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TCR and CD3 Antibody Cross-Reactivity in 44 Species

Melanie L. Conrad,[1]* William C. Davis,[2] Ben F. Koop[1]

[1]Centre for Biomedical Research, University of Victoria, Victoria, British Columbia, Canada V8W 3N5

[2]Department of Veterinary Microbiology and Pathology, Washington State University College of Veterinary Medicine, Pullman, Washington

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*Correspondence to: Melanie L. Conrad, Centre for Biomedical Research, University of Victoria, Box 3020 STN CSC, Victoria, BC, Canada V8W 3N5.

Email: conradml@gmail.com

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Abstract

The production of monoclonal antibodies is very costly, and antibodies are only available for a limited number of species. Until a more cost effective method of antibody production is found, identification of cross-reactive antibodies is an alternative approach that can provide investigators studying immunity in minor species with valuable antibody reagents. Flow cytometry was used to test 21 monoclonal antibodies (mAb), raised against αβ and γδ T cell receptors and CD3 from human and five animal species, for cross-reactivity in 44 different species including 16 species of nonhuman primates, marsupials, carnivores, lagomorphs, rodents, ruminants, swine, cetacean, horse, birds, a reptile, and fish. Fifteen of the mAbs cross-reacted with orthologous molecules in one or more species. Two antibodies, anti-human TCR γδ (B1.1), and anti-human CD3 (SP34) were found to costain in 13 species of nonhuman primates. This study has identified valuable new reagents for studying T cell populations in different animal species and for the first time characterized antibodies useful for studying γδ T cell populations in many species of primates. These antibodies may be used for further immunity research in species with less well-characterized immune systems. © 2007 International Society for Analytical Cytology

Key terms

monoclonal antibody; T cell receptor; αβ; γδ; CD3; WC1; cross-reactivity; primate; mammal; carnivore

AN interesting finding to emerge from T cell research is the disparity between peripheral blood γδ T cell populations in certain animals. Percentages of γδ T lymphocytes in peripheral blood vary so greatly between different species that species can be classified as γδ high or low (1). γδ low species include humans and mice (2–5%) (2,3) and γδ high species include chickens (15%) (4), swine (24%) (5), and cattle (20–40%) (6).

The high concentration of γδ T cells in ruminants and swine is attributable to the presence of a subpopulation of γδ T cells that express the workshop cluster 1 (WC1) molecule in ruminants and the orthologue in swine. WC1 and orthologues have only been identified in artiodactyls including ruminants, swine, and camelids (7,8). The current data suggest this is a unique population of γδ T cells that has evolved in artiodactyls. The concentration of WC1-γδ T cells identified in ruminants and swine is similar to the concentration seen in humans and mice (9,10). Analysis of γδ T cells in chickens has not revealed an explanation for the high concentration of γδ T cells. The lack of reagents has thus far limited analysis of the composition and function of γδ T cells in additional species. Identification of cross reactive mAbs would facilitate the conduct of further studies and an opportunity to delineate any functional differences in populations of γδ T cells present in species with high and low concentrations of γδ T cells in peripheral blood.

Comparative studies with mAbs developed against major histocompatibility and leukocyte differentiation antigens in humans and other species have demonstrated that some mAbs recognize epitopes conserved on orthologous molecules in two or more species. Cross-reactive mAbs that have been identified through collaborative

studies and international workshops have afforded an opportunity to accelerate identification of orthologous molecules in less well-studied species. They have also proven useful in documenting the specificity of mAbs that recognize species restricted epitopes on the same molecule (11–16).

This research represents an expansion of previous studies, specifically analyzing a panel of mAbs specific for TCR αβ, TCR γδ, and CD3 for cross-reactivity in 44 different species.

Materials and Methods

Animals

One to three millilitres of whole blood was collected in tubes containing EDTA as an anticoagulant and analyzed within 24 hours of blood draw. Samples for this research were obtained from a variety of sources including veterinary practices, zoos, animal care units, and primate research centers. To avoid subjecting zoo animals to undue stress, blood was collected opportunistically during routinely scheduled examinations or veterinary procedures, thus the number of animals analyzed per species varies. A list of the species tested for cross-reactivity and number of individuals tested is shown in Table 1 (Panels A–C).

Antibodies

Monoclonal antibodies (mAb) to human, mouse, rat, dog, bovine, and chicken TCR and CD3 were selected for the reason that peripheral blood samples were readily available from these species to use as positive controls. The mAbs were chosen according to previous cross-reactivity data shown on the TKP antibody reactivity database www.vetmed.wsu.edu/tkp and the NIH Non Human Primate Antibody Resource http://nhpreagents.bidmc.harvard.edu/NHP as well as availability of these reagents through commercial suppliers.

The antibodies used in this study are listed in Table 1 (Panels A–C). Antibodies were obtained from Biolegend (San Diego, CA), Caltag (Burlingame, CA), eBioscience (San Diego, CA), Peter Moore (University of California, Davis, CA), Southern Biotech (Birmingham, AL), Becton Dickinson (Mississauga, ON, Canada), Serotec (Raleigh, NC), and VMRD (Pullman, WA).

Cell Preparation and Staining

Labeling experiments were performed in duplicate to ensure labeling accuracy. If there was no difference between duplicate tests in the first two individuals, the rest of the individuals were labeled only once. Fifty microlitres of whole blood was placed in a 12×75 mm^2 flow cytometry tube and mixed with the appropriately titred amount of each primary conjugated mAb (anti-CD3 PE-Cy5.5, anti-TCR αβ FITC, or anti-TCR γδ PE) and incubated in the dark at room temperature for 15 min. For secondary conjugated mAb, Zenon reagents were used to manufacturer's specifications (Invitrogen – Molecular Probes, Burlington, ONT, Canada) and subjected to the same procedure as above. After labeling, the blood was lysed with 4 mL of 1× ammonium chloride lysis buffer (NH_4Cl 8.4 g/L, $KHCO_3$ 1 g/L, NaEDTA 0.032g/L) incubated at room temperature for 10 min. After lysis, the sample was centrifuged at 400*g* for 6 min, the supernatant was removed and the cell pellet resuspended in 1 mL of phosphate buffered saline and subsequently analyzed by flow cytometry.

The birds, reptiles, and fish in this study have nucleated red blood cells (RBC). Comparisons between nucleated and non-nucleated RBC samples showed comparable forward angle light scatter (FSC) versus side angle light scatter (SSC) profiles with the exception of a high number of RBC nuclei contaminants. Nucleated RBC samples were treated the same as above with the exception of increasing the flow cytometry cell count to 500,000 total cells. The purpose of increased cell count was to ensure that antibody signal could be detected amidst the contaminating RBC nuclei.

Flow Cytometry

Because of prohibitions on shipping blood samples across the Canadian/American border, flow cytometry was performed in two different cities using two different flow cytometers. The stained and lysed blood sample preparations were run on either a Beckman coulter FC 500 (Fullerton, CA) or a Becton Dickinson FACSCalibur (San Jose, CA). Samples were treated with the same method regardless of the instrument used; the voltage and compensation settings were established on each flow cytometer using human peripheral blood samples. No significant difference was seen between identically labeled human samples run on either machine. To reliably identify small populations of cells, 10,000 lymphocytes were counted for each sample.

Negative controls consisted of testing blood with no antibody added for autofluorescence. Positive antibody controls consisted of reacting a particular species' blood with its corresponding antibody. Human, mouse, rat, dog, cattle, and chicken antibodies were all tested in this manner (data not shown). Data for the cross-reactivity of TCR αβ antibodies in cattle and chicken were included in Table 1 (Panels B and C) as there are presently no antibodies that recognize pan αβ T cells in these species.

For an antibody to be deemed cross-reactive, several factors were considered including the size and granularity of the cell population, the fluorescence signal within the lymphocyte population and nonspecific binding in the periphery. Antibody cross-reactivity was sought in the gated lymphocyte population, which contains T cells, B cells, and NK cells. To assess the fluorescence within the lymphocyte gate, the size and morphology of the resulting cell population was taken into consideration. These two factors provided a means to visually decipher true cross-reactivity from artifact. Furthermore, if a positive reaction was detected within the lymphocyte population, an ungated histogram was also examined to rule out the possibility of nonspecific binding. Examples of positive and negative flow cytometry profiles taken from the horse data are shown in Figures 1A and 1B. F1

In the case of primate three color labeling, a CD3 gate was created from a SSC versus CD3-PC5 dot-plot and subse-

Table 1. Reactivity of 21 commercially available mAb against 44 different species

						TEST SPECIES															
						PRIMATES															
ANTIBODY	SUPPLIER	CLONE	SPECIES SPECIFICITY	ISOTYPE	LABEL	CH	GOR	WG	CM	JM	PM	LTM	OB	HB	MA	RG	RCM	CO	FL	AA	RT
Panel A																					
CD3	BD Pharmingen	SP34	Human	mIgG	1° PE-Cy5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
TCR αβ*	Serotec	R7 3	Rat	mIgG1	1° FITC	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
TCR γδ	eBioscience	B1.1	Human	mIgG1	1° PE	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	−
Number of individuals tested						1	2	1	2	2	2	2	2	2	2	2	1	2	2	1	1

						TEST SPECIES												
						CETARTIODACTYLA										CARNIVORA		
ANTIBODY	SUPPLIER	CLONE	SPECIES SPECIFICITY	ISOTYPE	LABEL	AL	WH	GO	SH	BO	ZE	OX	MS	CAR	BW	CAT	FOX	SL
Panel B																		
CD3	Biolegend	145-2C11	Mouse	hIgG1	1° PE-Cy5	−	−	N	N	/	/	−	−	−	N	−	−	N
CD3	Peter Moore	CA17.2A12	Dog	mIgG1	2° PE-Cy5	−	−	N	N	/	/	−	−	−	N	−	+	−
CD3	Southern Biotech	CT-3	Chicken	mIgG1	2° PE-Cy5	−	−	N	N	/	/	−	−	−	N	−	−	−
CD3	VMRD	MM1A	Bovine	mIgG1	2° PE-Cy5	−	−	−	−	/	+	−	−	−	−	−	−	−
CD3	BD Pharmingen	SK-7	Human	mIgG1	1° PE-Cy5	−	−	−	−	/	/	N	−	−	−	−	−	−
CD3	BD Pharmingen	SP34	Human	mIgG1	1° PE-Cy5	−	−	−	−	/	/	−	−	−	−	−	−	−
TCR αβ	Peter Moore	CA15.8G7	Dog	mIgG1	2° FITC	-	-	N	N	−	−	−	−	−	N	−	−	−
TCR αβ	Serotec	H57-597	Mouse	hIgG1	1° FITC	−	−	−	−	−	−	−	−	−	−	−	−	−
TCR αβ	Serotec	R7.3	Rat	mIgG1	1° FITC	−	+	−	−	−	−	−	−	−	−	−	−	−
TCR αβ	BD Pharmingen	T10B9	Human	mIgM	1° FITC	−	−	N	N	−	−	−	−	−	N	−	N	N
TCR αβ	BD Pharmingen	WT31	Human	mIgG1	1° FITC	−	−	−	−	−	−	N	−	−	−	−	−	−
TCR γδ	BD Pharmingen	11F2	Human	mIgG1	1° PE	−	−	−	−	/	/	N	−	−	−	−	−	−
TCR γδ	Caltag	5A6.E9	Human	mIgG1	1° PE	−	−	+	+	/	/	−	−	−	N	−	−	−
TCR γδ	eBioscience	B11	Human	mIgG1	1° PE	−	−	N	N	/	/	−	−	−	N	−	−	N
TCR γδ	Peter Moore	CA20.8H1	Dog	mIgG2a	2° PE	N	−	N	N	/	/	−	N	−	N	−	N	−
TCR γδ	VMRD	GACTB6A	Bovine	mIgM	2° FITC	−	−	N	N	/	+	−	+	−	N			
TCR γδ	VMRD	GB21A	Bovine	mIgG2b	2° PE	−	−	−	−	/	+	+	−	+	−	−	−	−
TCR γδ	Serotec	GL3	Mouse	hIgG1	1° PE	−	+	+	−	/	/	−	−	−	−			
TCR γδ	Southern Biotech	TCR-1	Chicken	mIgG1	1° PE	−	−	N	N	/	/	−	−	−	N	−	−	−
TCR γδ	Biolegend	UC7-13D6	Mouse	hIgG1	1° PE	−	−	−	−	/	/	−	−	+	N			
TCR γδ	Serotec	V65	Rat	mIgG1	1° PE	−	−	+	+	/	/	−	−	−	N	−	−	−
Number of individuals tested						1	4	6	4	7	1	1	1	4	1	2	1	4

(*Continued*)

Table 1. Reactivity of 21 commercially available mAb against 44 different species (*Continued*)

						TEST SPECIES														
						CARNIVORA (CONT)					AVES			MISCELLANEOUS						
ANTIBODY	SUPPLIER	CLONE	SPECIES SPECIFICITY	ISOTYPE	LABEL	SO	BE	RP	TI	LE	CH	DU	TU	EL	EO	KA	RB	PO	SN	TR
Panel C																				
CD3	Biolegend	145-2C11	Mouse	hIgG1	1° PE-Cy5	−	+	−	−	−	/	−	−	−	−	−	−	−	−	N
CD3	Peter Moore	CA 17 2A 12	Dog	mIgG1	2° PE-Cy5	−	−	−	−	−	/	−	−	−	−	−	−	N	−	−
CD3	Southern Biotech	CT-3	Chicken	mIgG1	2° PE-CY5	−	−	−	−	−	/	−	−	−	−	−	−	−	−	−
CD3	VMRD	MM1A	Bovine	mIgG1	2° PE-Cy5	−	−	−	−	−	/	−	−	−	−	−	−	−	−	−
CD3	BD Pharmingen	SK-7	Human	mIgG1	1° PE-Cy5	N	−	−	−	N	/	N	N	−	−	−	−	N	−	−
CD3	BD Pharmingen	SP34	Human	mIgG1	1° PE-Cy5	−	−	−	?[1]	−	/	−	−	−	−	−	−	−	−	−
TCR αβ	Peter Moore	CA15.8G7	Dog	mIgG1	2° FITC	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
TCR αβ	Serotec	H57-597	Mouse	hIgG1	1° FITC	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
TCR αβ	Serotec	R7.3	Rat	mIgG1	1° FITC	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
TCR αβ	BD Pharmingen	T10B9	Human	mIgM	1° FITC	−	−	−	−	−	?[2]	−	−	−	−	−	−	+	−	N
TCR αβ	BD Pharmingen	WT31	Human	mIgG1	1° FITC	N	−	−	−	N	−	N	N	−	−	−	−	N	N	−
TCR γδ	BD Pharmingen	11F2	Human	mIgG1	1° PE	N	−	−	−	N	/	N	N	−	−	−	−	N	−	−
TCR γδ	Caltag	5A6.E9	Human	mIgG1	1° PE	−	−	−	−	−	/	−	−	−	−	−	−	−	−	−
TCR γδ	eBioscience	B1.1	Human	mIgG1	1° PE	−	−	−	−	−	/	−	−	−	−	−	−	−	+	N
TCR γδ	Peter Moore	CA20.8H1	Dog	mIgG2a	2° PE	−	N	N	N	−	/	−	−	N	−	N	−	−	−	−
TCR γδ	VMRD	CACTB6A	Bovine	mIgM	2° PE	−	−	−	−	−	/	−	−	−	+	+	−	−	−	−
TCR γδ	VMRD	GB21A	Bovine	mIgG2b	2° PE	−	−	−	−	−	/	−	−	−	−	−	−	−	−	−
TCR γδ	Serotec	GL 3	Mouse	hIgG1	1° PE	−	−	−	?[1]	−	/	−	−	−	−	−	−	−	?[1]	−
TCR γδ	Southern Biotech	TCR-1	Chicken	mIgG1	1° PE	−	−	−	−	−	/	−	−	−	−	−	−	−	+	N
TCR γδ	Biolegend	UC7-13D5	Mouse	hIgG1	1° PE	−	−	−	−	−	/	−	−	−	−	−	−	−	−	−
TCR γδ	Serotec	V65	Rat	mIgG1	1° PE	−	−	−	−	−	/	−	−	−	−	−	−	−	−	−
Number of individuals tested						1	2	1	1	1	7	1	1	3	4	1	1	1	1	1

+, Positive staining patterns; −, clear negative results; N, antibody not tested in this species; ?[1], possible positive with further testing required; ?[2], Clear positive result achieved that could not be replicated; *, antibody only stained a subpopulation of cells. /, Indicates no testing because species-specific antibodies are already available for this particular molecule. Individual labeling was performed in duplicate to ensure labeling accuracy. If there was no difference between duplicate tests in the first two individuals, the remaining individuals were labelled only once. Panel A: Species tested: Primates: chimpanzee (CH), gorilla (GOR), white-handed gibbon (WG), cynomolgus (CM), Japanese (JM), pigtailed (PM) and lion-tailed (LTM) macaques, olive (OB) and hamadryas (HB) baboons, mandrill (MA), red guenon (RG), red-capped mangabey (RCM), colobus (CO), Francois langur (FL), aye-aye (AA), ring-tailed lemur (RTL). The number of individuals tested is indicated in the bottom row. Panel B: Species tested: Cetartiodactyla: alpaca (AL), warthog (WH), pygmy goat (GO), bighorn sheep (SH), domestic bovid (BO), zebu bovid (ZE), musk ox (OX), moose (MS), woodland caribou (CAR), beluga whale (BW). Carnivora: cat (CAT), swift fox (FOX), stellar sea lion (SL). As species-specific CD3 and TCR γδ antibodies are currently available for domestic bovid (BO) cross-reactivity testing was only performed for TCR αβ. The number of individuals tested is indicated in the bottom row. Panel C: Carnivora: sea otter (SO), grizzly bear (BE), red panda (RP), Siberian tiger (TI), amur leopard (LE). Aves: chicken (CH), Muscovy duck (DU), turkey (TU). Miscellaneous: elephant (EL), equine (EQ), kangaroo (KA), rabbit (RB), porcupine (PO), milk snake (SN), cutthroat trout (TR). As species-specific CD3 and TCR γδ antibodies are currently available for chicken (CH), cross-reactivity testing was only performed for TCR αβ. The number of individuals tested is indicated in the bottom row.

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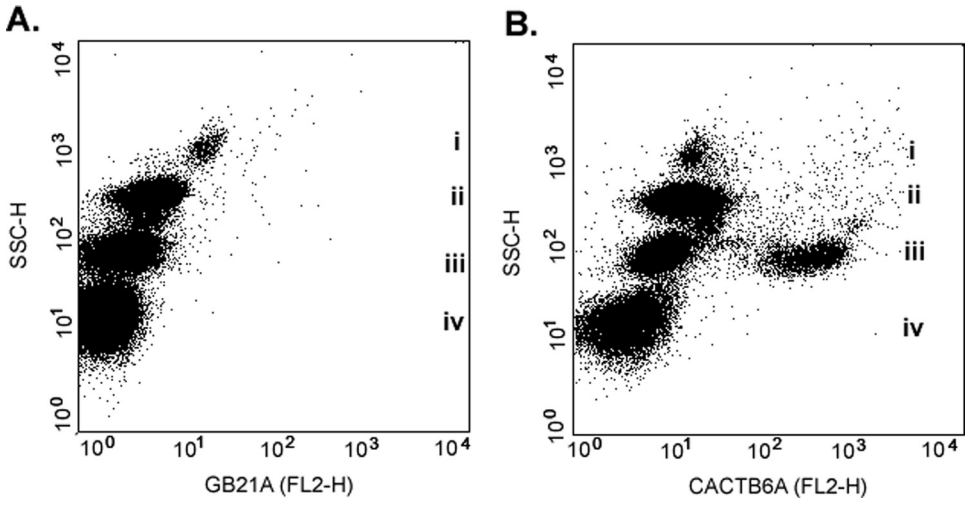

Figure 1. Representative dual parameter, single fluorescent, dot plot profiles illustrating the criteria for acceptance of cross-reactivity. **A**: Anti-bovine TCR γδ (GB21A), negative cross-reactivity. **B**: Anti-bovine TCR γδ (CACTB6A), positive cross-reactivity observed in the lymphocyte population only. Samples are whole blood lysis preparations from horse (*Equus caballus*). Numerals on the figure represent (i) granulocyte, (ii) monocyte, (iii) lymphocyte, and (iv) red blood cell/debris populations. Similar results were observed in all four animals tested. To demonstrate reproducibility, the tests from two randomly selected individuals were duplicated and no significant intra-individual differences were found.

quent analysis consisted of viewing TCR αβ-FITC and TCR γδ-PE populations gated with CD3.

Analysis of Flow Cytometry Data

Data were analyzed using WinMDI (© Joseph Trotter). FSC versus SSC was used for lymphocyte gating and analysis of antibody cross-reactivity.

Results

Forty-four species were used to test 21 mAbs for cross-reactivity; however, because of the opportunistic and sporadic nature of species sampling, the full panel of antibodies was not available at the time of testing for every species. As shown in Table 1 (Panels A–C), 3 anti-TCR αβ, 8 TCR γδ, and 4 CD3 mAb identified epitopes conserved on orthologous molecules in one or more species including the endangered swift fox (*Vulpes velox*) and the threatened woodland caribou (*Rangifer tarandus caribou*). Cross reactivity was observed in 100% of the primates tested (16/16 species) and 54% of the other species tested (15/28 species).

Cross-reactivity with multiple antibodies was found in the Siberian tiger, zebu, warthog, and primate samples (Table 1, Panels A–C); therefore, attempts were made to dual or triple stain these samples. The Siberian tiger blood sample was dual labeled with anti-human CD3 (SP34) and anti-mouse TCR γδ (GL3). However, as the sample was adversely affected during shipping, single positive staining could be detected but dual labeled populations could not be resolved.

Since there are presently no anti-TCR αβ antibodies available for either domestic cattle or zebu, both of these species were tested for cross-reactivity with the anti-TCR αβ antibodies used in this study (Table 1, Panel B). Unfortunately, no cross-reactivity was found. Further testing with the zebu cells revealed cross-reactivity with anti-bovine CD3 (MM1A), anti-bovine TCR γδ (GB21A), and anti-bovine γδ (CACTB6A). Other CD3 and TCR γδ antibodies were not tested in the zebu because of the robust labeling found with domestic cattle antibodies. Dual staining (CD3/TCR γδ) of the single zebu individual demonstrated that CACTB6A γδ T cells represented 11% of the peripheral blood CD3 population whereas GB21A γδ T cells represented 20%. The CACTB6A antibody is known to stain a subpopulation of WC1+TCR γδ cells, thus these numbers are comparable to domestic cattle numbers (6).

In the four warthog (*Phacochoerus africanus)* samples analyzed, cross-reactivity was found with both anti-rat TCR αβ (R7.3) (mean 2% ± 1% of total lymphocytes) and anti-mouse TCR γδ (GL3) (mean 7% ± 2% of total lymphocytes). As no cross-reactive CD3 antibody was found, only single staining could be utilized with these samples and percentages were calculated from the lymphocyte gate (B, T, and NK cells). It is likely that with a dual stained sample gated on CD3, the TCR percentages would have been higher.

In the primates, anti-rat TCR αβ (R7.3) and anti-human TCR γδ (B1.1) costained with CD3 (SP34) in the majority of primate species tested [(Table 1, Panel A), Fig. 2]. F2

None of the mAbs tested identified conserved epitopes on orthologous molecules on cells from alpaca, cat, duck, elephant, leopard, sea otter, rabbit, red panda, sea lion, turkey, or the beluga whale. In addition to this, anti-CD3 CT-3 and SK7, anti-TCR αβ CA15.8G7 and WT31, and anti-TCR γδ 11F2 and CA20.8H1 reacted only when tested on the species they were produced for. These antibodies appear to recognize species restricted epitopes.

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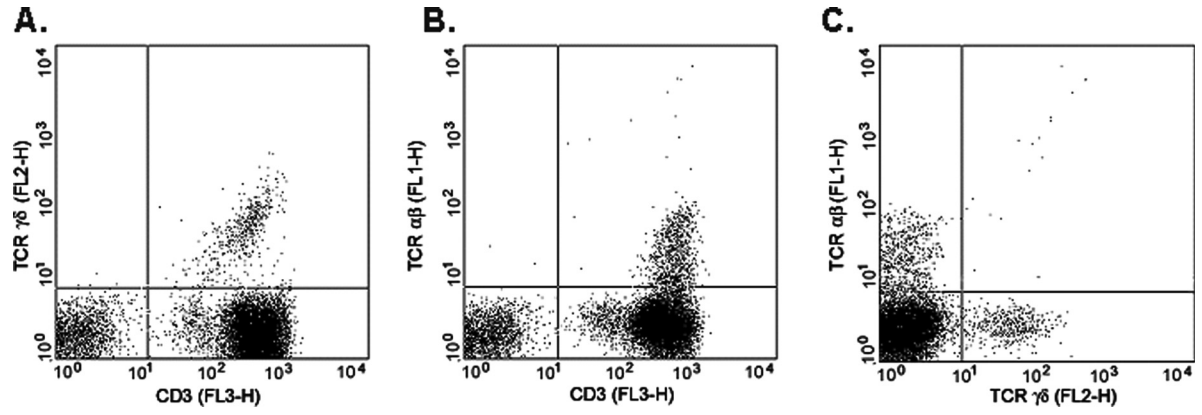

Figure 2. Representative dual parameter, dual fluorescent, dot plot profiles illustrating **A**: Lymphocyte gated population representing costaining with TCR γδ/CD3. **B**: Lymphocyte gated population representing costaining with TCR αβ/CD3. **C**: CD3 gated population representing the mutually exclusive labeling of TCR αβ and TCR γδ. Samples are whole blood lysis preparations from Hamadryas baboon. Similar results were observed in each of the two animals tested. To demonstrate reproducibility, the tests from both individuals were duplicated and no significant intra-individual differences were found.

Discussion

Distantly Related Species Cross-Reactivity—CACTB6A, GL3

Many mAb cross-reactions including bovine/zebu/musk ox/moose/caribou, human/primate, and canine/fox occurred in closely related species, Table 1 (Panels A–C); results such as these were expected. Nevertheless, some evolutionarily distant species also showed clear positive flow cytometric staining patterns; for instance, anti-human CD3 (SP34) reacted with Siberian tiger cells and anti-human TCR αβ (T10B9) reacted with porcupine, Table 1 (Panels A–C).

The antibodies exhibiting the broadest cross-reactivity between taxa in this study were the anti-bovine TCR γδ (CACTB6A) and anti-mouse TCR γδ (GL3). Table 1 (Panels B and C) demonstrate that anti-mouse TCRγδ (GL3) cross-reacts with lymphocytes in both warthog and pygmy goat. GL3 represents an antibody cross-reaction between rodents (mouse) and artiodactyls (warthog and pygmy goat).

Mice are common research animals for which many antibody reagents are available. The existence of many mouse antibodies, which recognize several different epitopes increases the probability that an evolutionary distant cross-reaction can be found, therefore, the GL3 mAb cross-reactivity is not entirely unexpected. In this case, knowledge of the specific epitope recognized by the antibody would allow a more thorough analysis of antibody reactivity. Unfortunately, the specific epitope for GL3 is not known (17). This highlights the importance of antibody epitope identification studies to verify that the cross reactive mAb recognize the bona fide orthologue. Although it will be difficult to validate specificity for the warthog, multiple anti-γδ mAbs are available in cattle (18) that can be used to verify GL3 that recognizes an epitope expressed on γδ T cells.

The second broadly cross-reactive mAb in this study is the anti-bovine TCR γδ, CACTB6A (Fig. 3). Interestingly, this antibody recognizes a subpopulation of γδ T cells that also coexpress a molecule called WC1. WC1 is a transmembrane protein and a member of the scavenger receptor cysteine rich family of molecules that has been found in artiodactyls such as sheep, cattle, pigs, and camelids but has yet to be identified in other species (8,18). Bovine and swine γδ subpopulations are characterized by the presence or absence of the WC1 and WC1 orthologues, respectively. Current hypotheses suggest that this γδ T cell molecule may be involved in cell signaling (19,20), innate immunity in young animals (21), or act as homing receptor for different tissues (7,8,22,23).

Anti-TCR γδ CACTB6A positively reacted with 4 of 25 species including 1 marsupial (kangaroo), 1 perissodactyl (horse), and 2 artiodactyls (zebu and moose). To our knowledge, CACTB6A is the first antibody identified that may recognize γδ T cells in the horse. CACTB6A has also been found to cross react with γδ T cells in many other species in the suborder Ruminantia including goat, water buffalo, Cape buffalo, white-tail deer, mule deer, black tail deer, elk, and bison (W.C. Davis unpublished observations).

The taxonomic tree in Figure 3 demonstrates that the broad cross-reactivity of anti-bovine CACTB6A is likely due to the fact that most species are closely related within the superorder Ruminantia (18). Considering the distant relationship between horse, kangaroo, and cattle, whose most recent ancestor is in the class Mammalia, another hypothesis must be generated to explain cross-reactivity. It is possible that CACTB6A recognizes a highly conserved epitope on the TCR γ chain in each species; however, further studies are required to demonstrate that the epitope identified is expressed on bona fide γδ T cell receptors.

Cross-Reactive Cell Populations—Quantification

Some organisms, such as the domestic cattle (*Bos taurus*) and zebu cattle (*Bos indicus*), demonstrate excellent antibody cross-reactivity patterns due to close species relationships. Analysis of the double stained CD3/γδ and CD3/CACTB6A

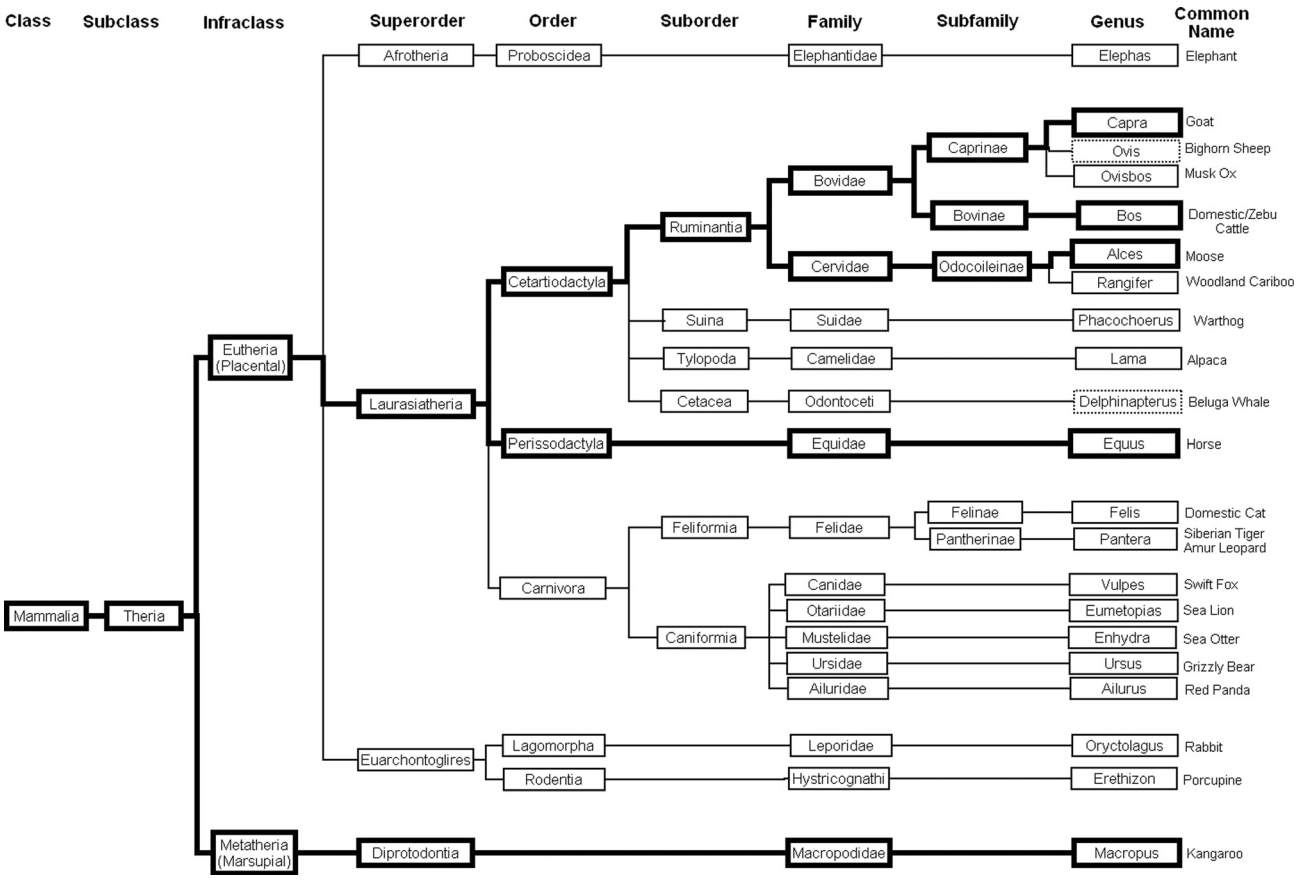

Figure 3. Taxonomic tree demonstrating anti-bovine CACTB6A cross-reactivity in all the mammalian species from this study. Dark boxes indicate cross-reactivity, dashed boxes indicate species not tested with this antibody. (Although CACTB6A cross-reactivity with goats was not tested in this study, the antibody is cross-reactive, W. C. Davis – unpublished observation).

zebu samples indicates that zebu T cell percentages are comparable with that of domestic cattle (9,24,25).

Considering other closely related species, domestic swine and warthogs (suborder Suina) are expected to have similar percentages of αβ and γδ T cells. Domestic swine are categorized as γδ high species, with peripheral blood percentages reported from 12 to 49% (5). Unfortunately, the warthog samples could not be tested with anti-swine antibodies as these antibodies were not included in the initial experimental design. Furthermore, due to the opportunistic nature of sampling, it was difficult to predict from which species blood samples would be received. Future studies involving warthog populations should involve the examination of cross-reactivity with domestic swine antibodies.

Although anti-swine antibodies were not tested, the cross-reactivity results from the warthog samples have nonetheless proved revealing. As single labeling of warthog samples with anti-mouse (GL3) stained 7% ± 2% of the TCR γδ cells in the lymphocyte gate (B, T, and NK cells), it is conceivable that with a dual stained sample gated on CD3, the TCR percentages would be higher. Efforts must be made to find an anti-warthog CD3, which will result in higher accuracy when calculating γδ T cell percentages.

In the case of the warthog samples stained with anti-rat TCR αβ (R7. 3), only 2% of the cells were stained. There are two possible explanations for this outcome: positive signal is the result of nonspecific staining or antibody R7.3 identifies a particular TCR αβ subpopulation in the warthog. As nonspecific staining was ruled out of all leukocyte gates by viewing an ungated histogram an argument can be made for the latter. Notably, anti-rat TCR αβ (R7.3) demonstrates a similar pattern of staining in the primate species tested. Further two color testing of specificity with anti-human αβ T cells mAbs will show whether R7.3 reacts with human αβ T cells.

Early in the study, three antibodies were found that cross-react with primate TCR αβ, γδ, and CD3. Anti-human TCR γδ (B1.1) exhibited positive staining in the lymphocyte population in addition to the anti-rat TCR αβ (R7.3) and anti-human CD3 (SP34) antibodies. Triple staining experiments demonstrated that both TCR αβ and TCR γδ costained with CD3 forming mutually exclusive populations, Figure 2. Antibodies cross-reacting with primate CD3 and TCR αβ have been previously been found in organisms such as cynomolgous macaques (26). This study demonstrates for the first time an antibody that recognizes the γδ TCR in 14 species of primates.

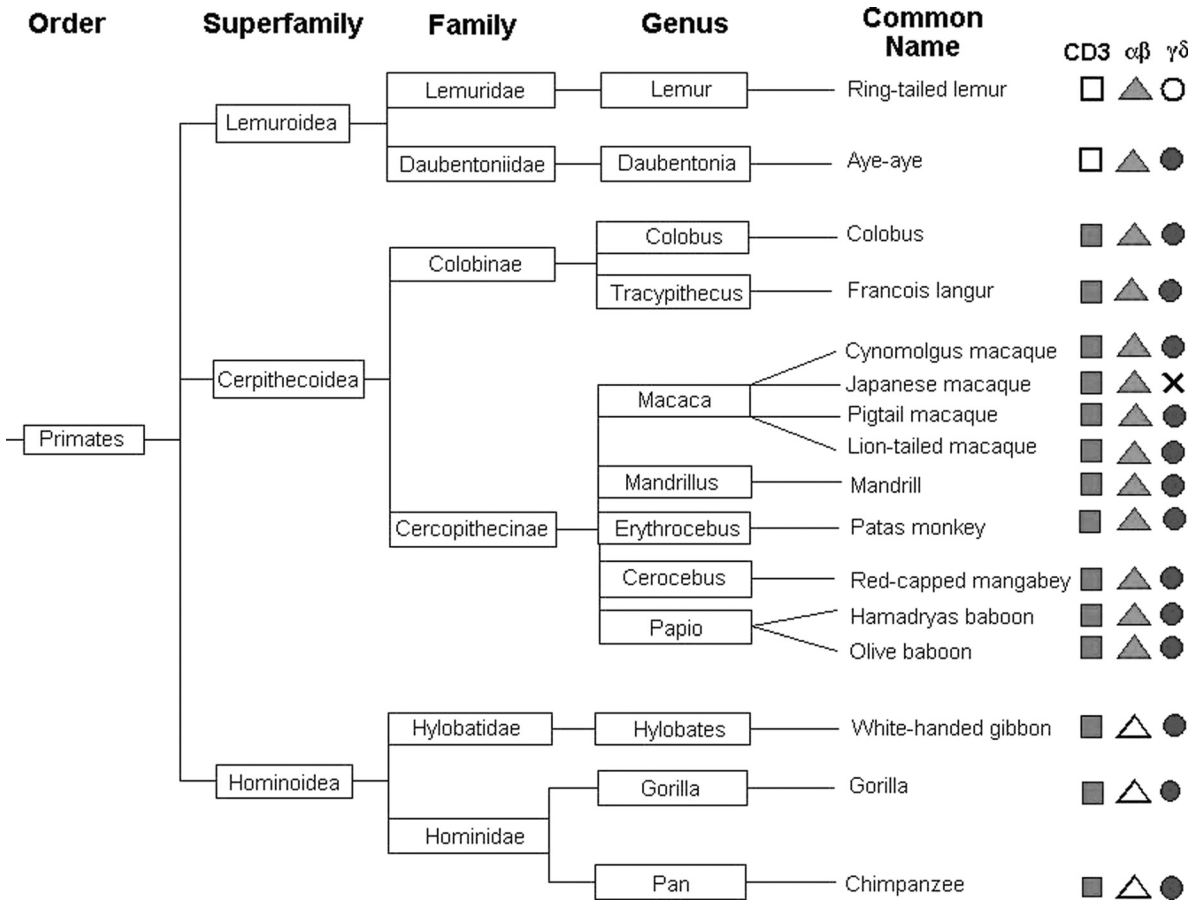

Figure 4. Taxonomic tree of the order Primates demonstrating the cross-reactivity profile with anti-rat TCR αβ (R7.3), anti-human TCR γδ (B1.1) costained with CD3 (SP34). Colored shapes indicate positive cross-reactivity, white shapes indicate no cross-reactivity. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

As primates are important research animals, the primate portion of the study shifted in focus to examine the extent of anti-TCR γδ B1.1, anti-TCR αβ R7.3, and anti-CD3 SP34 mAb cross-reactivity across many families in the order Primates (Fig. 4). Antibody cross-reactivity follows a genus specific pattern of staining; of the 12 genera tested, all were cross-reactive except the one or two taxonomic groups furthest from the species that the antibody was created for. For example, anti-human CD3 and anti-human TCR γδ were cross-reactive with all genera except those within the superfamily Lemuroidea, which is least related to humans. The opposite is demonstrated clearly in Figure 4 with the anti-rat TCR αβ antibody (R7.3); this antibody reacted with all genera except those in the superfamily Hominoidea, which is least closely related to rat. These data clearly demonstrate cross-reactive antibody molecules following a taxonomic structure.

Quantification of the 27 triple stained primate blood samples demonstrated that CD3+TCR γδ+ cells represent a mean of 7% ± 4% and CD3+TCR αβ+ cells represent a mean of 22% ± 11% of the CD3 labeled population. As primates are known to have ~95% peripheral blood αβ T cells (27), these data indicate that the cross-reactive anti-rat TCR αβ R7.3 may recognize a subpopulation of CD3 positive primate TCR αβ cells as it does in the warthog. Further examination of primate TCR αβ percentages indicates that R7.3 cross-reactivity also follows a genus specific pattern within the primate order (Fig. 4). No cross-reactivity was found within members of the family Hominoidea ($n = 4$). Members of the family Cercopithecoidea exhibited a mean 17% CD3+TCR αβ+ cells ($n = 21$, range 6–25%) and the family Lemuroidea exhibited a mean 33% TCR αβ+ T cells ($n = 2$, range 21–46%) gated on the lymphocyte population. It is worth noting that if a CD3 antibody were available for the family Lemuroidea, the TCR αβ populations would exhibit even higher numbers because of percentages calculated within a smaller CD3 gate. These data demonstrate that ancestral primates appear to have the highest amount of cross-reactivity with anti-rat TCR αβ (R7.3).

A point to consider regarding TCR subpopulation cross-reactivity is the fluorochrome on the primary labeled antibody. The R7.3 antibody used in this study was labeled with FITC. Sopper et al. (1997) noted in a rhesus cross-reactivity study that antibodies conjugated with FITC sometimes show very reduced or negative staining (28). If it is found that FITC

labeling does influence R7.3 cross-reactivity, perhaps a PE labeled R7.3 mAb would be a more useful candidate for enumeration studies.

This study found cross-reactive antibodies in 16 species of primates.

Because of the small sample size in this study a new experiment is being conducted to examine cell percentages in statistically relevant samples of the primates represented in this study.

Conclusion

This study has contributed to expanding the animal homologues section of the HLDA (16) by finding new cross-reactive anti-TCR and CD3 antibodies for 31 species. In addition, cross-reactive anti-TCR αβ and γδ antibodies costain with CD3 in five of six families of primates.

This research provides an essential cross-reactivity resource for investigators studying T cell immunity in less well-studied species. Until mAb production becomes more straightforward and cost effective, cross-reactivity studies will continue to be essential for expanding the repertoire of mAb currently in use. New reagents simplify immunity research in minor species and allow for an increased understanding of the immune system.

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