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A Monoclonal Antibody Selection for Immunohistochemical Examination of Lymphoid Tissues From Non-human Primates

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SUMMARY Non-human primates (NHPs) offer valuable animal models for basic research into human diseases and for the preclinical validation of new therapeutics. Detailed in situ examination of the involved cell types using immunohistochemistry is often hampered by the lack of cross-reactive antibodies (Abs). In the current study, we have tested a large panel of monoclonal antibodies raised against human leukocyte differentiation and activation markers for cross-reactivity on cryosections of lymphoid tissue from six NHP species. In total, we have tested 130 Abs against 69 antigens expressed in tissues from one great ape species (chimpanzee/*Pan troglodytes*), two Old World species (rhesus macaque/*Macaca mulatta* and cynomolgus macaque/*Macaca fascicularis*), and three New World species (common marmoset/*Callithrix jacchus*, cotton-top tamarin/*Saguinus oedipus*, and owl monkey/*Aotus trivogatus*). We have found a large panel of cross-reactive Abs: 93 of 102 (91%) in chimpanzee, 97 of 125 (78%) in rhesus macaque, 70 of 109 (64%) in cynomolgus macaque, 69 of 116 (60%) in common marmoset, 40 of 81 (49%) in cotton-top tamarin, and 35 of 80 (44%) in owl monkey. The availability of a reliable panel of cross-reactive markers is important to gaining further insight into immunological processes in disease-affected tissues from NHP species. (J Histochem Cytochem 57:1159–1167, 2009)

KEY WORDS

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NON-HUMAN PRIMATES (NHPs) provide important experimental models of immune-mediated inflammatory disorders in biomedical research, because of the outbred nature and the phylogenetic proximity with humans. Recent milestones in biotechnology have resulted in the development of species-specific therapeutics, such as humanized antibodies (Abs) or fully human Abs (Lutterotti and Martin 2008), which need to be evaluated in relevant disease models before they can be tested in patients. In cases in which rodent models are precluded by the lack of sufficient cross-reactivity, NHP models provide a useful alternative ('t Hart et al. 2004).

A variety of NHP species are used in biomedicine. Although biomedical research in great apes has been banned from Europe for a few years, chimpanzees have

been and still are productively used as models for human immunodeficiency virus (HIV) infection (Rutjens et al. 2003; Heeney et al. 2006) and for the development of certain vaccines, e.g., against hepatitis (Bukh 2004). Old World monkeys, such as rhesus macaques and cynomolgus macaques, are frequently used as experimental models in transplantation (Kean et al. 2006; Haanstra et al. 2007), tuberculosis (Capuano et al. 2003), malaria (Moreno et al. 2008), rheumatoid arthritis (Vierboom et al. 2007), and HIV infection (Ambrose et al. 2007). New World monkeys serve as important tools, for e.g., for Parkinson's disease (Eslamboli 2005), idiopathic colitis (Warren and Watkins 1994), malaria vaccine research (Herrera et al. 2002), and for modeling multiple sclerosis (MS) in experimental autoimmune encephalomyelitis (EAE) ('t Hart et al. 2000).

Studies of immunopathogenic mechanisms in each of these experimental models rely on the availability of reagents and techniques for ex vivo immunodiagnosis, such as flow cytometry analysis or immunohistochemistry. Previously, the cross-reactivity in flow cytometry

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of anti-human monoclonal antibodies (mAbs) with NHPs has been reported (Neubert et al. 1996; Brok et al. 2001). However, little is known about the cross-reactivity of mAbs on NHP tissue for usage in immunohistochemistry. In this study, we have investigated a set of 130 human-specific mAbs against 69 molecules used in immunohistochemistry for cross-reactivity with lymphoid tissue of six NHP species. The mAbs in this set were reactive against cluster of differentiation (CD) markers, cytokines, and other immunological markers, such as human leukocyte antigen (HLA) and Igs. The selection of CD markers included critical costimulatory molecules against which novel immunotherapeutics, such as CD40 (Laman et al. 1996,2002), are under development. Furthermore, the selection included markers distinguishing leukocyte cell types as T-cells, B-cells, natural killer cells, and macrophages. The selected cytokines, such as interferon-gamma (IFN- γ), interleukin (IL)-12, and IL-17A, play a central role in inflammatory responses. The latter is produced by the newly identified Th17 functional T-cell subset, which is presumed to be critical in several autoimmune diseases, including rheumatoid arthritis and MS (Ivanov and Linden 2009). Furthermore, IL-17A may be involved in neutrophil recruitment (Witowski et al. 2000; Yu et al. 2007).

Materials and Methods

Tissues

For the detection of cross-reactivity, lymph node and/or spleen samples were used. Material from the African chimpanzee (*Pan troglodytes*), as a representative species of the great apes, was used. The selected Old World species were the rhesus macaque (*Macaca mulatta*) and the cynomolgus macaque (*Macaca fascicularis*). As representatives of New World species, the common marmoset (*Callithrix jacchus*), the cotton-top tamarin (*Saguinus oedipus*), and the owl monkey (*Aotus trivivatus*) were chosen. All animals, except the cynomolgus macaques, were obtained from the outbred, genetically typed, breeding colonies at the Biomedical Primate Research Center (BPRC), Rijswijk, The Netherlands. The breeding policy is aimed at prevention of inbreeding using genetic typing and the regular introduction of new animals. Cynomolgus macaques were obtained from licensed breeders. Tissue samples were collected at necropsy and were snap frozen in liquid nitrogen and stored at -80°C until use. Human tonsil served as positive control tissue.

mAbs

All mAbs described in this study are commercially available unless stated otherwise. Samples were kindly donated by the participating companies: Becton Dickinson (BD) Bioscience (San Jose, CA); Dako (Glostrup, Denmark); Sanquin (Amsterdam, The Netherlands); Diaclone (Flem-

ing, France); Serotec (Düsseldorf, Germany); Abcam (Cambridge, MA); Cymbus Biotech (Hampshire, UK); Beckman Coulter (Fullerton, CA); Bender MedSystems (Vienna, Austria); Genzyme (Framingham, MA); Sanbio (Uden, The Netherlands); Innogenetics (Gent, Belgium); Santa Cruz Biotechnology (Santa Cruz, CA); U-Cytech (Utrecht, The Netherlands); Ebioscience (San Diego, CA); Nordic Labs (Tilburg, The Netherlands); Biosource (Nivelles, Belgium); and BMA Biomedicals (Augst, Switzerland). Other mAbs were kindly provided by Dr. D. Boraschi (Centro Ricerche Sclavo; Siena, Italy), Dr. R. Noelle (Dartmouth Medical School; Lebanon, NH), and Prof. Dr. G. Opdenakker (Rega Institute; Leuven, Belgium). BD Bioscience and Cymbus Biotech provided isotype-matched Abs as controls. All specificities and clone names are listed in Table 1.

Immunohistochemistry

Immunohistochemistry was performed as described in detail previously (Laman et al. 1998). Briefly, 6- μm frozen spleen and lymph node sections were cut and thawed and mounted on glass slides. Slides were kept overnight at room temperature, which is below the optimum temperature for most proteolytic enzymes, in a humidified atmosphere to allow controlled drying of the tissue. After the slides were air dried for 1 hr, they were fixed either in fresh acetone or 4% paraformaldehyde in PBS (pH 7.4), both containing 0.02% (v/v) H_2O_2 . Acetone-fixed slides were air dried for 10 min and subsequently washed in PBS, whereas paraformaldehyde-fixed slides were immediately washed in PBS. Tissue sections were incubated with primary Abs overnight at 4°C in a humidified atmosphere. Abs were tested in a dose titration that was based on the recommended dilution for human tissue and included a $10\times$ dilution of the provided Ab. The same dose titration was used for all species. If the staining in a given case was doubtful, a higher concentration was tested if sufficient Ab was available. Incubations with secondary and tertiary reagents were performed for 1 hr at room temperature. Between incubation steps, the slides were washed twice with PBS. Detection of primary unlabeled mouse anti-human Ab was followed by incubation with rabbit anti-mouse-Ig-horseradish peroxidase (HRP) (Dako), or in a three-step staining with anti-mouse-Ig-biotin (Dako) and HRP-labeled avidin-biotin-complex (Dako). HRP activity was revealed by incubation for 10 min at room temperature with 3-amino-9-ethyl-carbozole (Sigma, Zwijndrecht, The Netherlands), leading to a bright-red precipitate. Some positive stainings, i.e., IL-17A, IFN- γ , and CD40, were enhanced with the tyramide signal amplification kit (Invitrogen; Carlsbad, CA). Human tonsil was used as positive control tissue. Incubation with isotype-matched primary Abs of irrelevant specificity and omission of the primary Ab served as negative controls.

Table 1 Cross-reaction of anti-human antibodies on cryosections of non-human primate lymphoid tissue

CD marker	Clone	Provider ^a	Old World monkey			New World monkey		
			Great ape Chimpanzee	Rhesus macaque	Cynomolgus macaque	Common marmoset	Cotton-top tamarin	Owl monkey
CD2	CLB-T11/1 (6G4)	3	+	+	+	-	-	-
	RPA-2.10	1	+	+	+	-/+	-	-/+
	S5.2	1	+	+	+	+	+	-
	B-E2	4	+	+	+	+	+	-
	LT2	5	+	+	+	-	-	-
CD3	SK7	1	+					
	FN-18	6	+	+				
	CLB-T3/2 (16A9)	3	+	+	-	-/+	+	-
	SP34	1	+	+	-/+	-	-	-
CD4	SK3	1		-	-	-		
	MT477	1		-	-	-		
	CLB-T4/2 (6D10)	3	+	-	-	-	-	+
	SK3, SK4	1	+	-	-	-	-	+
	MT310	2	+	-	-	-	-	+
CD5	UCHT2	7	+	+	+	+	-/+	+
CD7	B-F12	4	+	+	-/+	-	+	+
CD8	DK25	2	+	+				
	SK1	1			-	-		
	LT8	5	+	+	+	-/+	-	+
	CLB-T8/4 (4H8)	3	+	+	+	+	-	+
	UCHT4	7	+	+	+	-	+	-
CD10	ALB1	8	+	-	-	+	+	+
CD11a	HI111	1	+	+	+	+	+	+
	G-25.2	1	+	+	+	+	+	+
	25.3	8	+	+	+	+	+	+
	B-B15	4	+	-/+	+	+	+	+
	R7.1	9	+	+	+	+	+	-
CD11b	D12	1	+	+	+	+		
CD13	B-F10	4	+	+	-/+	-/+	+	+
CD14	322A-1 (MY4)	8	+	+	-/+	+		
	B-A8	4	+	-/+	-/+	-	-/+	-
	NKP15	1	-	+	+	-	-/+	-
CD16	3G8	1	+	+	+	+	-	+
	B-E16	4	+	+	-	-	+	-
	L130	1	+	+	+	+	+	-
CD18	CLB-LFA-1/1 (54)	3	+	+	+	+	+	+
	6.7	1	+	+	+	+	+	+
	SJ25C1 (FITC)	1	+	-	-	-	-	-
CD19	4G7	1	+	-	-	-	+	-
	L26	2	+	+	+	+		
CD20	H299 (B1)	8	+	+	+	+	-	-
	B-H20	4	+	-/+	-	-	+	-
	BL13	8	+	+	+	+	+	+
CD21	B-LY4	1	+	+	+	+	+	+
	B-E5	4	+	+	+	+	+	+
	SN3	5	+	-/+	-/+	-	-	-
CD24	B-B10	4	+	+	-	-	-	-
	Tu69	7	-	-	-	-	-	-
	CLB-IL2R/1 (TB30)	3	+	+	-	-	-	-
CD25	2A3	1		+				
	BA5	2	-/+	+	+	-	-	+
	L272	1	+	-	-	-	-	-
CD26	L128	1	+	-	-/+	-	-	-
	CLB-CD28/1 (15E8)	3	+	+				
CD27	L293	1	+	+	+	-	-	-
	B-T3	4	+	+	+	+	+	-/+

Continued on next page

Table 1 (continued)

	Clone	Provider ^a	Old World monkey					
			Great ape Chimpanzee	Rhesus macaque	Cynomolgus macaque	Common marmoset	Cotton-top tamarin	Owl monkey
CD marker								
CD29	TDM29	7	+	+	+	+	+	+
	B-D15	4	+	+	+	+	-/+	+
CD30L	M81	10		+				
CD31	5.6E	8	+	+	+	-	+	-
CD33	D3HL60.251	8		+				
CD34	QBEEnd 10	3	+	-				
CD35	E-11	1	+	+	+	+	+	+
CD38	T16	8	+	-	-/+	-	+	-
	HB7	1	+	-	-	-	-	-
CD40	5C3	1	+	+	-	-	-	
	CLB-14G7	3	+	+				
	B-B20	7	+	+	+	+	+	+
CD44	B-F24	4	+	+	-	-/+	+	+
	NKI-P2	3	+	+	+	+	+	+
	F10-44-2	5	+	-	-	+	+	+
CD45	B-A11	4	+	+	-/+	+	+	-
CD45RA	B-C15	4	+	+	+	+	-	+
CD45RO	UCHL-1	1	+	-				
CD49d	HP2/1	8	+	+	+	-	-/+	-
CD49e	SAM1	8	+	+	+	+	-	-
CD55	CLB-CD97L1	3		-	-	+		
CD56	123C3	11	+	+				
	B-A19	4	+	-/+	-	-	-	-
	N901 (NKH-1)	8	-	-	+	+	+	-
	MEM188	7	-	+	+	-	+	-
	NCAM16.2	1	+	-	+	+	-	-
CD58	L306.4	1	-/+	-	-	-	-	-
	1C3	1	+	+	+	+	+	+
CD62L	FMC46	7	+	-	-	-	-	-
	DREG.55	9	+	+	+	+	-	+
CD64	10.1	1	+	+				
CD68	KP1	2	+	+	+	+		
CD70	CLB-2F2	3	+	+	-/+	-/+		
CD80	M24	12		+	+	+		
CD83	HB15A	8	+	+	+	+		
CD86	1G10	12	+	+	+	+		
	B-T7	4	+	+	-/+	-	+	-
	IT2.2	1	-	+	+	+	-	-
CD95	UB2	8		+		+		
	FAS6	3		+	+	+		
	APO1-1	9	-/+	-/+	+	-	-	-
	DX2	1	+	+	+	-	-	-
	BMS-140	9	+	+	+	-/+	-	-
CD97	CLB-CD97/1	3		+		+		
CD138	BB4	8	+	+	+	-	-	-
	CLB-1D4	3	+	-	-	-	-	-
CD154/CD40L	TRAP1	1	+	+	+	+ ^b		
	24-31	13		+	+	+		
CD169	HSN1 (7D2)	5		+	+	+		
Cytokines								
IFN- α	MC-16	14		+	+	+		
IFN- γ	MD-1	14	+					
	MD-2	14		+	+	+		
TNF- α	61E71	14	-	+	-/+	+		
IL-1 α	Vmp18	15		+		+		
IL-1 β	Vmp20	15		+		+		
IL-2	80-3418-01	10				+ ^b		

Table 1 (continued)

	Clone	Provider ^a	Old World monkey			New World monkey		
			Great ape Chimpanzee	Rhesus macaque	Cynomolgus macaque	Common marmoset	Cotton-top tamarin	Owl monkey
Cytokines								
IL-4	QS-4	14		+	+	+		
	1842-01	10		+	-/+	+		
IL-6	BE-8	15		+	+	+		
IL-10	B-S10	16	+	+	+	+		
IL-12p40/p70	C8.6	1		+	-/+	+		
IL-17A	eBio64CAP17	17		+		+		
Miscellaneous								
HLA-ABC	G46-2.6	1	+	+	+	-	-	-
	W6/32	7	+	+	+	+	+	+
HLA-DP, DQ, DR	CR3/43	2		+	+	+		
HLA-DR	TAL-1B5	2	+	+				
	B-F1	4	+	+	+	+	+	+
	CLB-HLA-DR (1E5)	3	+	+	+	+	+	+
	L243	1	+	+	+	+	+	+
HLA-DQ	SPV-L3	7	+	+	+	+	+	+
IgM	NI179	18			+	+		
IgG	-	19		+	+	+		
MMP-9	2D9	20		+		+		
MRP 14	27E10 (early)	21		+	+	+		

^aProviders: 1, Becton Dickinson; 2, Dako; 3, Sanquin; 4, Diaclone; 5, Serotec; 6, Abcam; 7, Cymbus Biotech; 8, Beckman Coulter; 9, Bender MedSystems; 10, Genzyme; 11, Sanbio; 12, Innogenetics; 13, gift from Dr. R. Noelle; 14, U-Cytech; 15, gift from Dr. D. Boraschi; 16, Santa Cruz; 17, Ebioscience; 18, Nordic Labs; 19, Biosource; 20, gift from Prof. Dr. G. Opendakker; 21, BMA Biomedicals.

^bOnly cross-reactive when paraformaldehyde is used as fixative.

+, cross-reactive; -, no specific staining; -/+, needs more investigation; blank boxes, not tested. CD, cluster of differentiation; HLA, human leukocyte antigen; IFN- γ interferon-gamma, IL, interleukin.

Immunohistochemistry Analysis

Cross-reactivity is expressed as follows: - = no specific staining; -/+ = doubtful and needs more investigation; + = specific staining.

Ethics

All NHP tissues that were used for this study were obtained from purpose-bred animals at the BPRC or purchased from licensed breeders. All tissues were from animals from other experimental studies; no monkeys were sacrificed solely for the purpose of this study. All study protocols were reviewed and approved according to Dutch law on animal experimentation.

Results

Table 1 shows the Abs clustered according to their reactivity with CD markers, cytokines, and miscellaneous markers, such as HLA and Igs. For most markers, we have tested Ab clones obtained from several companies. Cross-reactivity was scored as: no specific staining (-), doubtful (-/+), or specific staining (+). Doubtful means that some staining is observed, but the specificity is unclear. To further investigate the cross-reactivity of these Abs, the titration of the mAb should be extended or another staining method will be necessary, but this was outside the scope of the current study.

In total, we have tested 130 mAbs against 69 markers: 105 clones against 50 CD markers, 13 clones against 11 cytokines, and 12 clones against 8 miscellaneous markers. The isotype controls and primary Ab omission were negative on all tissues tested (Figure 1). All Abs were directed against human antigens, and, as expected, were positive on human control tissue (data not shown). All mAbs were first tested on acetone-fixed tissue. In addition, mAbs of particular interest that failed to stain cryosections were next tested on paraformaldehyde-fixed tissue. For example, an mAb against IL-2 that showed no cross-reactivity on acetone-fixed tissue did display cross-reactivity on paraformaldehyde-fixed common marmoset tissue.

Table 2 summarizes the number and percentages of cross-reactive mAbs tested per specificity per species. Predictably, the highest cross-reactivity was found in chimpanzees: 93 of 102 tested mAbs showed cross-reactivity (91%). A somewhat lower percentage of cross-reactive mAbs was found with the Old World species, i.e., 78% in rhesus macaques and 64% in cynomolgus macaques. An again lower, but still high, percentage of mAbs were found to cross-react with New World species. In the common marmoset, cotton-top tamarin, and owl monkey the cross-reactivity was, respectively, 60%, 49%, and 44%.

Figure 1 shows a selection of cross-reactive mAbs on the spleen of a rhesus macaque and a common

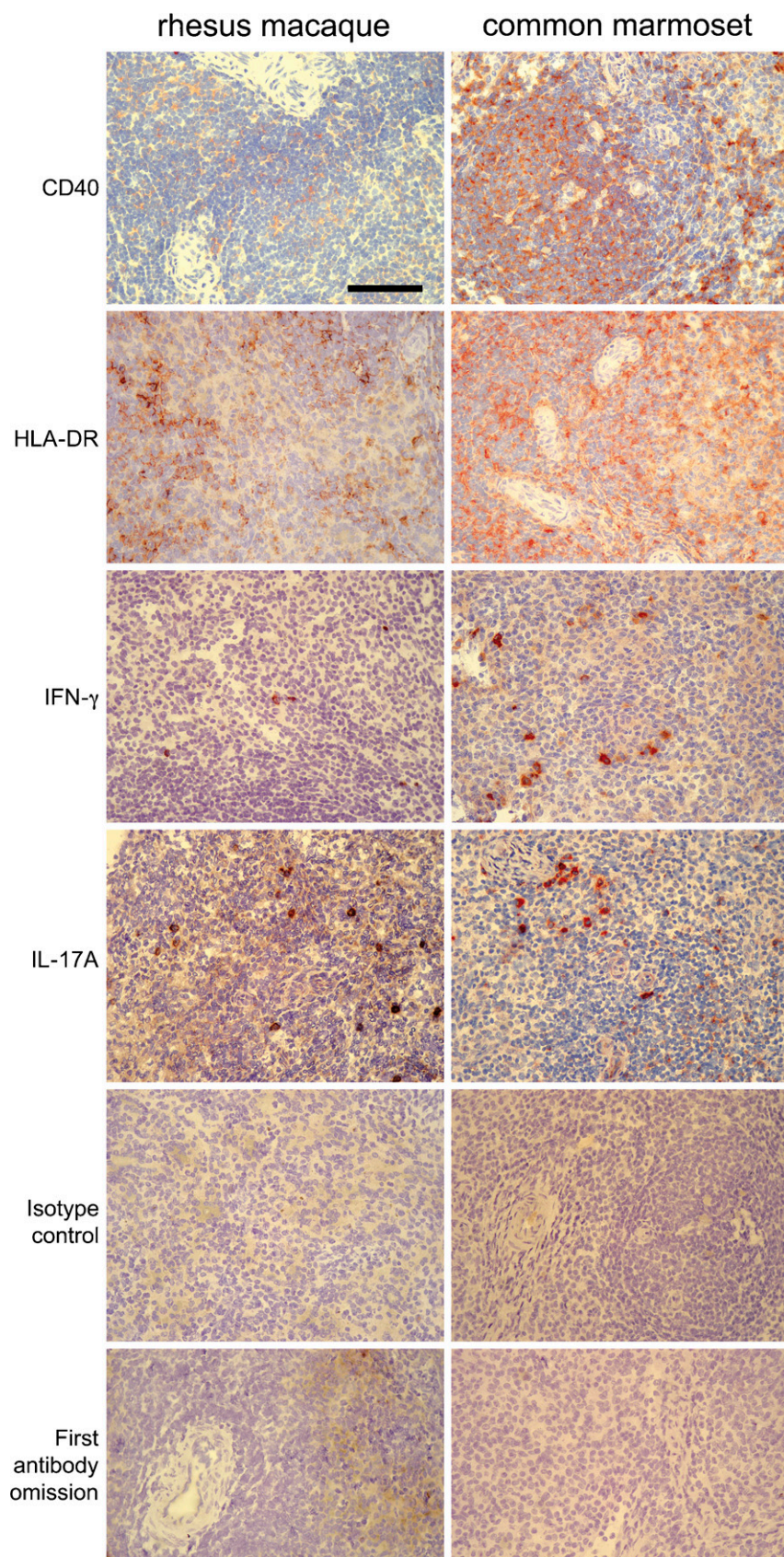


Figure 1 Representative selection of cross-reactive monoclonal antibodies on spleen cryosections of rhesus macaque and common marmoset. Spleen sections were stained for CD40, HLA-DR, IFN- γ , and IL-17A. Controls included isotype control and first antibody omission. Bar = 100 μ m.

Table 2 Number and percentage of cross-reactive monoclonal antibodies

	Great ape		Old World monkey		New World monkey		
	Chimpanzee	Rhesus macaque	Cynomolgus macaque	Common marmoset	Cotton-top tamarin	Owl monkey	
CD markers							
Tested (n)	92	103	91	93	75	74	
+	84	75	55	47	35	30	
+ (%)	91	73	60	51	47	41	
Cytokines							
Tested (n)	3	11	8	12			
+	2	11	5	12			
+ (%)	67	100	63	100			
Miscellaneous							
Tested (n)	7	11	10	11	6	6	
+	7	11	10	10	5	5	
+ (%)	100	100	100	91	83	83	
Total							
Tested (n)	102	125	109	116	81	80	
+	93	97	70	69	40	35	
+ (%)	91	78	64	60	49	44	

marmoset, i.e., mAbs directed against CD40, HLA-DR, IFN- γ , and IL-17A.

Discussion

NHP models are invaluable models for research in biomedicine. For the study of immunopathological mechanisms in these models, mAbs for flow cytometry and immunohistochemistry are indispensable. A selection of mAbs directed against human antigens for flow cytometry analysis of NHP cells has previously been reported (Neubert et al. 1996; Brok et al. 2001). The purpose of the current study was to compose a panel of cross-reactive mAbs that could be used for immunohistochemical analysis of lymphoid tissues from six NHP species. Cross-reactive mAbs can be used for immunopathological examination of NHP disease models, which subsequently will increase our knowledge about NHP models and human diseases.

We have found a large panel of cross-reactive Abs for each species, with the highest level of cross-reactivity in chimpanzees, followed by monkeys of Old World origin and of New World origin. The decreasing level of cross-reactivity reflects the evolutionary distance, which has been estimated at 5 million years for chimpanzees, 25 million years for macaques, and 35 million years for marmosets (Enard and Pääbo 2004). We assessed the cross-reactivity in three categories, but not the intensity of the staining, although we did observe that some clones had more-intense staining than did other clones. We did not include this observation in the results, because a subjective scoring of intensity grades may be confusing. Furthermore, the intensity of a staining can be enhanced today by several techniques, and the intensity may vary between batches.

The markers shown in Figure 1 were chosen because CD40, HLA-DR, IFN- γ , and IL-17A are important in

immune-mediated diseases. CD40 is a member of the tumor necrosis factor receptor family and is expressed on antigen-presenting cells, such as B-cells, macrophages, and dendritic cells, and also on endothelial cells and fibroblasts. CD40 is a costimulatory molecule that binds to CD154 (CD40L) expressed on T-cells. Binding of CD40L to CD40 on B-cells induces B-cell proliferation and isotype switching. Interaction of CD40L with CD40 on monocytes induces the production of cytokines and nitric oxide. Furthermore, CD40 activation can prevent apoptosis of B-cells and monocytes. CD40 is involved in several immune-mediated diseases (Laman et al. 1996; Grewal and Flavell 1998; Vogel and Noelle 1998). Blocking of CD40 prevents EAE in common marmosets (Boon et al. 2001; Laman et al. 2002) and kidney allograft rejection in rhesus macaques (Haanstra et al. 2003). HLA-DR is associated with several disease susceptibilities, such as rheumatoid arthritis and MS (Fernando et al. 2008). In the common marmoset, in which HLA-DR is called Caja-DR, Caja-DRB*W1201 is essential for the activation of Th1 cells that induce EAE (Brok et al. 2000). IL-17A and IFN- γ are both pro-inflammatory cytokines produced by T-cells. IFN- γ is also produced by natural killer cells, and IL-17A may also be produced by non-T-cells such as microglia (Kawanokuchi et al. 2008) and lymphoid tissue inducer-like cells (Takatori et al. 2009). IL-17A is suggested to be involved in several immune-mediated inflammatory diseases, such as rheumatoid arthritis, psoriasis, and MS (Ivanov and Linden 2009). The IL-17-producing T-cells, called Th17, are now being recognized as a functional CD4⁺ T-cell subset in addition to Th1 and Th2, as critically reviewed recently (Steinman 2007). In MS, IL-17A may play a role in blood-brain barrier transmigration of inflammatory cells (Kebir et al. 2007).

Some mAb clones cannot be used for immunohistochemistry, but can be used for flow cytometry analysis (Brok et al. 2001) or vice versa. For example, the anti-CD4 clones MT310 and SK3, as well as the anti-CD3 clone SP34, can be used for flow cytometry analysis of common marmoset mononuclear cells, but cannot be used for immunohistochemistry on common marmoset tissue. Vice versa, clone B-B15 against CD11a can be used for immunohistochemistry of common marmoset tissue, but not for flow cytometry analysis of common marmoset cells. Representation and accessibility of Ab epitopes on live cells in cell suspensions can be quite different from that in frozen sections of solid tissue subjected to fixation, even with relatively mild fixatives such as acetone (Laman et al. 1991). Another explanation is that the activation and differentiation status of leukocytes might differ between blood and lymphoid tissue.

Although rhesus macaques and cynomolgus macaques are closely related Old World species, differences in cross-reactivity can be found. For example, the anti-CD3 clone CLB-T3/2 showed cross-reactivity in rhesus macaque tissue, but not in cynomolgus macaque tissue. Differences between New World species were also observed. As an example, clone CLB-T8/4 against CD8 showed cross-reactivity in common marmoset and owl monkey tissue, but not in cotton-top tamarin tissue. In contrast, clone UCHT4 directed against CD8 showed cross-reactivity in cotton-top tamarin tissue, but not in common marmoset and owl monkey tissue. This demonstrates that although species are closely related, differences in epitope conservation do occur.

Seven of the 69 markers seem to be highly conserved between humans and NHPs. Antibodies directed against CD11a, CD18, CD21, CD29, CD35, HLA-DR, and HLA-DQ were cross-reactive with tissue of all six NHPs. CD21 is the complement receptor 2 and CD35 is the complement receptor 1, suggesting a conservation of complement receptors between humans and NHPs. CD21 is expressed on B-cells and is the receptor for Epstein-Barr virus (EBV). Conservation of CD21 suggests that these NHPs are susceptible to infection with EBV-related herpes viruses, which is indeed compatible with the literature (Johannessen and Crawford 1999). CD11a and CD18, which together form lymphocyte function-associated antigen-1, as well as the very late antigen-4 component, CD29, are expressed on leukocytes and are involved in adhesion. This may suggest a conservation in the adhesion system between humans and NHPs.

For some markers, no cross-reactive mAbs were found for a species. For example, no cross-reactive anti-CD3 or anti-CD4 mAb could be found for the common marmoset. This was expected because in earlier studies, it was observed that Abs against human CD3 did not cross-react with rhesus monkeys, necessitating the generation of a specific Ab, known as FN18 (Nooij et al. 1986). A

polyclonal Ab that recognizes multiple epitopes may overcome the problem of no cross-reactivity with mAbs. For example, a polyclonal Ab against CD3 (Dako) can be used to stain common marmoset tissue. To obtain Abs that recognize these species-specific epitopes, new Abs are being developed. For instance, Ito and colleagues recently generated mAbs recognizing common marmoset lymphocytes (Ito et al. 2008).

In summary, we have identified a large panel of cross-reactive mAbs for in situ analysis of NHP lymphoid tissues and organs affected by inflammation, infection, or tumors. These mAbs will serve as important tools in future investigations of leukocyte differentiation and activation status in NHPs and will aid in dissecting the mechanisms underlying the efficacy of novel vaccination and immunotherapeutic regimens.

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