytes had a substantial enhancing effect on proliferation (Table 1); when cultures were conducted in round-bottomed plates, which maximized cell contact, monocytes were not necessary for proliferation and sometimes had a suppressive effect (B734) (Table 1).

Inhibition of mixed leucocyte cultures by mAb

Both mAbs IL-A26 and IL-A27 inhibited proliferation in MLC in five out of six allogeneic combinations tested (Table 2). PBM from animals which were unresponsive to stimulation by mAb alone were used in some tests, and culture conditions that did not result in stimulation of proliferation by the mAb alone were used in all tests. The suppression of proliferation occurred using the same concentration of mAb (30 μg/ml) that stimulated

Table 1. Influence of monocytes on blastogenesis of PBM cultured in flat-bottomed or round-bottomed plates with mAb IL-A26, IL-A27, or IL-A28*

Animal no.	MAb used as stimulator	Responding PBM population and culture vessel†			
		Flat-bottomed plate		Round-bottomed plate	
		With monocytes	Without monocytes	With monocytes	Without monocytes
B641	Medium	3134±2417	618±314	3792±175	5863±1389
	IL-A26	74,296±12,488	1617±920	44,623±2295	42,489±2773
	IL-A27	55,581±6755	2059±525	39,519±1756	46,418±4988
	IL-A28	79,543±8166	5906±5211	50,396±11,549	47,142±11,077
B734	Medium	1706±276	1139±145	2743±1065	3499±1135
	IL-A26	1355±301	1302±334	5313±1361	12,080±3076
	IL-A27	3706±673	1440±869	6263±1961	44,409±9489
	IL-A28	6770±2363	1432±735	10,624±3078	40,656±12761

* Results are mean±SD of c.p.m.

† PBM with monocytes contained 5–20% monocytes; PBM without monocytes contained less than 0·1% monocytes.

Table 2. Representative examples of inhibition of proliferation in MLR by mAb IL-A26 and IL-A27*

MmAb added to the culture	Allogeneic combination			
	B734 versus D96		D96 versus B734	
	Control	±Stimulators	Control	±Stimulators
None	461±235	67,309±31,055	1899±1771	32,842±5619
IL-A26	1245±1051	1806±30	413±28	4971±696
IL-A27	1691±263	19,156±2678	664±14	3437±1037

* Results are mean±SD c.p.m.; cultures were conducted in flat-bottomed plates. Control cultures were without stimulator cells present.

proliferation of PBM from some of the cattle under different culture conditions. For the two allogeneic leucocyte combinations presented (Table 2), inhibition was 97% and 85% for IL-A26, and 72% and 90% for IL-A27. The mAb IL-A28 also inhibited proliferation of PBM in response to alloantigens by about 50%.

DISCUSSION

Three mAbs described herein identify pan T-cell-specific differentiation antigens of bovine lymphocytes. All three mAbs reacted with cell-surface molecules important in the regulation of proliferation by T cells, as demonstrated by their ability to induce proliferation of PBM and to inhibit proliferation in allogeneic MLC. Based on a number of criteria, mAb IL-A26 is believed to react with the bovine homologue of CD2, called herein BoT2, which is expressed by both mature and immature T cells. In contrast, the antigen recognized by mAbs IL-A27 and IL-A28 is expressed only by mature T cells, as shown by the distribution of cells stained in thymus sections.

The high level of reactivity with the mature T cells by IL-A27/28 is similar to that of mAb to human pan T-cell differentiation antigens CD3 (T3), CD5 (T1) and CD6 which largely react with mature T cells (Reinherz & Schlossman, 1980; Reinherz *et al.*, 1979; Van Agthoven *et al.*, 1981; Reinherz *et al.*, 1982). CD3, CD5 and CD6 can be distinguished by differences in molecular mass (Reinherz & Schlossman, 1980; Knowles 1986), expression of CD5 and CD6 by some B cells (Antin *et al.*, 1986; Boyd *et al.*, 1985), although mAb SPV-14 also believed to recognize CD6 apparently does not react with B cells (Yssel *et al.*, 1987) and the ability of mAb to CD3 but not to CD5 to inhibit proliferative responses of lymphocytes to alloantigens (Platsoucas & Good, 1981). We have been unable to characterize the molecule recognized by IL-A27/28 by immunoblotting, affinity columns, or immunoprecipitation including the use of cross-linking reagents. This suggests that the binding affinities of the mAb to antigen were weak and/or were easily disrupted by detergents, possibly due to reactivity of the mAb with conformational determinants. The lack of reactivity of IL-A27/28 with B cells indicates the greatest similarity with mAb to CD3 and CD6.

The cellular distribution of the molecules recognized by IL-A26 and the T-cell differentiation antigens BoT4 and BoT8, was similar to that of CD2, CD4 and CD8, respectively, on human

peripheral T lymphocytes and thymocytes. In addition, cells reactive with IL-A27/28 had a similar distribution to those expressing CD3 and CD6 in humans relative to CD2, CD4 and CD8 or their bovine homologues (Verbi *et al.*, 1982; Van Agthoven *et al.*, 1981; Reinherz & Schlossman, 1980; Lanier, Ruitenber & Phillips, 1986; Yssel *et al.*, 1986). The proportion of bovine thymocytes which were negative for all the T-cell markers (about 20%) was, however, somewhat higher than that in humans. At least part of the IL-A26/27/28$^-$ population was located in the outer cortex of the thymus. In addition, a small population (less than 5% thymocytes) of IL-A26$^-$ cells, identified by another mAb, IL-A29, can be detected in the medulla (W. I. Morrison and N. D. MacHugh, unpublished observations).

The results presented herein suggest that the majority of thymocytes which have dual expression of BoT4 and BoT8 do not react with IL-A27/28, although it was not possible to demonstrate this directly. These findings, together with the distribution of positive cells in tissues, suggest that most of the cells which have dual expression of BoT4 and BoT8 are located in the thymic cortex, whereas the majority of cells which are IL-A27/28$^+$ and have singular expression of BoT4 or BoT8 are in the medulla. By analogy with the expression of human differentiation antigens, we expect the IL-A27/28$^+$ thymocytes to include the functionally mature T cells (De La Hara *et al.*, 1986; Taribio *et al.*, 1986).

The detection of a population of cells that is IL-A26$^+$ and IL-A27/28$^+$ but lacks BoT4 and BoT8 is also in agreement with findings in humans. Studies of CD3$^+$ CD4$^-$ CD8$^-$ cells in man have shown that they may be precursors of the CD4$^+$ or CD8$^+$ cells (De La Hara *et al.*, 1986; Taribio *et al.*, 1986). We have obtained evidence that the IL-A26$^+$ IL-A27/28$^+$ BoT4$^-$ BoT8$^-$ cells in the peripheral blood of cattle are functionally competent, in that we have identified two cytotoxic T-cell clones with this phenotype. Unlike the situation in man, these clones are clearly class I MHC restricted (Teale *et al.*, 1986; Morrison *et al.*, 1986) and thus appear to resemble the BoT8$^+$ cells. Further studies will be required to determine whether or not this is a general feature of cells within this subpopulation and to elucidate the functional properties of the IL-A26$^+$ IL-A27/28$^-$ cells in peripheral blood.

Although the finding that IL-A26 induced proliferation of PBM from some animals and inhibited proliferation in MLC is similar to results using mAb to CD2 with human PBM (Huet *et al.*, 1986; Meuer *et al.*, 1984; Olive *et al.*, 1986; Reinherz, 1985; Van Wauwe *et al.*, 1981), some differences are apparent between the bovine and human systems. The effects of IL-A26 on PBM differed from the responses induced by mAb to the homologous molecule in man, CD2, in that stimulation via CD2 requires co-stimulation by mAb to two different epitopes (Huet *et al.*, 1986; Olive *et al.*, 1986). Several factors could account for this difference, including variation in the abilities of the mAb to cross-link the antigen, differences in the affinity of the mAb for antigen, or a difference in the epitope recognized by IL-A26 which may be uniquely different from those recognized by mAb to CD2. It is thought that the conformational change of the CD2 molecule after binding with mAb might account for variations in the abilities of mAb to CD2 to induce proliferation (Huet *et al.*, 1986). There may be structural variations between these homologous molecules of man and cattle and therefore in conformational changes induced following binding with mAb. Other mAbs to BoT2 of the IgG1, G2a or G2b isotypes, including mAbs CH128A and CH61A (Davis *et al.*, 1987) did not induce proliferation (C. L. Baldwin, unpublished observations).

Other functional differences, as well as similarities, occurred with the mAbs described herein and those previously published for mAb to human T-cell antigens. All three mAbs could induce proliferation of monocyte-depleted PBM, which differs from some reports using mAb to CD2 and CD3 (Cueppens, Bloemmen & Van Wauwe, 1985; Huet *et al.*, 1986; Tsoukas *et al.*, 1985). Monocyte-depleted cultures described herein were conducted in round-bottomed plates and with the addition of 2-ME. Previously, 2-ME has been shown to substitute for monocytes in the induction of proliferative responses of bovine PBM to mitogens (Goddeeris *et al.*, 1986a) and may play a similar role here. Furthermore, proliferation only occurred in the absence of monocytes when cultures were conducted in round-bottomed plates which maximize cell contact, including that with any residual monocytes, and thereby potentiate lymphokine production and responsiveness. Moreover, under these circumstances (culture in round-bottomed plates) the presence of 5–10% monocytes occasionally suppressed proliferation, which is consistent with their potent regulation of proliferative responses of bovine PBM to autologous leucocytes (Goddeeris *et al.*, 1987) and further emphasizes the variable effects of maximizing cell contact. Although we do not know the basis for it in cattle, the lack of ability to stimulate proliferation of PBM from all cattle is similar to results in the human system with mAb to CD3 (Cueppens *et al.*, 1985). The requirements for induction of proliferation by mAb to T-cell differentiation antigens remain to be defined precisely for cattle and may be found to differ among species.

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Note added in proof

Recently another mAb, IL-A57, produced in our laboratory, has been shown to inhibit binding of IL-A27/28 to lymphocytes and has been used to precipitate a molecule of 120,000–130,000 MW (N. D. MacHugh and A. Bensaid, unpublished data), suggesting that these three mAb may recognize the bovine homologue of CD6 (Yssel *et al.* 1987).

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