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Cross-reactivity of mAbs to human CD antigens with cells from cattle

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Abstract

A panel of 377 commercially available mAbs were submitted to the animal homologue section of the 8th International Workshop on Human Leukocyte Differentiation Antigens (HLDA8, Adelaide, Australia) for cross-reactivity studies on different animal species. In this study we describe the results of testing the mAbs on cattle cells by flow cytometry and Western blot. Eight commercial suppliers participated, providing mAbs to a total of 144 CD antigens plus controls. Fifty-two mAbs were identified as potentially staining cattle cells in the first round screen. In the second phase, 38 mAbs were confirmed as staining cattle cells. This included some that may recognise polymorphic determinants and others with atypical distribution patterns compared to humans. mAb to human CD9, CD11a, CD14, CD18, CD21, CD23, CD29, CD44, CD45R, CD47, CD49d and CD172a cross-reacted with bovine cells and mAb to CD22, CD88, CD119 and CD163 stained CD antigens that have not previously been identified in cattle.

Keywords: Cattle; CD; Cross-reactivity

1. Introduction

The testing of mAbs for cross-species reactivity has proven useful for identifying reagents to previously unrecognised antigens in several different species. Three International Workshops on Ruminant Antigens (Howard et al., 1991; Howard and Naessens, 1993; Naessens and Hopkins, 1996) have revealed, as expected due to their close evolutionary relationship, a high degree of cross-species reactivity of mAbs to cattle, sheep and goat antigens. Studies of mAbs to ruminant antigens on cells from non-ruminants also revealed several cross-reactions with canine, swine and human cells (Schuberth et al., 1996; Vilmos et al., 1996).

To date the most comprehensive studies of cross-reactivities of human anti-CD mAb for bovine molecules have involved the testing of mAbs submitted to the International Workshops on Human Leukocyte Differentiation Antigens (HLDA) (Sopp and Howard, 1997; Sopp et al., 2001). In these, large panels of antibodies, representative of most of the available 'anti-CD' antigens mAbs, were screened on bovine cells and about 10% were found to cross-react. In many cases the potentially useful cross-reactive mAbs were not easily available and consequently the reagents have not been fully characterised. Those previously recognized are in Table 1. To combat this problem a panel of 377 commercially available mAbs (including controls) was organised by representatives of the Veterinary Immunology Committee of the International Union of Immunological Societies (VIC-IUIS) for testing on a wide range of animal species. This panel included the widest possible

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Table 1
Summary of previous studies of human CD mAbs on cattle cells

Typical	No. of mAbs identified		Atypical	No. of mAbs identified	
	HLDA 5	HLDA 6		HLDA 5	HLDA 6
CD11a	1		CDw17		1
CD14	9	2	CD19		1
CD18	2	2	CD35	1	
CD21	8	3	CD37	1	
CD23		1	CD49c	1	
CD27	1	2	CD50	1	
CD29	7	2	CD54	1	
CD39		1	CD55		1
CD44		3	CD56		1
CD47		2	CD66	3	3
CD49a	2	1	CD71		1
CD49b	3		CD74		1
CD49d	3		CD81	1	
CD49e	2	1	CD86		3
CD51/CD61	5		CD88	2	
CD62L	3		CD102	1	
CD62P	1		CD105		1
CD63	3		CD117		5
CDw78	1		CD120b		1
CD98	1		CD122		1
CD100	1	2	CD165		1

Typical: staining patterns consistent on cattle cells compared to human cells; Atypical: different cellular distribution in cattle compared to humans; HLDA 5: summary of results from 5th International Human Leucocyte Differentiation Antigen Workshop (Sopp and Howard, 1997); HLDA 6: summary of results from 6th International Human Leucocyte Differentiation Antigen Workshop (Sopp et al., 2001).

range of CD mAbs available from the donors. In this study we describe the testing of the mAbs on cattle cells where the aim was to identify, as far as possible, commercially available mAbs that react with the cattle homologue of the human antigen.

2. Materials and methods

2.1. Monoclonal antibodies

The full list of mAbs and their suppliers are listed in the chapter "Summary of the Animal homologue section of HLDA8" of this volume. All mAbs were used at a concentration specified by the suppliers and diluted in buffer (PBS supplemented with 1% (w/v) BSA and 0.1% (w/v) NaN_3). Most mAbs were of murine origin, purified and unconjugated but a few were directly conjugated with FITC or R-PE. A small minority were rat Ig. The anti-bovine mAbs CC42 (CD2, Davis and Splitter, 1991), CC-G33 (CD14, Sopp et al., 1996), CC21 (CD21, Naessens et al., 1990) and CC15 (WC1, Morrison and Davis, 1991) were used as biotin conjugates in two colour flow cytometry (FCM). These mAbs were used to identify the main sub-populations in peripheral blood (PB): CD2 for αβ-T cells and some γδ-T cells, CD14 for monocytes, CD21 for B cells and WC1 for $CD2^-$ γδ-T cells.

2.2. Preparation of cells

Peripheral blood samples were prepared by density gradient centrifugation using standard techniques to yield mononuclear cells (PBMC) or by red blood cell lysis using ammonium chloride buffer to give a leucocyte suspension (WBC) (Sopp et al., 2001) and resuspended in buffer. Cells from two animals were assayed at both laboratory sites (Institute for Animal Health and the University of Massachusetts) in both the first and second round studies.

2.3. Staining cells for FCM

The first round studies involved single colour FCM and the second phase included one and two colour FCM. Standard indirect staining techniques using fluorochrome conjugates were employed (Sopp et al., 2001) except for the directly labelled mAbs. Goat $F(ab')_2$ anti-mouse Ig FITC and goat anti-rat FITC (Southern Biotech, Birmingham, AL, USA) were used as secondary reagents where appropriate for the HLDA8 mAb panel and streptavidin–phycoerythrin conjugate (Southern Biotech) was used to detect the biotinylated mAbs. The cells were assayed using a FACSCalibur (Becton Dickinson, San Jose, CA).

2.4. Analysis of FCM data

Data were analysed using CellQuest (Becton Dickinson) or FCS Express (De Novo Software, Ontario, Canada). Mononuclear cells and polymorphonuclear cells were distinguished by gating using low angle light scatter (forward scatter, FSC) and orthogonal light scatter (side scatter, SSC) and each cell type was analysed separately. Selection of mAbs that stained bovine cells in the first round was determined by comparison with control mAbs. After background (control mAb) subtraction, any mAbs that stained cells at $\geq$1% of total gated cells were assessed and the staining patterns examined. All of these mAbs were included in the second phase unless the observed staining patterns and proportion of stained cells were in total discordance with the published human data at which point it was concluded to be an erroneous reactivity. In the second round, one colour FCM was repeated on the restricted panel of mAbs and more detailed analyses were performed by two colour FCM. In this, the two-parameter plots of data obtained with mAb against humans molecules and those mAb known to be reactive with bovine markers were studied in detail for consistency of their pattern of reactivity with those reported for human cells.

2.5. Western blot analysis

Whole cell lysates from bovine or human PBMC were prepared as described (Werling et al., 2004). Briefly, cells were harvested, counted, and washed once with ice cold PBS (300 × *g*, 10 min, 4 °C), transferred to an Eppendorf tube and pelleted (2000 × *g*, 10 min, 4 °C). Cell pellets (8×10^7) were lysed in 500 μl of lysis buffer, Mammalian Protein Extraction Reagent (M-PERM, Pierce, Oxnard, CA), supplemented with 50 mM sodium fluoride, 1 mM sodium vanadate, 0.5 mM PMSF, 10 μg ml^{-1} aprotinin, 10 μg ml^{-1} leupeptin (all reagents from Roche Diagnostics, Rotkreuz, Switzerland). The cellular extract was sonicated for 15 s on ice, allowed to sit for 20 min, and then centrifuged at 15,000 × *g* for 10 min. The supernatant was removed and boiled for 3 min with 5× Laemmli buffer. Twenty microliters of each sample was loaded onto a 10% SDS-PAGE gel, and run at 100 V for 1.5 h in a MiniProtean chamber (BioRad, Reinach, Switzerland). Cell proteins were blotted onto nitrocellulose (ECL; Amersham, Arlington Heights, IL) at 40 V for 4 h. The nitrocellulose was blocked with 5% milk powder in PBS with 0.05% Tween 20 (TPBS) overnight, washed, and incubated for 2 h at room temperature with the primary antibody and appropriate controls all diluted 1:200 in TPBS. Blots were washed five times with TPBS and incubated for 1 h with horseradish peroxidase-conjugated anti-rat IgG or anti-mouse IgG antibody, respectively (Amersham, diluted 1:3000 in TPBS). Immunoreactive bands were developed using a chemiluminescent substrate (ECL; Amersham). Antibody specificity was verified by comparing the size of positive bands with published molecular weights under denaturing conditions.

3. Results and discussion

3.1. FCM—first round screening

Fifty-two mAbs from the first phase studies were selected for further analysis in the second round (Table 2). Many of the mAbs gave distinctive staining and were good candidates for cross-reactive mAb. However for others, where the expected target population was small, there was significant doubt whether the staining was genuinely against the bovine homologue and not just unusually high non-specific background staining. For some mAb PB cells were not ideal targets and it is possible that a few genuinely cross-reactive mAb may have been missed. As an example, CD34 positive cells are rare in peripheral blood except in neonates so we cannot exclude the possibility that one or more of the CD34 mAb were cross-reactive. With the limited amount of mAb supplied to the study it was not possible to test a wide range of targets. Another potential problem that was not easy to overcome was the possibility that mAbs were not used at their optimal concentration on bovine cells. However, it is fully expected that the optimal dilution for use on human cells, determined by the supplier and used here, would be suitable in most cases.

3.2. Second round analyses

The aims of the second round included: (1) repeating the results of the first round to eliminate spurious data; (2) to look more critically at the staining patterns, in both one and two colour FCM and compare these with published data of human CD antigens; (3) to attempt to identify the MW of the antigen recognized by Western blotting and compare this with the human molecule.

3.3. FCM—second round studies

Twenty-six mAbs (indicated by +) in Table 2 reacted with bovine cells where the staining pattern was consistent with recognising the bovine homologue of

Table 2
Results and interpretation of second round FCM assays

CD	Clone	Supplier	Percentage of indicated sub-population stained by mAb				Site assessment		Overall assessment
			%CD2	%WC1	%CD21	%CD14	IAH[a]	UMASS[b]	
CD1a	B17.20.9	Coulter	3.4	6.6	39.5	40.4	+	–	P
CD9	RH1A	VMRD	7.9	60.1	19.1	89.5	+	+	+
CD9	LT86A	VMRD	12.7	66.2	43.8	90.3	+	+	+
CD9	MM2/57	Serotec	8.6	61.2	15.9	78.8	+	+	+
CD10	SN5c	Serotec	2.6	2.9	8.0	13.7	+/?	–/?	A
CD10	ALB1	Coulter	NT	NT	NT	NT	NT	+	P
CD11a	HUH73A	VMRD	74.9	69.6	54.6	98.7	+	+	+
CD14	CAM36A	VMRD	2.4	2.5	9.3	92.4	+	+	+
CD14	TÜK4	Dako	1.4	2.8	7.6	92.0	+	+	+
CD14	M5E2	BD Pharmingen	1.2	3.5	7.6	87.9	+	+	+
CD16	3G8	Coulter	0.9	2.5	3.8	4.1	–	–	–
CD18	BAQ30A	VMRD	67.0	85.1	78.0	99.5	+	+	+
CD18	H20A	VMRD	50.0	52.0	63.7	99.4	+	+	+
CD18	7E4	Coulter	36.8	41.9	66.3	98.2	+	+	+
CD18	MHM23	Dako	88.9	87.7	79.8	99.6	+	+	+
CD21	LB21	Serotec	3.6	6.4	91.1	5.4	+	+	+
CD21	BL13	Coulter	3.1	7.2	87.9	5.2	+	–	P
CD22	Mc64-12	Serotec	4.0	4.9	43.9	9.3	+	+	+
CD23	9P.25	Coulter	2.7	5.8	49.2	20.7	+	NT	+
CD25	B1.49.9	Coulter	2.7	6.3	23.1	41.2	+/?	–	?
CD27	LT27	Serotec	4.3	6.6	38.3	22.9	+/?	+/?	?
CD28	YTH913.12	Serotec	1.4	9.2	8.0	8.9	–	–	–
CD29	3S3	Serotec	10.7	9.9	13.2	36.0	–	+	P
CD29	K20	Dako	33.2	16.1	21.5	72.9	+	–/?	+
CD32	AT10	Serotec	9.5	7.3	11.2	33.5	+/?	–	A
CD32	2 E1	Coulter	3.5	5.7	42.5	49.2	+	–	P
CD44	BAG40A	VMRD	92.2	91.4	57.9	98.7	+	+	+
CD44	LT41A	VMRD	83.4	96.3	40.9	95.5	+	+	+
CD45	DH16A	VMRD	55.8	30.0	19.8	19.9	+	+	+
CD47	HUH69A	VMRD	99.8	98.8	99.8	99.9	+	+	+
CD47	HUH71A	VMRD	99.9	99.9	100.0	99.9	+	+	+
CD49d	HP2/1	Coulter	59.9	58.8	76.9	95.9	+	–/?	+
CD49d	P4G9	Dako	38.3	31.8	37.6	61.3	+	–/?	+
CD49d	9F10	BD Pharmingen	44.4	40.5	58.3	89.2	+	+	+
CD51/61	23C6	Serotec	1.1	2.3	4.0	3.5	–	–	?
CD59	MUC93A	VMRD	1.0	2.5	4.6	3.3	–	–	P
CD61	PM6/13	Serotec	2.1	3.5	6.3	5.5	–	–/+	?
CD61	Y2/51	Dako	2.2	4.9	5.8	4.1	–	–	?
CD62L	FMC46	Dako	52.8	90.0	48.2	91.6	+	–	P
CD 62P	AK-6	Dako	1.6	3.7	7.1	5.6	–	–	?
CD69	TP1.55.3	Coulter	2.3	4.6	10.6	4.0	?	–	–
CD88	S5/1	Serotec	29.9	15.5	65.1	80.5	+	+	A
CD88	W17/1	Serotec	47.0	74.5	22.9	76.0	+	NT	A
CD119	BB1E2	Serotec	23.1	6.4	82.8	78.5	+	–/?	+
CD120a	H398	Serotec	1.3	2.5	4.6	3.9	–	–	–
CD163	Ber-MAC3	Dako	1.5	3.7	6.7	78.2	+	+/?	+
CD165	AD2-13H12	Serotec	3.6	4.9	38.7	16.6	?	–	A
CD166	3A6	Serotec	4.4	3.5	6.7	25.8	?	–/?	?
CD172a	DH59B	VMRD	2.6	4.8	30.4	97.6	+	+	+
CD200	MRC-OX-104	Serotec	3.7	3.5	12.2	2.8	–	+	A/P
CD236R	Ret40f	Dako	1.3	3.4	6.0	3.2	–	–	–
CD247	G3	Serotec	1.8	2.5	5.5	1.9	–	–	–
G1 control	Dako	Dako	1.2	4.2	5.7	2.4			

Table 2 (*Continued*)

CD	Clone	Supplier	Percentage of indicated sub-population stained by mAb				Site assessment		Overall assessment
			%CD2	%WC1	%CD21	%CD14	IAH[a]	UMASS[b]	
IgM control	Dako	Dako	1.9	4.0	6.2	3.3			
G2a control	Dako	Dako	1.6	3.5	5.6	2.0			
G2b control	Dako	Dako	1.9	3.4	6.1	2.9	Background controls		

(+) Stained cattle cells; (−) no reaction; (A) atypical staining pattern compared to humans; (P) may recognise a polymorphic determinant; (?) insufficient evidence to make a conclusion.

[a] Data generated at the Institute for Animal Health.

[b] University of Massachusetts.

the human CD antigen. This included mAbs to CD9 (although strong staining of PMN is atypical), CD11a, CD14, CD18, CD21, CD22, CD23, CD29, CD44, CD45R, CD47, CD49d, CD119, CD163 and CD172a; examples of staining patterns are shown in Fig. 1. Within this group were mAbs Tük4 and M5E2 (CD14), BL13 (CD21), 9P25 (CD23), K20 (CD29) and 9F10 (CD49d)—the cross-reactivity of these has been previously reported (Tük4 and M5E2 (CD14), BL13 (CD21), K20 (CD29), 9F10 (CD49d): Sopp and Howard, 1997; 9P25 (CD23): Sopp et al., 2001).

With some mAbs the staining patterns of bovine cells were inconsistent with those reported in humans (marked 'A' in Table 2) and it is not possible to conclude that they recognise bovine homologues. However the staining patterns suggested that genuine reactions and not non-specific binding were seen and thus these mAb should be re-examined, possibly on a broader range of targets. CD10 mAbs generally stained a small proportion of PBM but failed to significantly stain PMN (Fig. 2) in contrast to reports in humans where PMN are recognised. CD32 mAbs also failed to stain PMN (Fig. 2) this is different to reports in humans and also dissimilar to results with mAbs raised against bovine CD32 (Zhang et al., 1994). CD88 mAbs (W17/1 and S5/1) strongly stained a sub-population of PBM (Fig. 2) but once again did not stain PMN, this observation was also reported by Sopp and Howard (1997). S5/1 recognised a protein by Western blot of the expected size (approximately 30–45 kDa) adding to the evidence that the cattle homologue of CD88 was identified (Table 3). mAb AD2-13H12 which recognises human CD165 and is reported to react with thymocytes and thymic epithelial cells weakly stained most bovine PB (Fig. 2). In summary, most of the mAbs that recognised bovine cells with an atypical staining pattern compared to humans did so because of their non-reactivity with PMN, this indicated that either bovine PMN are significantly different in their surface marker expression compared to human PMN or alternatively the bovine cells are altered for surface marker expression during or as a result of the preparation method.

An unexpected result of this study was the apparent abundance of mAbs that may recognise polymorphic epitopes of antigens—this was characterised by staining of bovine cells in one or more animals at one laboratory but not the second laboratory. Polymorphisms in cattle have been reported for CD4 and CD5 (Morrison et al., 1991) but are relatively rare. It is unlikely that technical errors occurred but it will be necessary to repeat the results on a diverse range of cattle types before solid conclusions can be made. The staining pattern for each of the mAbs; B17.20.9 (CD1a), ALB1 (CD10), BL13 (CD21), 2E1 (CD32), MUC93A (CD59), FMC46 (CD62L) and MRC-OX-104 (CD200) marked 'P' in Table 2 were distinctive and with the exception of CD10, CD32 (see above) and CD200 were consistent with the mAb recognising the bovine homologue of the human antigen.

Table 3
Results of Western blot assays of human and cattle cell lysates

CD	Clone	MW (kDa) of band	
		Human	Cattle
CD9	RH1A	30–45	30–45
CD9	LT86A	?	–
CD10	SN5c	–	–
CD11a	HUH73A	97–220	–
CD22	Mc64-12	97–220	–
CD23	9P.25	45–50	–
CD27	LT27	–	–
CD44	BAG40A	66–97	–
CD44	LT41A	66–97	–
CD49d	HP2/1	97–220	–
CD69	TP1.55.3	20–45	–
CD88	S5/1	30–45	30–45
CD88	W17/1	30–45	–
CD119	BB1E2	50 (?)	–
CD163	Ber-MAC3	45–66 (?)	–
CD247	G3	–	–

(?) Uncertain results/faint bands, – = no band detected.

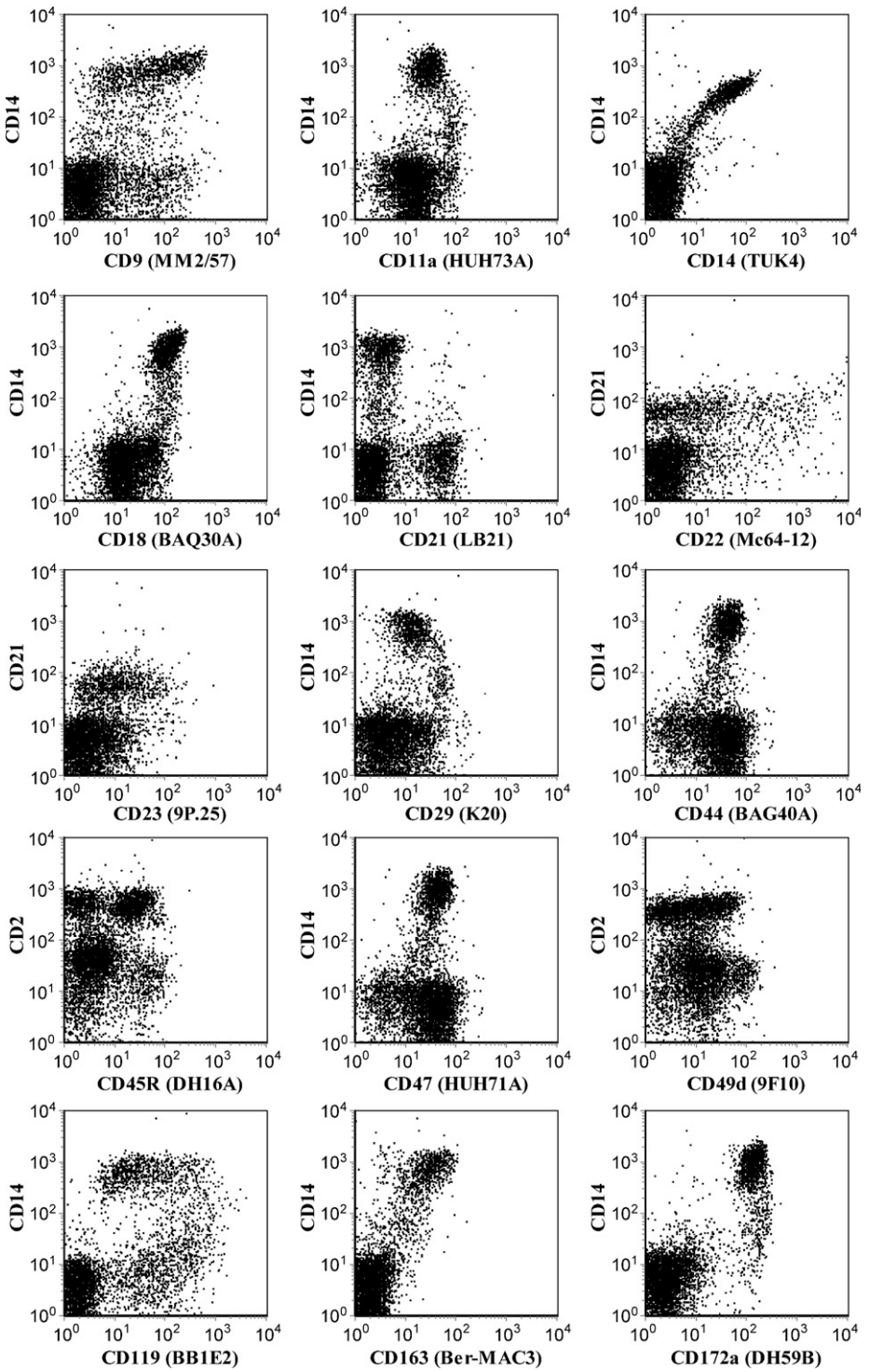

Fig. 1. Two colour immunofluorescence staining of bovine PBMC. mAbs to human CD antigens (indicated on *x*-axis with clone names in parentheses) and mAbs to characterised bovine antigens (indicated on *y*-axis). mAbs to bovine antigens were biotinylated and detected using streptavidin–phycoerythrin; mAbs to human antigens were stained with goat anti-mouse FITC. Fluorescence was assayed by FCM.

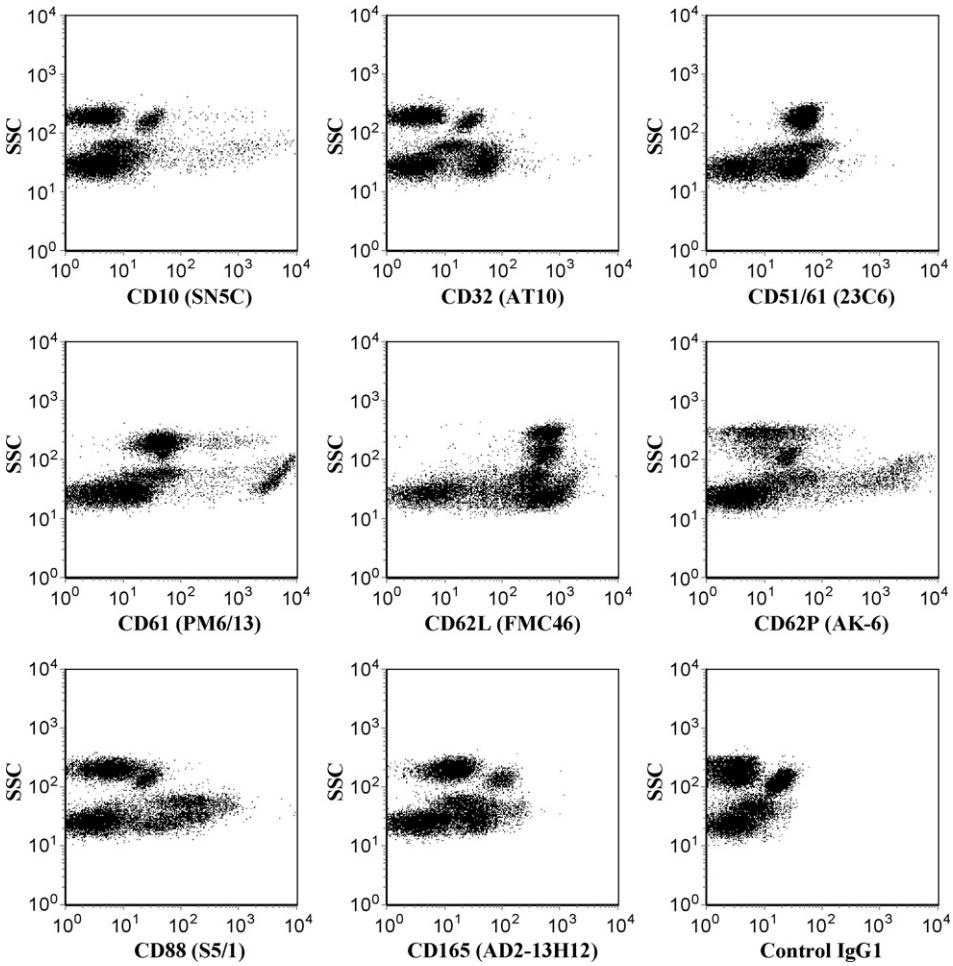

Fig. 2. Dual parameter correlated FCM data of bovine WBC stained with mAbs to human CD antigens (*x*-axis, clone name in parentheses) against 90° light scatter (SSC) *y*-axis. Mononuclear cells are resolved from PMN in terms of lower levels of SSC ($<10^2$ units). mAbs were stained with goat anti-mouse FITC.

A total of seven mAbs: B1.49.9 (CD25), LT27 (CD27), 23C6 (CD51/61, Fig. 2), PM6/13 (CD61, Fig. 2), Y2/51 (CD61), AK-6 (CD62P, Fig. 2) and 3A6 (CD166) gave staining patterns in one or more assays to suggest that the bovine homologue may have been recognised. It is highly likely that the mAbs recognising platelet associated antigens (CD51, CD61 and CD62P) were genuinely reactive—in the first round they stained WBC where platelets would have been abundant and associated with monocytes but in the second round staining was not evident on PBM where very few platelets would have been present. mAbs recognising CD25, CD27 and CD166, all activation associated antigens, also gave conflicting results in some assays. This may have reflected the different number of activated cells in each sample, however it will be necessary to acquire more data before conclusions are possible on these mAbs.

3.4. Western blot analyses

Most of the mAbs selected for assay by Western blot (WB, Table 3) were chosen because they recognised potentially new molecules in cattle or where mAbs raised against cattle molecules are not available. It is recognised that a negative reaction by WB does not necessarily mean that a mAb does not react because denaturation of proteins can prevent recognition of antigens. In contrast, a positive reaction is good evidence of a genuine reaction. Ten of the 16 selected mAb recognised human cell lysates and in most cases the bands identified were in accordance with published MW data. Two of these mAb (RH1A (CD9) and S5/1 (CD88)) also reacted with cattle cell lysates (Fig. 3). In both cases the MW of the identified bands were similar sizes to the bands in the human cell lysates. This taken together with the FCM data presents very strong

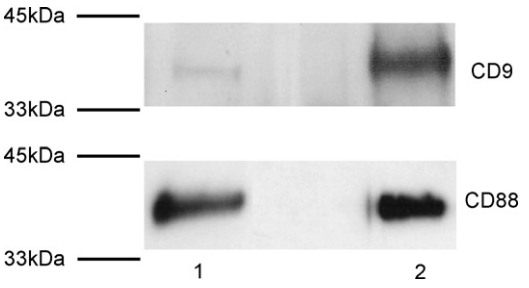

Fig. 3. Western blot assay of bovine (lane 1) and human (lane 2) PBMC lysate. mAb RH1A (CD9) and S5/1 (CD88) produced visible bands of similar size in both the human and bovine samples. The bands were approximately 30–45 kDa calculated from molecular weight standards (not shown).

evidence that the bovine homologues of CD9 and CD88 are identified by these mAbs.

3.5. Summary

There are a wide range of well-characterised mAbs available for, and raised against, bovine cell surface markers but a large number are not available commercially due to the relatively small market for these products. One of the principle aims of the study was to identify reagents for cattle that are immediately available to all researchers through commercial outlets—this was successfully achieved and the mAbs are listed in Table 1. Another primary aim was to identify new reagents for studies of bovine immunology. This yielded four mAb that reacted with previously unrecognised bovine CD antigens:

- *CD22*. mAb Mc64-12 recognises human CD22 which is present in early stages of B cell development and is found on most mature B cells but not plasma cells. CD22 is a member of the IgSF domain-containing sialic acid binging proteins called the sialoadhesin family. In cattle, all of the cells that expressed CD21 (primarily B cells) were also stained by the mAb (Fig. 1). CD2+, CD14+ and WC1 cells + were not stained. This represents good evidence that the mAb recognises the bovine homologue of CD22.
- *CD88*. mAbs S5/1 and W17/1 recognise human CD88 which is expressed on most cells of the myeloid lineage and other cells in the lung, liver and smooth muscles. CD88 is also known as C5a receptor and has seven transmembrane domains. With bovine cells the two mAbs directed against human CD88 stained CD14+ monocytes, CD21+ B cells at a low level and a small sub-population of CD2+ T cells but failed to stain PMN. This is atypical of the cellular distribution in human PB, however, the staining patterns with both mAbs in cattle were distinctive and unlike non-specific binding. In addition, mAb S5/1 recognised a protein band in both human and cattle cell lysates that was similar to other data reported for humans (Fig. 3). Together this evidence suggests that the mAbs recognise the bovine homologue of CD88.
- *CD119*. mAb BB1E2 stains human CD119—the IFNγ receptor, expression is restricted mainly to monocytes, B cells and epithelial cells. In cattle only monocytes and the majority of B cells were stained by the mAb. This is good evidence that BB1E2 also stains bovine CD119.
- *CD163*. CD163, also referred to as M130, a member of the scavenger receptor cysteine-rich family (SRCR) is exclusively expressed on cells of the monocyte lineage. The human CD163 mAb Ber-Mac3 stained only monocytes in cattle PBMC and this is consistent with identifying the bovine homologue.

The data presented here ranges from very good evidence of genuine cross-reactive mAbs, where staining is strong with bovine cells and fully consistent with that using human cells, to data where there are several discrepancies in reactivity patterns. We stress the need for investigations of all the mAbs to prove without doubt that they recognise the bovine homologues. This may include molecular and functional analyses. The reagents identified will be valuable tools for the field of bovine immunology.

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