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IDENTIFICATION OF MONOCLONAL ANTIBODY REAGENTS FOR USE IN THE STUDY OF THE IMMUNE RESPONSE TO INFECTIOUS AGENTS IN CAMEL AND WATER BUFFALO

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ABSTRACT

Progress in elucidating the mechanisms regulating the immune response to infectious agents and derived vaccines in domestic species, especially in camels and water buffalo, has been impeded by the lack of monoclonal antibody (mAb) reagents needed to study the immune response in the species of interest. As a first step to address this problem, we conducted a study to determine how many existing mAbs developed against leukocyte differentiation molecules (LDM) in various species recognise conserved epitopes on orthologous (identical) molecules in two or more species of Artiodactyla. Analysis of 490 monoclonal antibodies raised against LDM in cattle, goat, sheep, llama, pig, dog, and human revealed that many epitopes have been conserved on orthologous molecules in the course of evolution in closely related species in the suborder Ruminantia such as in cattle, bison, and water buffalo, and fewer on more distantly related species such as goat and sheep. Only a few of the epitopes conserved in Ruminantia were conserved in the suborders Suiformes (pigs) and Tylopoda (llamas and camels). The highest level of conservation in all suborders was found with major histocompatibility complex (MHC) class I (MHC I) and class II (MHC II) molecules. These findings show the potential as well as the limitations of screening existing mAbs for research in less use studied species. Importantly, the findings also provide further insight into the composition of the immune system in Artiodactyla and factors to be considered when studying the immune response to infectious agents and vaccines in the different suborders of Artiodactyla.

Key words: Buffalo, camel, immune response, infectious agents, monoclonal antibody

Although considerable progress has been made in characterisation of the immune system in economically important species such as cattle, goat, sheep, pig, and horse, limited progress has been made in camelid and water buffalo, species of economic importance to countries in South America, Africa, the mid east, parts of Europe, Asia, and certain Island nations. This is in part associated with the limited number of investigators trained in the area of immunology, limited funding for research, and most critically, the lack of monoclonal antibody (mAb) reagents needed to study the immune response to infectious agents and parasites in these species. One approach that has been considered to address this problem has been to screen existing sets of mAbs developed against (LDM) for mAbs that recognise conserved epitopes on orthologous (identical) molecules. The rationale and support for this approach has been that previous investigations (Davis *et al,* 1987; 2001) and surveys conducted as part of workshops on LDM in ruminants and pigs (Davis and Ellis, 1991; Haverson *et al*, 2001) have shown some of the mAbs do recognise conserved epitopes. More recently, a comparative study has shown that mAbs to some cytokines also recognise conserved epitopes (Pedersen *et al,* 2002). These observations have suggested that it would be useful to extend the comparative studies to identify additional mAbs that recognise conserved epitopes on LDM in less studied species. Such an endeavor could reduce the need to develop reagents for some important molecules and allow investigators to focus

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on developing mAbs to fill gaps in reagent sets for use in a species of interest.

In the present study we screened sets of mAbs that were generated against LDM in cattle, goats, sheep, pigs, llamas, humans, and dogs to determine how many existing mAbs recognise conserved epitopes on orthologous molecules in these and other less studied species such as bison, water buffalo and camel.

Methods and Materials

Animals : Cattle *(Bos taurus)*, goat *(Capra hircus)*, sheep *(Ovis aries)*, pig *(Sus scrofa)*, rabbit *(Oryctalogus cuniculus)*, llama *(Lama glama)*, and alpaca *(L. pacos)* in use for teaching and/or research were used as a source of blood for the present study. Camels *(Camelus dromedarius and C. bactrianus)*, from which blood was obtained were maintained by private owners in Washington and Missouri. Bison *(Bison bison)* blood was obtained from experimental animals maintained at the National Animal Disease Centre, Ames, Iowa. The animals at Washington State University and National Animal Disease Centre were housed and maintained according to the Institutional Animal Care and Use Committee guidelines and the Association for Assessment of Laboratory Animal Care.

Monoclonal antibodies : The monoclonal antibodies used in the present study are listed in table 1. Many of the mAbs have been described in workshops held over the past few years. Others are currently under investigation. Links to summaries of workshops conducted in ruminants, pigs, and horses can be found at http://www.vetmed.wsu.edu/tkp. The web program contains a database on leukocyte differentiation molecules characterised in humans and other species where orthologous molecules have been identified. The web program also contains a database of mAbs reactive with MHC and LDM in non-human species that are available through investigators or commercial sources.

Cell preparation : Blood was collected in acid citrate dextrose (ACD) to a final concentration of 15-20% ACD. The blood was distributed into sterile 50 ml polypropylene centrifuge tubes and centrifuged at 1500 RPM for 30 min. at room temperature to sediment the cells. Following centrifugation, plasma was removed. In the case of bovine, bison, water buffalo, goat, sheep, and pig, blood was resuspended in Tris-buffered ammonium chloride (NH_4Cl, 0.87 % w/v, pH 7.4) to lyse erythrocytes. As soon as the erythrocytes were lysed, the blood was centrifuged at 1500 RPM for 8 min. to pellet the leukocytes. The cell pellets were resuspended in phosphate buffered saline (PBS) containing 20% ACD (PBS-ACD) and then subjected to 2 cycles of centrifugation and washing in PBS-ACD to remove platelets. The cells were then resuspended at 2×10^7 cells/ml in PBS-ACD and kept at 4°C until used in flow cytometry (FC).

For llama and camel, erythrocytes were removed by density gradient centrifugation. Blood was diluted to 50% ACD and then placed in glass centrifuge tubes (50 ml). Blood was underlaid with 10 ml Histopaque (specific gravity 1.119) and centrifuged at 2000 RPM for 25 min., at room temperature. Leukocytes and platelets were collected from the interface and washed in PBS-ACD by two cycles of centrifugation and re-suspension in PBS-ACD. After the final wash, the cell pellets were resuspended in 1 ml of distilled H_2O for 4-8 sec. to lyse erythrocytes and then resuspended in 50 ml of PBS-ACD. The cells were then sedimented and resuspended to 2×10^7 cells/ml and kept at 4°C until used.

For flow cytometry, cells were distributed in 96-well V-bottom microtitre plates (50 µl/well) containing 50 µl of mAb (0.7 µg) and incubated for 15 min. at 4°C. Cells were subjected to 3 cycles of centrifugation and re-suspension in PBS-ACD-containing 0.5% horse serum. After the final wash, the cells were resuspended in 100 µl of second step reagent (fluorescein conjugated goat anti-mouse IgG and IgM) and then incubated in the dark for 15 min. at 4°C. The cells were then washed twice in PBS-ACD and fixed in 2% PBS buffered formaldehyde.

Cell culture : To analyse the patterns of reactivity of molecules only expressed on activated cells, mononuclear cells from all species were isolated by density gradient centrifugation using Accupaque separation medium (density 0.086). Following washing in PBS-ACD, cells were placed in RPMI-1640 tissue culture medium containing 13% calf serum, 2-mercatoepthanol, and antibiotics and a polyclonal activator, concanavalin A (5 µg/ml) and incubated for 24 hr in a CO_2 incubator at 37°C. Cells were harvested and prepared as described above and labeled with the mAbs and second step reagent.

Flow cytometery

A Becton Dickinson FACScan equipped with Cell Quest software (Becton Dickinson Immunocytometry Systems, San Jose CA) was used to collect the data. FCS Express software (De Novo Software, Thornton, Ontario) was used to analyse the data. At the time of data collection, electronic gates were placed on granulocytes, monocytes and lymphocytes (as displayed in dot plot profile, side light scatter vs

Table 1. Monoclonal antibodies cross-reactive with Artiodactyla leukocyte differentiation molecules.

mAb	Ig Isotype	Specificity	Species reactivity								
			Bo	Bi	WB	Cp	Ov	Pg	Lm	Cm1	Cm2
1	2	3	4	5	6	7	8	9	10	11	12
B5C	G2b	MHC Cl I^b	+	+	NT	+	+	-	+	+	+
PT85A	G2a	MHC Cl I^b	+	+	NT	+	+	+	+	+	+
H1A	G2a	MHC Cl I^b	+	+	NT	+	+	+	+	+	+
H11A	G2a	MHC Cl I^b	+	+	NT	+	+	+	+	+	+
H58A	G2a	MHC Cl I^b	+	+	+	+	+	+	+	+	+
H34A	G2b	MHC Cl II^b	+	+	NT	+	+	P	-	-	+
H42A	G2a	MHC Cl II^b	+	+	+	+	+	+	+	+	+
TH12A	G2a	MHC Cl II^b	+	+	NT	P	-	-	-	-	-
TH14B	G2a	MHC Cl II^b	+	+	+	+	+	+	+	+	+
TH21A	G2b	MHC Cl II^b	+	+	NT	+	+	+	+	-	-
TH22A5	G2a	MHC Cl II^b	+	+	NT	+	+	+	+	+	+
TH81A5	G2a	MHC Cl II^b	+	+	+	+	+	+	+	+	+
PG173A	M	MHC Cl II^{*p}	+	NT	NT	+	+	+	NT	+	NT
TPF232A	G1	MHC Cl II^{*b}	+	+	NT	+	+	+	+	-	-
BAQ150A	G3	MHC Cl II^b	+	+	NT	+	-	+	-	-	I
7B10§	M	MHC Cl II	Wk	NT	NT	+	NT	+	NT	+	NT
TH97A	G2a	$CD1^b$	+	NT	NT	+	+	-	-	-	-
BAQ95A	G1	$CD2^b$	+	+	+	+	-	-	-	-	-
BAT18A	G1	$CD2^b$	+	+	NT	+	-	-	-	-	-
BAT42A	G1	$CD2^g$	+	+	NT	+	-	-	-	-	-
BAT76A	G2a	$CD2^g$	+	+	NT	+	-	-	-	-	-
CH61A	G1	$CD2^b$	+	+	NT	-	-	-	-	-	-
CH128A	G1	$CD2^b$	+	+	+	-	-	-	-	-	-
CH132A	M	$CD2^b$	+	-	NT	-	-	-	-	-	-
CH134A	G1	$CD2^b$	+	+	NT	-	-	-	-	-	-
MUC2A	G2a	$CD2^s$	+	+	NT	+	+	-	+	-	-
CACT31A	M	$CD2^b$	+	+	+	+	-	-	-	-	-
PGBL6A§	G2a	$CD2^p$	-	-	NT	-	-	+	+	+	-
MM1A	G1	$CD3^b$	+	+	+	-	-	-	-	-	-
CACT138A	G1	$CD4^b$	+	+	+	-	-	-	-	-	-
GC1A	G2a	$CD4^g$	-	-	NT	+	+	-	-	-	-
GC17A	M	$CD4^g$	+	-	NT	+	+	-	-	-	-
GC50A1	M	$CD4^g$	+	+	+	+	+	-	+	+	+
ILA11A	G2a	$CD4^b$	+	+	+	-	-	+	-	-	-
CC17A	G1	$CD5^b$	+	-	+	+	+	-	-	-	-
ST1	G2a	$CD5^s$	-	-	NT	-	+	-	-	-	-
LT3A	G1	$CD5^{*l}$	-	-	NT	-	-	-	+	-	-
BAQ82A	M	$CD6^b$	+	-	NT	-	-	-	-	-	-
BAQ83A	G2b	$CD6^b$	+	+	NT	-	-	-	-	-	-
BAQ91A	G1	$CD6^b$	+	+	+	+	P	-	-	-	-
CACT141A	G2b	$CD6^b$	+	+	+	-	-	+	-	-	-
BAQ111A	M	$CD8a^b$	+	+	NT	P	-	-	-	-	-
BAT82A	G1	$CD8b^g$	+	+	NT	+	+	-	-	-	-
CACT80C	G1	$CD8a^b$	+	+	+	+	+	-	-	-	-
CACT88A	G3	$CD8a^b$	+	+	+	-	+	-	-	-	-

1	2	3	4	5	6	7	8	9	10	11	12
TH82A	G1	CD8β[*b]	+	+	+	+	+	-	-	-	-
ST8	M	CD8a[s]	+	+	+	+	+	-	-	-	-
7C2B	G2a	CD8[s]	+	+	+	+	+	-	-	-	-
17D	G1	CD4[s]	-	-	NT	+	+	-	-	-	-
B18A	G3	CD9[b]	+	-	+	-	-	-	-	-	-
BAQ86A	G1	CD9[b]	+	+	NT	-	-	-	-	-	-
LT86A	G2a	CD9[l]	+	+	NT	-	-	+	+	+	-
RH1A	G3	CD9[b]	+	-	+	-	-	-	+	-	+
BAQ11A	G1	CD11a[b]	+	+	NT	+	+	-	+	-	-
HUH73A	IgG1	CD11a[h]	+	+	NT	+	+	-	+	+	+
LND73A	G1	CD11a[b]	+	+	NT	-	-	-	+	+	-
LT48A	G2a	CD11a[*l]	-	-	NT	+	-	-	+	+	+
MUC76A	G2a	CD11a[s]	P	+	NT	+	+	+	-	-	-
LT35	G1	CD11a[*l]	-	-	NT	-	-	-	+	+	+
BAQ147A	M	CD11b[b]	+	-	NT	-	+	-	-	-	-
CAM13A	G1	CD11b[g]	+	+	NT	+	+	-	-	-	-
MM10A	G2b	CD11b[b]	+	+	NT	-	-	-	-	-	-
MM12A	G1	CD11b[b]	+	+	+	+	+	-	+	-	-
MM13A	G1	CD11b[b]	+	+	+	-	-	-	-	-	-
LND51A	G2b	CD11b[b]	+	+	NT	-	-	-	-	-	-
LND77A	G1	CD11b[b]	+	+	NT	-	-	-	-	-	-
LND88A	G1	CD11b[b]	+	+	NT	+	+	-	-	-	-
LT93A	G2a	CD11c[*l]	-	-	NT	-	-	-	+	+	-
BAQ153A	M	CD11c[b]	+	+	+	+	+	-	-	-	-
CACTB11A	G1	Similar but not CD11a[b]	+	+	NT	+	+	-	-	-	-
MM11A	G1	Similar but not CD11b[b]	+	-	NT	+	+	-	-	-	-
CAM36A	G1	CD14[g]	+	+	NT	+	+	+	+	+	+
CAM66A	M	CD14[g]	+	+	NT	+	+	-	-	-	+
MM61A	G1	CD14[b]	+	+	NT	+	+	-	-	-	-
biG10§	G1	CD14	Wk	NT	NT	+	NT	+	NT	+	NT
biG10§	G1	CD14	Wk	NT	NT	+	NT	+	NT	+	NT
BAQ30A	G1	CD18[b]	+	+	+	+	+	+	+	+	+
BAT75A	G1	CD18[g]	+	+	+	+	-	-	-	-	-
HUH82A	IgG2a	CD18[h]	+	+	NT	-	+	+	+	+	+
PNK-1§	G1	CD18[p]	NT	NT	NT	-	NT	+	NT	+	-
BAQ15A	M	CD21[b]	+	-	NT	+	+	-	-	-	-
GB25A	G1	CD21[g]	+	+	NT	+	+	-	-	-	-
LCT21A	G1	CD21[b]	+	+	NT	+	-	-	-	-	-
CACT116A	G1	CD25[b]	+	+	+	+	+	-	-	-	-
CACT260A	M	CD25[b]	+	+	NT	-	-	-	-	-	-
LCTB2A	G3	CD25[b]	+	+	+	+	+	-	-	-	-
GB112A	G1	CD25[g]	+	+	+	+	+	-	-	-	-
CACT114A	G2b	CD26[b]	+	+	I	-	-	-	-	-	-
FW4-101	G1	CD29[s]	+	+	NT	+	+	+	+	+	+
CAPP2A	G1	CD41[b]	+	+	+	+	+	-	-	-	-
GB84A	G1	CD42d[*b]	+	+	+	+	+	-	-	-	-
BAT31A	G1	CD44[s]	+	+	+	+	+	-	-	-	-
BAG40A	G3	CD44[g]	+	+	+	+	+	+	-	-	-

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CACTB45A	G1	CD44[*b]	+	+	NT	-	+	-	-	-	-
CACTB48A	G1	CD44[*b]	+	+	NT	+	+	+	-	-	-
CACTB40A	G1	CD44[b]	+	+	NT	+	+	+	+	-	+
GB34A	M	CD44[g]	+	+	NT	+	+	+	-	-	-
GB50A	G1	CD44[g]	+	+	NT	+	+	+	-	-	-
LT36A	G2b	CD44[l]	+	+	NT	-	+	+	+	+	+
LT41A	G2a	CD44[l]	+	+	NT	+	+	+	+	+	+
IL-A118[§]	G1	CD44[b]	+	NT	NT	-	NT	NT	NT	+	NT
IL-A148[§]	G3	CD44[b]	+	NT	NT	-	NT	NT	NT	+	NT
CACTB51A	G2a	CD45[b]	+	-	+	-	-	-	-	-	-
LT12A	G2a	CD45[*l]	-	-	NT	-	-	-	+	+	+
LT13A	G2a	CD45[*l]	-	-	NT	-	-	-	+	+	+
BAG36A	G1	CD45R[g]	+	+	NT	+	+	-	-	-	-
GC6A	M	CD45R[g]	+	+	NT	+	+	-	-	-	I
GS5A	G1	CD45R[g]	+	+	NT	+	+	-	-	-	I
LCT2A	G2a	CD45R[b]	+	+	+	-	-	-	-	-	
LCT27A	G1	CD45R[b]	+	+	+	-	-	-	+	-	I
DH16A	M	CD45RB[d]	+	-	NT	+	+	-	-	-	-
GC42A	G1	CD45R0[g]	+	+	NT	I	I	-	-	-	-
GC44A	G3	CD45R0[g]	+	+	NT	I	I	-	-	-	-
GC62A	M	CD47[g]	+	+	NT	+	-	-	+	-	-
TH17A	M	CD47[b]	+	+	+	+	+	+	+	-	-
218	G2b	CD49d[s]	+	+	NT	+	+	+	+	-	+
BAQ92A	G1	CD62L[b]	+	-	+	-	+	-	-	-	-
DU1-29	G1	CD62L[s]	+	-	NT	+	+	-	-	-	-
DH59B	G1	CD172a[d]	+	+	+	P	+	+	+	+	+
GB21A	G2b	gd TCR-N24 d chain[g]	+	+	+	+	-	-	-	-	-
CACT148A	M	gd TCR-N21 d chain[b]	+	-	+	-	-	-	-	-	-
CACTB14A	G1	gd TCR-N6 CL[b]	+	+	+	+	+	-	-	-	-
CACTB6A	M	gd TCR-N6 g chain?[b]	+	+	+	+	+	-	-	-	-
CACT19C	M	gd TCR N6 CL[b]	+	-	NT	+	-	-	-	-	-
CACTB81A	G1	gd TCR-N7 g chain?[b]	+	-	+	-	-	-	-	-	-
CACT22B	M	gd TCR-N7 CL[b]	+	+	NT	+	+	-	-	-	-
CACTB44A	G1	gd TCR-N7 CL[b]	+	+	+	-	-	-	-	-	-
86D1	G1	gd TCR-N7 CL[b]	+	+	+	+	+	+	-	-	-
CACT75A	M	gd T cell sub[b]	+	-	+	+	-	-	-	-	-
GC52A	G1	gd T cell sub[g]	+	+	NT	+	+	-	-	-	-
TPB16A	G1	gd T cell sub[b]	+	-	NT	-	+	+	-	-	-
TPB30A	G1	gd T cell sub[b]	+	+	NT	-	-	-	-	-	-
TPN4A	G1	gd T cell sub[b]	+	+	NT	NT	+	-	+	-	+
ILA29A§	G1	WC1[b]	+	+	NT	+	+	-	-	P	-
TPN19A	G1	WC1[b]	+	+	NT	+	+	-	+	+	+
CACTB28A	G1	WC1[b]	+	+	NT	+	+	-	+	+	+
BAQ128A	G1	WC1[b]	+	+	NT	+	+	-	+	+	+
B7A1	M	WC1-N1[b]	+	+	+	+	+	-	-	-	-
CGB24A	G1	WC1-N1 CL[b]	+	+	NT	+	+	-	-	-	-
BAQ4A	G1	WC1-N2[b]	+	+	+	+	+	-	-	+	I
CACTB32A	G1	WC1-N3[b]	+	+	+	P	P	-	-	-	-

1	2	3	4	5	6	7	8	9	10	11	12
CACTB1A	G1	WC1-N3CL[b]	+	+	+	+	+	-	-	-	-
CACTB15A	G1	WC1-N3 CL[b]	+	+	+	+	+	-	-	-	-
CACTB18A	G1	WC1-N3 CL[b]	+	+	NT	-	+	-	-	-	-
BAQ99A	G1	WC1-N3 CL[b]	+	+	NT	+	+	-	-	-	-
BAQ89A	G1	WC1-N4[b]	+	+	+	+	+	-	-	-	-
BAQ159A	G1	WC1-N4 CL[b]	+	+	+	+	+	-	-	-	-
BAQ90A	G3	WC1-N11[b]	+	-	+	+	+	-	-	-	-
BAQ113A	G1	WC1-N11 CL[b]	+	+	NT	+	+	-	-	+/-	+
CACTB31A	G2b	WC1-N22[b]	+	+	+	+	+	-	-	-	-
GB54A	G2a	WC1-N25[b]	+	+	+	+	+	-	-	-	I
GB45A	G1	WC1-N26[b]	+	+	NT	+	+	+	+	-	-
BAG25A	G1	WC1 subg	+	+	+	+	+	-	-	-	-
LCTB19A	G1	Pan L = LCTB39A[b]	+	+	+	-	+	+	-	-	-
C11[§]	G1	T	NT	NT	NT	-	NT	+	NT	+	NT
TPF203A	M	T[b]	+	+	NT	+	-	-	-	-	-
PG107A[§]	M	T sub[p]	+	NT	NT	+	NT	+	NT	+	NT
B1B	M	T + M[b]	+	?	NT	-	-	-	-	-	-
CVR18A	G1	T + M[g]	+	+	NT	+	+	-	-	-	-
BAQ155A	G1	IgM associated[b]	+	+	+	+	+	-	-	-	-
BAQ129A	M	IgM[b]	+	-	+	-	+	-	-	-	-
BIG73A	G1	IgM[b]	+	+	NT	-	-	-	-	-	-
PIG45A2	G2b	IgM[p]	+	+	+	+	+	+	-	-	-
BIG715A	G1	G1[b]	+	+	NT	+	+	-	+	-	-
BIG25A	G1	IgM[b]	+	+	NT	-	-	+	-	-	-
28BO27A	M	IgG[b]	+	+	NT	-	-	-	-	-	-
BIG501E	G1	l light chain[b]	+	?	+	+	+	-	-	-	-
BAS9A	M	B-B1[s]	+	+	+	+	+	-	-	-	-
BAQ44A	M	B-B2[b]	+	+	+	+	+	-	-	I	-
CH127A	M	B-B5[b]	+	+	NT	-	-	-	-	-	-
GC65A	M	B-B6[b]	+	-	NT	+	+	-	-	-	-
LCTB16A	G1	B-B14[b]	+	+	+	+	-	+	-	-	-
GB26A	M	B + T sub[g]	+	+	NT	+	+	-	+	+	+
GC34A	M	B + M[g]	+	+	NT	+	+	-	+	-	-
GB53A	G1	B + Gr + M[b]	+	+	NT	+	+	-	-	-	-
BAQ151A	G1	M[b]	+	+	NT	-	+	-	-	-	-
LND37A	G1	M[b]	+	+	NT	+	+	-	+	+	+
LND68A	G1	M[b]	+	+	NT	+	+	-	+	+	+
MM29A	M	M[b]	+	+	NT	-	-	-	-	-	-
GC81A	M	Gr + L sub[g]	+	+	NT	+	-	-	-	-	-
RCV112A	G3	Gr + L sub = GC81A[g]	+	+	NT	+	+	-	+	-	
PG68A	G1	Gr[p]	+	-	NT	+	+	+	+	-	-
GS23A	G3	Gr + endo[g]	+	+	NT	-	-	-	-	+	+
CH138A	M	Gr + endo[b]	+	+	+	-	-	-	-	-	-
MM20A	G1	Gr + endo = CH138A[b]	+	+	NT	-	-	-	-	-	-
RCV59A	M	Gr + endo[g]	+	+	NT	+	-	-	-	-	-
PT25A[§]	G3	Pan leukocyte[p]	+	NT	NT	+	NT	+	NT	+	NT
BAGB27A	G1	Pan leukocyte + endo[b]	+	+	NT	+	-	-	-	-	-
CACTB22A	G1	Pan leukocyte + endo[b]	+	+	+	-	+	-	-	-	-

1	2	3	4	5	6	7	8	9	10	11	12
RCV98A	G2a	Pan leukocyte +endo[g]	+	+	NT	+	+	+	-	-	-
RCV106A	M	Pan leukocyte + endo[g]	+	+	NT	+	+	+	+	+	+
GB20A	G1	Platelets[g]	+	I	+	+	+	-	-	-	-
CACT7A	M	ACT1[b]	+	-	+	+	+	-	-	-	-
CACT101A	M	ACT1 CL[b]	+	+	+	+	+	-	-	-	-
CACT26A	G1	ACT2[b]	+	+	+	+	+	-	-	-	-
CACT63A	G1	ACT2 CL[b]	+	+	NT	-	-	-	-	-	-
CACT77A	M	ACT2 CL[b]	+	+	NT	+	-	-	-	-	-
CACT100A	G1	ACT4[b]	+	+	+	-	-	-	-	-	-
CACT111A	M	ACT6[b]	+	+	+	-	-	-	-	-	-
CACT65A	M	ACT8[b]	+	+	+	+	-	-	-	-	-
LCTB22A	M	ACT11[b]	+	+	NT	-	-	-	-	-	-
LCTB28A	G2a	ACT13[b]	+	+/-	+	-	-	-	-	-	-
LCTB50A	G2a	ACT14[b]	+	-	+	-	-	-	-	-	-
GB110A	M	ACT16[g]	+	+	+	+	+	-	-	-	
GB127A	M	ACT17[g]	+	-	+	+	+	-	-	-	
CACT195A	M	ACT27[b]	+	+	NT	+	-	-	-	-	-
CACT164A	M	ACT undesignated[b]	+	+	NT	+	-	-	-	-	-
CACT282A	M	ACT undesignated[b]	+	+	NT	+	+	-	-	-	-
TPN18A	G1	ACT undesignated[b]	+	+	NT	-	-	-	-	-	-
TPN23A	G1	ACT undesignated[b]	+	+	NT	-	-	-	-	-	-
LH9A	M	ACT undesignated[l]	-	-	NT	-	-	-	+	+	+
ILA142[§]	G1	Unknown[b]	+	NT	NT	-	NT	-	NT	+	NT
B1.g6[§]	G2a	β2-microglobulin	NT	NT	NT	+	NT	?	NT	+	NT

MHC = major histocompatibility complex class I and class II. L = lymphocyte, M = monocyte/macrophage, Gr = granulocyte, CL = cluster, P = polymorphic, ACT = molecule expressed on activated lymphocytes, TCR = T cell receptor, endo = endothelium, I = pattern of labeling inconsistent with pattern of labeling noted on bovine leukocytes, * = predicted specificity, § = as reported in separate study (see text for reference), Bo = *Bos taurus,* Bi = *Bison bison*, Cp = *Capra hircus*, Ov = *Ovis aries*, WB = *Bubalis bubalis*, Pg = *Sus scrofa*, Lm = *Lama glama* and *L. pacos,* Cm1 = *Camelus dromedarius,* Cm2 = *C. bactrianus*. The target species used for generating the mAbs used in this study are indicated by a superscript abbreviation shown with the specificity of the mAb (h = human, b = bovine, g = goat, s = sheep, l = lama, p = pig).

A plus (+) sign indicates that a mAb recognises a conserved epitope on an orthologous molecule. A negative sign indicates no reactivity. An **I** indicates that the pattern of reactivity differed from the standard pattern of labeling observed on bovine leukocytes. **P** indicates the epitope is polymorphic in the species indicated. **Wk** indicates that the pattern of reactivity was weak and **NT** indicates that cells were not available for testing.

forward light scatter, Fig 1) to exclude platelets and debris from analysis. Two parameter dot plot profiles (side scatter vs fluorescence Fig 1) were prepared from leukocyte preparations from each species. The profiles obtained for each mAb were then compared to determine whether the labeling pattern was the same or different from the pattern of reactivity obtained with bovine, goat, or llama leukocytes.

Results

Previous studies revealed that the specificity of mAbs can be predicted based on the pattern of labeling of leukocytes detected by flow cytometry (Fig 1) (Davis *et al,* 1990; 1995). Monoclonal antibodies that recognise epitopes on the same molecule yield the same pattern of labeling and form clusters. Because of variations in the level of expression of a given molecule on one or more populations of leukocytes, the patterns of labeling obtained with mAbs that recognise different molecules are unique. This observation has been used to cluster mAbs that recognise different molecules (Lanier *et al,* 1983; 1986). Verification of the specificity of mAb clusters has been established by determining the molecular weight of the mAb-defined molecule and/or identification of the gene encoding the molecule (Tavernor, 1993). Further studies have shown that the patterns of expression of orthologous molecules have been conserved cross species (Davis *et al,* 1995; 2000). This has permitted the use of flow cytometry to identify mAbs that recognise conserved epitopes on orthologous molecules. In the present study the

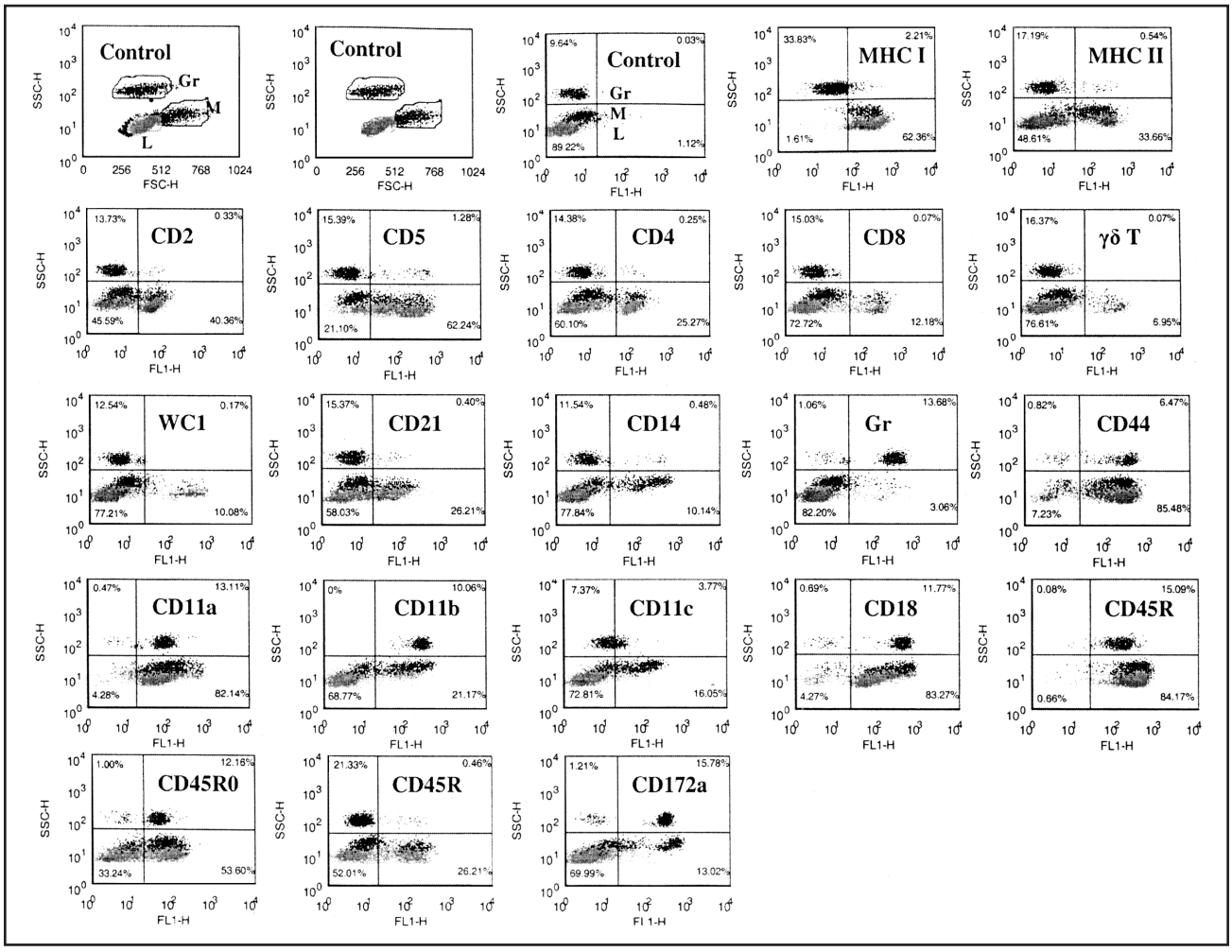

Fig 1. Representative dot plot profiles obtained with leukocytes from cattle and mAbs specific for MHC and LDM. CD, cluster of differentiation, is a term used to describe sets of mAb that define a given LDM in humans. Each unique molecule has been given a numerical designation following characterisation and validation in studies conducted in international workshops. The terminology has been adopted for designation of orthologous molecules identified in other species. The nomenclature is used generically to discuss the properties of any CD molecule, regardless of species. A prefix is added when discussing the characteristics of a given CD molecule (i.e.: bovine = BoCD#, caprine = CaCD#, etc). mAbs that yielded the same pattern of labeling in other species were considered to recognise orthologous molecules. Gr = granulocytes, M = monocytes, L = lymphocytes. The pattern of expression of each CD molecule defined with a labeled antibody is shown in the respective panels.

pattern of labeling obtained with bovine leukocytes was used as the standard for comparison of the labeling patterns obtained with bison, water buffalo, goat, sheep, pig, llama, and camel leukocytes and determining whether one or more mAbs recognise the orthologous molecule in two or more species.

The initial screening of 490 mAbs generated against human, cattle, goat, sheep, llama, and pig leukocyte differentiation molecules yielded 190 mAbs reactive with bovine, goat, sheep, pig, and/or llama leukocytes (Table 1). These were used in further studies to look for mAbs reactive with leukocyte differentiation molecules in bison and camels. The data on leukocytes from water buffalo were obtained from previous studies (Davis *et al,* 2001; Vilmos *et al,* 1996). Data are also included from a previous study, with mAb submitted to pig and ruminant LDM workshops, that was conducted to identify mAb that cross reacted with dromedary camel LDM (Muriuki *et al*, 1998). Analysis revealed that the majority of the mAbs developed against bovine leukocyte differentiation molecules reacted with leukocytes from bison. Many of the mAbs that were used in the panel also reacted with water buffalo leukocytes. A few of the mAbs generated against goat, sheep, llama, or pig leukocyte differentiation molecules reacted with bovine, bison and water buffalo. Only a few of the mAbs reacted with camel leukocytes.

In particular, mAbs were found that recognised epitopes conserved on either or both dromedary and bactrian leukocytes: CD4, CD11a, CD11c, CD14, CD18, CD29, CD44, CD45, CD49d, and CD172a. Some additional mAbs were found that have not been completely characterised (Table 1). The highest frequency of cross reactivity was noted with mAbs specific for MHC class I and class II molecules. The majority of the epitopes detected with the selected anti-MHC mAbs were conserved on cells from cattle, goats, sheep, pigs, llamas and camels.

Discussion

The results obtained in the present study show the potential and limitations of identifying cross reactive mAbs for use in camels and water buffalo. Many epitopes on orthologous molecules have been conserved in the course of evolution of closely related species, as shown here, using mAbs generated primarily against LDM in cattle, goats, and sheep. The screening of mAbs made against human, pig, dog, and llama yielded very few mAbs that recognise conserved epitopes on ruminant and camel leukocytes. Of interest though, a few mAbs made against llama LDM did recognise epitopes conserved on camel LDM. The data indicate that it would be worthwhile to screen additional mAb made against ruminant LDM for mAb useful for research in bison and water buffalo. The screening of mAbs developed against pig LDM and other non-ruminant species show that it is unlikely that screening will yield very many mAbs for use in ruminants or camelids. The screening of mAbs against llama LDM, however, suggests it would be useful to screen any mAb generated against llama LDM for mAbs that recognise LDM in camels. This endeavor could yield additional useful mAbs. It appears though, that a concerted effort will have to be made to make many of the mAbs needed for immunological research in camels.

Until recently, very little information has been available on the composition of the immune system in the surviving species in the suborder Tylopoda (llamas and camels). However, studies conducted in llamas (Davis *et al,* 2000) and studies presented here now provide some insight into the composition of the immune system in llamas and camels and reveal the similarities and differences of their immune systems with those in species present in the other two suborders of Artiodactyla, Ruminantia (cattle, goats, and sheep) and Suiformes (pigs). The cumulative studies have revealed unique differences not found in other orders of mammals. Differences have been noted in the composition of ab and gd T lymphocytes in the pig, gd T lymphocytes in pigs, ruminants, and camelids, and B lymphocytes in camelids. In species that have been examined thus far, $CD4^+$ (T helper) and $CD8^+$ (T cytotoxic) ab T lymphocytes are mutually exclusive subpopulations, each comprised of naïve and memory T lymphocytes. *In vitro* stimulation with recall antigens in immunised animals elicits an antigen specific proliferative memory T lymphocyte response in either or both $CD4^+$ and $CD8^+$ populations. Double positive lymphocytes only occur in the thymus during T cell maturation. Expression of either CD4 or CD8 is lost during maturation before migration into blood and secondary lymphoid tissue. In contrast, three subpopulations of ab T lymphocytes are present in pigs $CD4^+$, $CD8^+$, and one that is positive for CD4 and CD8. This population differs from the double positive lymphocyte population present in the thymus. *In vitro* studies have shown that the population in pigs is comprised primarily of memory T lymphocytes. The proportion of double positive cells increases with age. It is not clear when memory T lymphocytes begin to express both CD4 and CD8 (Zkuckermann and Husmann, 1996; Zuckermann and Gaskins, 1996). Analysis of the gd T population in ruminants (Davis *et al,* 1996) and pigs (Davis *et al,* 1998) and more recently in camelids (Davis *et al,* 2000) has shown that it is comprised of two complex populations that differ in tissue distribution and possibly function. One is similar to gd T cells described in humans and rodents. It is characterised by the expression of CD2, CD3, CD5, and CD6. Subsets of this population co-express CD4 or CD8 (Kaufmann, 1996). The second population is characterised by absence of CD2, CD4, CD8, and CD6 and the expression of two unique molecules, WC1 and GD3.5 in cattle (Wijngard, 1994; Jones *et al,* 1996) and the orthologue of WC1 and SWC6 in pigs (Davis *et al,* 1998, Binns 1994; Carr *et al,* 1994). The orthologue of WC1 has been identified in camels and llamas with cross reactive mAbs (Davis *et al,* 2000; Zidan *et al*, 2000). The molecule WC1 is a member of the scavenger receptor cysteine rich superfamily (SRCRSF) of molecules that contain one or more copies of a highly conserved ~ 110 aa motif. The CD5 and CD6 are also members of SRCRSF of molecules (Wijngaard *et al,* 1994; Aruffo *et al,* 1997). However, it appears that the genes encoding WC1 and its orthologues are only present in suborders of Artiodactyla (Davis *et al,* 2000). Studies in cattle (MacHugh *et al,* 1993) and sheep (Walker *et al,* 1994) have shown that multiple isoforms of the WC1 are encoded by different members of the WC1 gene family and that isoforms may be expressed on mutually

exclusive or overlapping subpopulations of $WC1^+$ gd T lymphocytes. Analysis of the $V_{\tilde{a}}$ gene segment usage has shown that the $WC1^+$ and $WC1^-$ populations of gd T lymphocytes are distinct lineages (Davis *et al,* 1996; Hein and Dudler, 1997). The complexity of the WC1 gene family has not been determined in pigs and camelids. Comparative studies have shown that the frequency of the $WC1^+$ population of gd T lymphocytes is high peripheral blood (30 – 60%) in young cattle, sheep, goats, and pigs and low in secondary lymphoid tissues (5 – 10%). The $WC1^-$ population is low in peripheral blood (3 – 5%) and high in spleen (20 – 60%). The distribution is similar to $WC1^+$ gd T lymphocytes in other secondary lymphoid tissues. Studies in llamas suggest that the frequency of $WC1^+$ gd T lymphocytes in peripheral blood and secondary lymphoid tissue is similar in young and adult animals (10 – 16%) (Davis *et al,* 2000). More camels need to be tested to see if the composition of gd T cells is similar to that in llamas.

As in ruminants and pigs, the majority of B lymphocytes in peripheral blood of camelids express surface immunoglobulin, sIgM. It is possible that subsets express other classes of immunoglobulins. Through cross reactivity with antibodies specific for human immunoglobulin, evidence has been obtained that, in camels and llamas, b lymphocytes produce membrane associated and secreted forms of IgM, IgG_1, IgA, and IgD immunoglobulins (Neoh *et al,* 1973). Studies have also shown that camelids produce two additional classes of immunoglobulin comprised of heavy chains without light chains IgG_2 and IgG_3 (Hamers *et al,* 1993; Hamers and Muyldermans, 1998). These classes of immunoglobulin are without the C1 constant domain that binds light chains (V_H, C_H2, C_H3) (Nguyen *et al* 1999; 2002). Approximately 75% of IgG_2 & IgG_3 antibodies in camelid serum are comprised of these immunoglobulins. The observation that different V_H gene segments are used by the four and that two chain forms of immunoglobulins has suggested two lineages of B lymphocytes may exist in camelids. Analysis of B lymphocytes in llamas with mAbs that detect molecules expressed on mutually exclusive subsets of B lymphocytes support this possibility (Davis *et al,* 2000). In contrast to cattle, the frequency of b lymphocytes is high in newborn llamas (4 – 12% and 27 – 73%, respectively).

In summary, the objective of the present study was to screen existing mAbs to LDM to identify mAbs that could be used to study the immune response to infectious agents and vaccines in camel and water buffalo. A large set of mAbs were found that reacted with water buffalo LDM. It should now be possible to conduct studies in water buffalo without the need for an extensive effort to generate a full set of mAbs to LDM in water buffalo. For camels, only a few mAbs were found. A more direct approach will be needed to develop mAbs for use in camels.

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News

SCIENTISTS FIND MORE BONES OF BIG CAMELS

Hunters stalked giant camels as tall as some modern-day elephants in the Syrian desert tens of thousands of years ago and archaeologists behind the find are wondering where the camels came from and what caused them to die off. The enormous beasts existed about 100,000 years ago and more of the bones, first discovered last year, have been found this year in the sands about 150 miles north of the capital, Damascus.

A photo illustration of a modern-day camel and the ancient giant camel (background). More bones of the giant camel were unearthed in the Syrian desert.

(Source:Albert Aji, Associated Press Writer, DAMASCUS, Syria)

CAMEL MOBILE LIBRARY SERVICE IN KENYA

The Camel Library Service is a library outreach program for people who are unable to use the static libraries in marginal areas in the country. The Kenya National Library Service launched it on October 14th 1996, and it is operational in Garissa town in North Eastern Province of the country. Following its successful implementation, the program was replicated in Wajir town on April 13th 1999. The Camel Library Service is meant to serve the Pastoralists in these areas which are geographically isolated because they experience difficulties in using directly the available library facilities at the static library branches.This project was set up to provide access to books and other publications to the pastoralists. There is no other means of access to information in the vast plains, to fight illiteracy, to support formal education, to support vulnerable groups to access books, knowledge and encourage education, to provide information to be used for leisure, knowledge and research, to stimulate public interest in books and promote reading for knowledge, information and enjoyment and to promote the use of non-motorised transport - the Camel.

WORLD'S BIGGEST CAMEL MILK PRODUCER: FACTS

The world population of camels is currently estimated at some 20 million. Somalia is believed to have the world's largest herd, with almost as many camels as humans. World production of camel milk available/used for human consumption is officially put at 1.3 million tonnes – 500 times less than cow's milk. The generally accepted figure for global camel milk production (most of which goes to the calf) is 5.4 million tonnes. Lactating she-camels each produce between 1,000 and 2,000 litres of milk for a period of anywhere between eight and 18 months.The world's biggest camel milk producer is Somalia, followed by Saudi Arabia.

(Source:FAO Publication on Camel cheese production)