Identification of Monoclonal Antibody Reagents for Use in the Study of Immune Response 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 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 mAbs developed against leukocyte differentiation molecules (LDM) in various species recognized conserved epitopes on orthologous (identical) molecules intow or more species of *Artiodactyla*. Analysis of 490 monoclonal antibodies raised against leukocyte differentiation molecules (LDM) in cattle, sheep, goats, lamas, pigs, dogs and humans revealed that many epitopes have been conserved in the speices in the course of eveolution in closely related species in the suborder Ruminatia (cattle, buffalo, bison) and fewer related species (sheep and goats). Only few of the epitopes conserved in Rumintia were conserved in the pig, lama and camels. The highest level of conservation in all suborders was found with major histocompatibility complex class 1 (MHC1) and class 11 (MHC11) molecules. These findings show the potential as well as the limitations of screening existing monoclonal antibodies to investigate disease conditions. These findings show the potential as well as limitations of screening existing mAbs for mAbs useful for research in less well 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 suborder of *artiodactyla.*

Introduction

Although considerable progress has been made in characterization of the immune system in economically important species such as cattle, goats, sheep, pigs, and horses, limited progress has been made in camelids 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 recognize conserved epitopes on orthologous (identical) molecules. The rationale and support for this approach has been that previous investigations[1;2] and surveys conducted as part of workshops on LDM in ruminants and pigs [3-13] have shown some of the mAbs do recognize conserved epitopes. More recently, a comparative study has shown mAbs to some cytokines also recognize conserved epitopes[14]. These observations have suggested that it would be useful to extend the comparative studies to identify additional mAbs that recognize conserved epitopes on LDM in less well studied species. Such an endeavor could reduce the need to develop reagents for some important molecules and allow investigators to focus 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, lamas, humans, and dogs to determine how many existing mAbs recognize conserved epitopes on orthologous molecules in these and other less well studied species such as bison, water buffalo and camel.

Methods and Materials

Animals: Cattle (*Bos Taurus*), goats (*Capra hircus*), sheep (*Ovis aries*), pigs (*Sus scrofa*), rabbits (*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 studies. For camels (*Camelus dromedarius* and *C. bactrianus*), blood was obtained from animals maintained by private owners in Washington and Missouri. Bison (*Bison*

bison) blood was obtained from experimental animals maintained at the National Animal Disease Center, Ames Iowa. The animals at Washington State University and National Animal Disease Center 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 characterized 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 minutes 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 (NH4Cl, 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 minutes 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 x 10^7 cells/ml in PBS-ACD and kept at 4^oC until used in flow cytometry (FC).

For lama and camel, erythrocytes were removed by density gradient centrifugation. Blood was diluted to 50% ACD and then placed in glass centrifuge tubes (50ml). Blood was underlaid with 10ml Histopaque (specific gravity 1.119) and centrifuged at 2000 RPM for 25 minutes, 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 one ml of distilled H_2O for four to eight

seconds to lyse erythrocytes and then resuspended in 50 ml of PBS-ACD. The cells were then sedimented and resuspended to 2 x 10^7 cells/ml and kept at 4^oC until used.

For flow cytometry, cells were distributed in 96-well V-bottom microtiter plates (50 µl/well) containing 50 µl of mAb (0.7 µg) and incubated for 15 minutes 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 minutes at 4^oC. The cells were then washed twice in PBS-ACD and fixed in 2% PBS buffered formaldehyde.

Cell culture: To analyze 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 .086)**.** Following washing in PBS-ACD, cells were placed in RPMI-1640 tissue culture medium containing 13% calf serum, 2-mercatoethanol, 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 analyze 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 forward light scatter, Fig. 1) to exclude platelets and debris from analysis. Two parameter dot plot profiles (side scatter vs fluorescence 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 lama leukocytes.

Results

Previous studies revealed the specificity of mAbs can be predicted based on the pattern of labeling of leukocytes detected by flow cytometry (Fig. 1)[15;16]. Monoclonal antibodies (mAbs) that recognize 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 recognize different molecules are unique. This observation has been used to cluster mAbs that recognize different molecules[17;18]. 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[19]. Further studies have shown the patterns of expression of orthologous molecules have been conserved cross species[16;20]. This has permitted the use of flow cytometry to identify mAbs that recognize conserved epitopes on orthologous molecules. In the present study the 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, lama, and camel leukocytes and determining whether one or more mAbs recognize the orthologous molecule in two or more species. As summarized in Table 1, a plus sign indicates a mAb recognizes 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 the pattern of reactivity was weak. This term is used only to report data obtained from other studies[5;21]. NT indicates cells were not available for testing.

The initial screening of 490 mAbs generated against human, cattle, goat, sheep, lama, and pig leukocyte differentiation molecules yielded 190 mAbs reactive with bovine, goat, sheep, pig, and/or lama 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[2;5]. 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 [21]. 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, lama, 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 recognized 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 characterized (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, lamas 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 lama yielded very few mAbs that recognize conserved epitopes on ruminant and camel leukocytes. Of interest though, a few mAbs made against lama LDM did recognize 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 lama LDM, however, suggests it would be useful to screen any mAb generated against lama LDM for mAbs that recognize 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 (lamas and camels). However, studies conducted in lamas[20]and studies presented here now provide some insight into the composition of the immune system in lamas and camels and reveal the

similarities and differences of their immune systems with the immune systems 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 αβ and γδ T lymphocytes in the pig, γδ 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) αβ T lymphocytes are mutually exclusive subpopulations, each comprised of naïve and memory T lymphocytes. In vitro stimulation with recall antigens in immunized 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 αβ 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 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 [22;23]. Analysis of the γδ T population in ruminants [24] and pigs [25] and more recently in camelids [20] has shown it is comprised of two complex populations that differ in tissue distribution and possibly function. One is similar to γδ T cells described in humans and rodents. It is characterized by the expression of CD2, CD3, CD5, and CD6. Subsets of this population co-express CD4 or CD8 [26]. The second population is characterized by absence of CD2, CD4, CD8, and CD6 and the expression of two unique molecules, WC1 and GD3.5 in cattle [27;28] and the orthologue of WC1 and SWC6 in pigs [25;29;30]. The orthologue of WC1 has been identified in camels and lamas with cross reactive mAbs [20;31]. 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. CD5 and CD6 are also members of SRCRSF of molecules [27;32]. However, it appears that the genes encoding WC1 and its orthologues are only present in suborders of Artiodactyla [20]. Studies in cattle [33] and sheep [34] 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 $WC1^+$ γδ T lymphocytes.

Analysis of the V_γ gene segment usage has shown that the $WC1^+$ and $WC1^-$ populations of γδ T lymphocytes are distinct lineages [24;35]. The complexity of the WC1 gene family has not been determined in pigs and camelids. Comparative studies have shown the frequency of the $WC1^+$ population of γδ 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 $WC1^+$ γδ T lymphocytes in other secondary lymphoid tissues. Studies in lamas suggest the frequency of $WC1^+$ γδ T lymphocytes in peripheral blood and secondary lymphoid tissue is similar in young and adult animals (10 – 16%) [20]. More camels need to be tested to see if the composition of γδ T cells is similar to that in lamas.

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 lamas, B lymphocytes produce membrane associated and secreted forms of IgM, IgG_1, IgA, and IgD immunoglobulins [36]. Studies have also shown camelids produce two additional classes of immunoglobulin comprised of heavy chains without light chains IgG_2 and IgG_3 [37;38]. These classes of immunoglobulin are missing the C1 constant region domain that binds light chains (V_H, C_H2, C_H3)[39;40]. Approximately 75% of antibodies in serum are comprised of these immunoglobulins. The observation that different V_H gene segments are used by the four and two chain forms of immunoglobulins has suggested two lineages of B lymphocytes may exist in camelids. Analysis of B lymphocytes in lamas with mAbs that detect molecules expressed on mutually exclusive subsets of B lymphocytes support this possibility [20]. In contrast to cattle, the frequency of B lymphocytes is high in newborn lamas (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 react 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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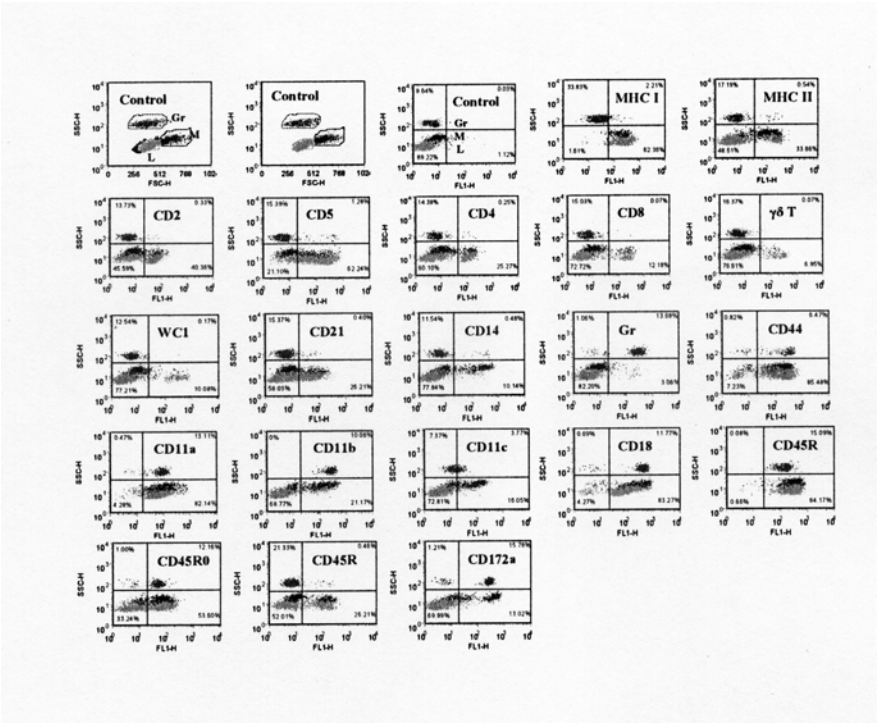

Figure 1: Representative dot plot profiles obtained with leukocytes from cattle and mAbs specific for MHC and LDM. CD, cluster of

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 characterization 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 recognize 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.

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
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	CD8α[b]	+	+	NT	P	-	-	-	-	-
BAT82A	G1	CD8β[g]	+	+	NT	+	+	-	-	-	-
CACT80C	G1	CD8α[b]	+	+	+	+	+	-	-	-	-
CACT88A	G3	CD8α[b]	+	+	+	-	+	-	-	-	-
TH82A	G1	CD8β*[b]	+	+	+	+	+	-	-	-	-
ST8	M	CD8α[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]	+	+	+	+	+	-	-	-	-
BAG40A	G3	CD44[g]	+	+	+	+	+	+	-	-	-
BAT31A	G1	CD44[s]	+	+	+	+	+	-	-	-	-
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	γδ TCR-N24 δ chain[g]	+	+	+	+	-	-	-	-	-
CACT148A	M	γδ TCR-N21 δ chain[b]	+	-	+	-	-	-	-	-	-
CACTB14A	G1	γδ TCR-N6 CL[b]	+	+	+	+	+	-	-	-	-
CACTB6A	M	γδ TCR-N6 γ chain?[b]	+	+	+	+	+	-	-	-	-

CACT19C	M	γδ TCR N6 CL[b]	+	-	NT	+	-	-	-	-	-
CACTB81A	G1	γδ TCR-N7 γ chain? [b]	+	-	+	-	-	-	-	-	-
CACT22B	M	γδ TCR-N7 CL[b]	+	+	NT	+	+	-	-	-	-
CACTB44A	G1	γδ TCR-N7 CL[b]	+	+	+	-	-	-	-	-	-
86D1	G1	γδ TCR-N7 CL[b]	+	+	+	+	+	+	-	-	-
CACT75A	M	γδ T cell sub[b]	+	-	+	+	-	-	-	-	-
GC52A	G1	γδ T cell sub[g]	+	+	NT	+	+	-	-	-	-
TPB16A	G1	γδ T cell sub[b]	+	-	NT	-	+	+	-	-	-
TPB30A	G1	γδ T cell sub[b]	+	+	NT	-	-	-	-	-	-
TPN4A	G1	γδ 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	-	-	-	-
CACTB1A	G1	WC1-N3 CL[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 sub[g]	+	+	+	+	+	-	-	-	-
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	λ 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]	+	+	+	-	+	-	-	-	-
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 a mAb recognizes 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 the pattern of reactivity was weak and NT indicates cells were not available for testing.

تعرف على الأجسام المضادة الوحيدة المنشأ واستخدامها لدراسة الاستجابة المناعية للمسببات الوبائية في الإبل والجاموس

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ملخص البحث: إن التقدم في توضيح آليات الاستجابة المناعية للمسببات المعدية واللقاحات المشتقة والمستخدمة في الفصائل الحيوانية المستأنسة وعلى وجه الخصوص الإبل والجاموس أصبح غير ملموساً وكان هذا العائق الذي يعترض هذا التقدم نتيجة لنقص إنتاج ومعرفة أنواع الأجسام المناعية وحيدة المنشأ والتي تستخدم في دراسة الجهاز المناعي وميكانيكية الاستجابة المناعية لهذه الحيوانات. ولمعالجة هذه المشكلات أجريت هذه الدراسة وذلك لتحديد ومعرفة نوعية الأجسام المضادة وحيدة المنشأ والتي سبق معرفة نوعيتها وتفاعلها مع المستقبلات المناعية الموجودة على أسطح خلايا الدم البيضاء للفصائل الحيوانية المختلفة والتي تحمل رؤوس وجزيئات أنتيجينية متماثلة لفصيلتين أو أكثر من هذه الحيوانات. في هذه الدراسة تم اختبار 490 نوعا من أنواع متعددة المنشأ للأجسام المضادة وحيدة المنشأ والتي سبق معرفة نوعيتها وتفاعلها مع المستقبلات المناعية الموجودة على أسطح خلايا الدم البيضاء للإنسان والماشية والأغنام والماعز والإبل والخنازير والكلاب والتي أفادت بوجود علاقة أنتيجينية وطيدة بينها وبين المستقبلات الأنتيجينية الموجودة

على أسطح خلايا الدم البيضاء للفصائل الحيوانية المندرجة تحت مجموعة المجترات مثل الأبقار والجاموس وقليل منها له علاقة بمثيلتها الموجودة على أسطح خلايا الدم البيضاء لفصائل الأغنام والماعز. كما وجد عدد قليل منها له علاقة أنتيجينية بينها وبين المستقبلات الأنتيجينية الموجودة على أسطح خلايا الدم البيضاء للخنازير والإبل بأنواعها. ومن هذه الدراسة أيضاً اتضح أنه يوجد مستوى عالٍ من الإيجابية للمستقبلات المناعية من نوع (MHCI and MHCII) الأنتيجينية الموجودة على أسطح خلايا الدم البيضاء لجميع الفصائل المختبرة. وقد بينت هذه الدراسة تقدما ملموساً في مدى معرفة وتحديد أنواع الأجسام المضادة وحيدة المنشأ والتي يمكن أن تستخدم في دراسة الجهاز المناعي ومعرفة العوامل ذات التأثير على ميكانيكية الاستجابة المناعية للإبل والجاموس والتي يجب أن توضع في الاعتبار حيث أن لها تأثير على آليات الاستجابة المناعية للمسببات الوبائية المعدية وكفاءة اللقاحات المنتجة للاستخدام للوقاية من الأمراض.