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Comparison and Characterization of Immunoglobulin G Subclasses among Primate Species

MICHAEL H. SHEARER,[1] ROBYN D. DARK,[2] JAMES CHODOSH,[3] AND RONALD C. KENNEDY[1]*

Departments of Microbiology and Immunology, University of Oklahoma Health Sciences Center,[1] and Ophthalmology and Molecular Pathogenesis of Eye Infection Research Center, Dean A. McGee Eye Institute,[3] Oklahoma City, Oklahoma 73104, and East Central University, Department of Biology, Ada, Oklahoma 74820[2]

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Little information is available on the immunoglobulin G (IgG) subclasses expressed in the sera of nonhuman primate species. To address this issue, we compared the IgG subclasses found in humans (IgG1, IgG2, IgG3, and IgG4) to those of nonhuman primates, such as baboons and macaques. Cross-reactive antihuman IgG subtype-specific reagents were identified and used to analyze purified IgG from sera by solid-phase enzyme-linked immunosorbent assay. Protein A-purified human IgG obtained from sera was composed of IgG1, IgG2, IgG3, and IgG4, whereas baboon and macaque IgG was composed of IgG1, IgG2, and IgG4. Protein G-purified human IgG was composed of IgG1, IgG2, IgG3, and IgG4, whereas baboon and macaque IgG was composed of IgG1, IgG2, and IgG4. To test the possibility that baboon and macaque IgG3 is actually present, but is outcompeted for binding to proteins A and G by the other more abundant IgG subclasses, we repurified the IgG from sera that did not bind either protein A or protein G. We found a baboon IgG3 population in the sera that did not bind protein A, but bound protein G. No IgG3 subtype was detectable in macaque sera. These data suggest that baboon sera, like human sera, contain four IgG subtypes, whereas macaque sera exhibit only three of the human subclass analogs. In addition, the IgG subtype-specific reagents were shown to be useful in determining the IgG subclass distribution following vaccination of baboons with hepatitis B surface antigen.

The use of animals to model human disease has long been accepted as a substitute for using humans (reviewed in references 24 and 37). The value of animals to the immunologist lies in their ability to produce immunological responses to infection and immunization that are similar to those of humans. Small animals, such as mice, rats, and rabbits, are commonly used to evaluate the immunogenicity of vaccines and can provide important and cost-effective information in large experimental groups. However, small experimental animals may not be good predictors of human responses. In general, those animals most closely related to humans mimic more accurately the human disease state and immunological response to infection and vaccination (24).

Phylogenetically, the great apes are most closely related to humans (reviewed in reference 37). The great apes include chimpanzees, orangutans, gorillas, and gibbons. Next in evolutionary distance are the Old World monkeys, which include mandrills, savannah baboons, gelada baboons, mangabeys, African green monkeys, and macaques. The most distantly related primate species compared to humans are the New World monkeys, which include the marmosets, *Aotus* monkeys, capuchin monkeys, and squirrel monkeys.

One of the best-studied hominid nonhuman primates is the chimpanzee. Chimpanzees represent valuable models of human disease. Examples of situations in which chimpanzees have been used to model human infection and disease include the human hepatitis A, B, and C viruses; human immunodeficiency virus (HIV); respiratory syncytial virus (RSV); and leprosy (1, 4, 9, 20, 22, 36). A variety of Old and New World monkeys are susceptible to infection with various human pathogens, but the issues of pathological consequence and mimicry of the human disease state are less clear. Examples of these include hepatitis A virus, hepatitis E virus, Epstein-Barr virus, RSV, pertussis, measles virus, anthrax, *Helicobacter pylori*, group B streptococcus, malarial parasites, HIV or simian immunodeficiency virus (SIV), and Ebola virus (3, 4, 6, 10, 12, 14, 15, 18, 19, 21, 25, 27–30, 41, 43, 46, 47, 51, 53, 57, 58).

Although the great apes are the most suitable model for studies of the safety and efficacy of vaccines and treatment modalities destined for human use, issues of cost, availability, and endangered species status make the use of great apes less feasible. Thus, Old World monkeys represent an attractive alternative.

In this study, we compare human immunoglobulin G (IgG) subclasses to those of two Old World monkeys, the baboon (*Papio cynocephalus*) and the macaque (*Macaca fascicularis*). By utilizing anti-human IgG subtype cross-reactive reagents and a combination of affinity purification methods, we demonstrate that, similar to humans, baboons exhibit the IgG1, IgG2, IgG3, and IgG4 subclasses, while macaques exhibit only IgG1, IgG2, and IgG4. In addition, we utilized the cross-reactive IgG subtype reagents to examine the humoral immune response to vaccination with hepatitis B surface antigen (HBsAg) in baboons. Our results indicate that the predominant antibodies with responses to HBsAg (anti-HBs) were IgG1 and IgG2, with the detection of some IgG4.

MATERIALS AND METHODS

Direct ELISA. Briefly, individual wells of 96-well microtiter plates were coated in triplicate with 50 μl of purified human IgG myeloma subtypes (The Binding Site Limited, San Diego, Calif.; Calbiochem-Novabiochem Co., San Diego, Calif.) at a concentration of 4 μg/ml in borate-buffered saline (BBS). Three different sets of individual myeloma proteins were examined. After coating, nonspecific binding sites were blocked with 5% normal goat serum (NGS) diluted in BBS, followed by washing the wells with BBS supplemented with 0.05% Tween 20 (BBS-T). Fifty microliters of serial dilutions of four horseradish peroxidase (HRP)-labeled anti-human IgG subtype-specific reagents (The Binding Site Limited and Calbiochem-Novabiochem Co.) diluted in NGS was then added to the

* Corresponding author. Mailing address: Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, 800 N. Research Parkway, Suite 462, Oklahoma City, OK 73104. Phone: (405) 271-5630. Fax: (405) 271-6339. E-mail: ronald-kennedy@ouhsc.edu.

TABLE 1. Reactivity of HRP-labeled anti-human IgG subtype reagents with purified human myeloma proteins[a]

Probe[b]	Anti-IgG antibody	Coat	OD_{410} with myeloma[c]			
			IgG1	IgG2	IgG3	IgG4
1	1	1	0.225 (0.011)[d]	0.039 (0.002)	0.009 (0.000)	0.034 (0.002)
		2	0.188 (0.009)	0.008 (0.000)	0.150 (0.008)	0.024 (0.001)
		3	0.226 (0.011)	0.007 (0.000)	0.012 (0.001)	0.031 (0.002)
	2	1	0.018 (0.001)	0.574 (0.029)	0.053 (0.003)	0.023 (0.001)
		2	0.012 (0.001)	0.250 (0.013)	0.019 (0.001)	0.019 (0.001)
		3	0.024 (0.001)	0.271 (0.014)	0.017 (0.001)	0.018 (0.001)
	3	1	0.010 (0.001)	0.013 (0.001)	1.798 (0.090)	0.019 (0.001)
		2	0.010 (0.001)	0.010 (0.001)	2.000 (0.100)	0.054 (0.003)
		3	0.028 (0.001)	0.007 (0.000)	2.000 (0.100)	0.011 (0.001)
	4	1	0.010 (0.001)	0.025 (0.001)	0.021 (0.001)	1.072 (0.054)
		2	0.008 (0.000)	0.013 (0.001)	0.019 (0.001)	0.757 (0.038)
		3	0.013 (0.001)	0.017 (0.001)	0.011 (0.001)	0.875 (0.044)
2	1	1	0.857 (0.043)	0.166 (0.008)	0.050 (0.003)	0.112 (0.006)
		2	0.740 (0.037)	0.016 (0.001)	0.031 (0.002)	0.068 (0.003)
		3	0.804 (0.040)	0.013 (0.001)	0.035 (0.002)	0.128 (0.006)
	2	1	0.009 (0.000)	0.315 (0.016)	0.015 (0.001)	0.016 (0.001)
		2	0.012 (0.001)	0.076 (0.004)	0.013 (0.001)	0.014 (0.001)
		3	0.004 (0.000)	0.075 (0.004)	0.005 (0.000)	0.015 (0.001)
	3	1	0.009 (0.000)	0.009 (0.000)	0.179 (0.009)	0.013 (0.001)
		2	0.013 (0.001)	0.012 (0.001)	0.225 (0.011)	0.009 (0.000)
		3	0.008 (0.000)	0.006 (0.000)	0.227 (0.011)	0.001 (0.000)
	4	1	0.017 (0.001)	0.020 (0.001)	0.016 (0.001)	0.851 (0.043)
		2	0.020 (0.001)	0.016 (0.001)	0.019 (0.001)	0.513 (0.026)
		3	0.015 (0.001)	0.016 (0.001)	0.011 (0.001)	0.661 (0.033)
3	1	1	2.000 (0.100)	0.115 (0.006)	0.015 (0.001)	0.104 (0.005)
		2	2.000 (0.100)	0.016 (0.001)	0.024 (0.001)	0.054 (0.003)
		3	2.000 (0.100)	0.010 (0.001)	0.020 (0.001)	0.113 (0.006)
	2	1	0.018 (0.001)	0.995 (0.050)	0.017 (0.001)	0.044 (0.002)
		2	0.023 (0.001)	0.144 (0.007)	0.014 (0.001)	0.030 (0.002)
		3	0.011 (0.001)	0.184 (0.009)	0.007 (0.000)	0.027 (0.001)
	3	1	0.013 (0.001)	0.015 (0.001)	0.313 (0.016)	0.040 (0.002)
		2	0.009 (0.000)	0.012 (0.009)	0.010 (0.001)	0.188 (0.009)
		3	0.001 (0.000)	0.004 (0.000)	0.005 (0.000)	0.163 (0.008)
	4	1	0.024 (0.001)	0.023 (0.001)	0.021 (0.001)	1.657 (0.080)
		2	0.020 (0.001)	0.019 (0.001)	0.021 (0.001)	1.418 (0.071)
		3	0.016 (0.001)	0.015 (0.001)	0.011 (0.001)	1.565 (0.078)

[a] Three HRP-labeled anti-human IgG subtype-specific reagents were tested by direct ELISA for reactivity against three sets of purified myeloma IgG subtypes.
[b] Three HRP-labeled probes were purchased from Calbiochem-Novabiochem Co., and The Binding Site, Inc. All probes were diluted (1:1,000) according to the manufacturer's instructions.
[c] Three myeloma lots were purchased from Calbiochem-Novabiochem Co. and used to coat the solid phase at 4 μg/ml. The data are means of triplicate determinations at OD_{410} minus background.
[d] Numbers in parentheses represent the standard errors of the means.

wells and incubated. We evaluated three different sets of HRP–anti-human IgG subtype reagents. The plate was washed again with BBS-T, and 100 μl of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) substrate with 0.01% hydrogen peroxide was added. The enzyme-substrate reaction was terminated by the addition of 100 μl of 10% sodium dodecyl sulfate (SDS). The plates were read in an automated enzyme-linked immunosorbent assay (ELISA) plate reader at an optical density of 410 nm (OD_{410}). These methods have been described previously (45). The optimal dilution that demonstrated individual subtype specificity for the human IgG myeloma proteins was selected for each of the HRP–anti-human IgG subtype reagents. The same procedure was used to test protein A- and protein G-purified IgG preparations; however, the coat concentration was increased to 40 μg/ml.

Protein A and protein G affinity purifications. Purification of IgG from pooled sera (10 ml each) was performed by passing the sera over either protein A or protein G immobilized on Sepharose. Individual affinity columns were prepared by washing with BBS, followed by a mock elution with 0.1 M glycine-HCl (pH 3.0), and then were equilibrated with 1.5 M glycine–3 M NaCl buffer at pH 9.0 (binding buffer). Pooled sera were mixed with an equal volume of binding buffer and passed over the column five times. Unbound material was removed by washing with binding buffer. Bound IgG was eluted in 1-ml fractions by using 0.1 M glycine-HCl (pH 3.0). The fractions were read at OD_{280}, and fractions (≥0.1) were pooled and dialyzed against BBS overnight. The protein concentration was determined by taking the absorbance value at OD_{280} and using an extinction coefficient of 13.6 for a 1.0% solution. This entire purification process was

TABLE 2. Reactivity of antihuman IgG subtype-specific probes with protein A- or protein G-purified human, baboon, and macaque IgG[a]

Method	IgG Coat	OD_{410} with HRP-labeled probe[b]			
		Anti-IgG1	Anti-IgG2	Anti-IgG3	Anti-IgG4
Protein A	Human	0.650 (0.033)	0.494 (0.025)	0.515 (0.026)	0.489 (0.024)
	Baboon	0.299 (0.015)	0.241 (0.012)	0.056 (0.003)	0.136 (0.007)
	Macaque	0.141 (0.007)	0.151 (0.008)	0.022 (0.001)	0.151 (0.008)
Protein G	Human	0.556 (0.028)	0.406 (0.020)	1.496 (0.075)	0.716 (0.036)
	Baboon	0.228 (0.011)	0.167 (0.008)	0.084 (0.004)	0.163 (0.008)
	Macaque	0.303 (0.015)	0.339 (0.017)	0.084 (0.004)	0.168 (0.008)

[a] Purified IgG preparations were used to coat the solid phase of ELISA plates and directly probed with a pretitrated amount of HRP-labeled antisubtype-specific reagent.
[b] Values represent the means of triplicate determinations at OD_{410} minus background. Numbers in parentheses represent the standard errors of the means.

repeated with pooled baboon and macaque sera. The purity of the IgG preparations was assessed by SDS-polyacrylamide gel electrophoresis (reference 26 and data not shown).

Indirect ELISA. The distribution of human and baboon anti-HBs IgG subtypes was examined indirectly with the use of the cross-reactive anti-human IgG subtype reagents. Briefly, HBsAg (Merck & Co., Inc., West Point, Pa.) was used to coat the solid phase of ELISA microplate wells at 200 ng in 50 μl of BBS. The unreacted sites were blocked with 5% NGS and washed with BBS-T, and a 1:50 dilution of sera from a human and baboons that had been vaccinated with HBsAg was added to the HBsAg-coated wells. The antisubtype-HRP-labeled reagents were added, and the ELISA was developed as described above.

Immunizations. Baboons received three intramuscular injections of recombinant HBsAg (Recombivax HB) as an alum precipitate at monthly intervals as has been described elsewhere (54, 55). Serum was obtained prior to immunization and after the third injection.

RESULTS

IgG subclass determination. We evaluated three different sets of anti-human IgG subtype-specific reagents. We also utilized three different sets of purified human IgG myeloma proteins representing the four subclasses. In order to test the HRP-labeled probes for reactivity, a direct ELISA was performed with the purified human IgG myeloma proteins representing the different subclasses. Of the three anti-IgG1-HRP probes, probe 3 demonstrated the best reactivity with all three human IgG1 preparations based on OD_{410} (Table 1). In addition, background levels of reactivity to the other (IgG2, IgG3, and IgG4) human myeloma proteins were minimal compared to those of the probes with reactivity to the IgG1 preparations. Of the anti-IgG2 probes, probe 3 reacted the best, with an OD of 0.995 for the IgG2 preparation (designated coat 1). None of the three anti-IgG2-HRP probes demonstrated good reactivity with the IgG2 myeloma proteins, representing coats 2 and 3 (OD_{410}, <0.30). Background reactivity with probe 3 against IgG1, IgG3, and IgG4 was minimal (OD_{410}, <0.05). The anti-IgG3-HRP probes demonstrated that probe 1 reacted the best with IgG3 myeloma proteins designated coats 2 and 3 (OD, 2.000). Background reactivity to the other IgG subclasses was minimal (<0.100). Of the anti-IgG4-HRP probes, probe 3 exhibited the highest reactivity to the three IgG4 preparations and minimal reactivity to the other IgG myeloma proteins, with all three coats exhibiting strong reactivity. Based on these data, we selected the anti-IgG-HRP probes and the respective IgG myeloma proteins as controls for subsequent studies.

Cross-reactivity of the anti-human IgG subtype probes with baboon and macaque IgG. Human, baboon, and macaque IgGs were individually purified from sera by protein A and protein G affinity chromatography. Each of the four anti-human IgG-HRP subtype probes reacted with protein A- and protein G-purified human IgG at OD_{410} values ranging from 0.4 to 1.4 (Table 2). With protein A-purified baboon IgG, the anti-IgG1 (0.299), anti-IgG2 (0.241), and anti-IgG4 (0.136) probes were reactive, while the anti-IgG3 probe appeared unreactive (0.056). Similarly, protein A-purified macaque IgG exhibited reactivity with the anti-IgG1 (0.141), anti-IgG2 (0.151), and anti-IgG4 (0.151) probes, but not with the anti-IgG3 (0.022) probe. The protein G-purified baboon IgG demonstrated similar reactivities, with the anti-IgG1 (0.228), anti-IgG2 (0.167), and anti-IgG4 (0.163) probes showing reactivity, while the anti-IgG3 (0.084) probe appeared unreactive. Similar results were obtained with protein G-purified macaque IgG (Table 2). These results demonstrated that baboons and macaques exhibit IgG1, IgG2, and IgG4 human-like subclasses, but appear to lack IgG3.

Repurification of sera. To test the possibility that baboon and macaque IgG3 is actually present in sera and either may be outcompeted for binding to the protein A and/or G by the more abundant IgG subtypes or does not bind protein A or protein G, we performed additional studies. Specifically, we repurified the sera that did not bind protein A on protein G and repurified the sera that did not bind protein G on protein A. Human sera that did not bind protein G contained IgG1 (0.536), IgG2 (0.361), IgG3 (0.374), and IgG4 (0.590) populations that bound to protein A (Table 3). The human sera that did not bind to protein A contained a significant population of IgG3 (1.066) that bound to protein G, but little IgG1 (0.122), IgG2 (0.044), or IgG4 (0.063). Interestingly, the baboon sera also exhibited an IgG3 (1.142) population in the sera that did not bind protein A, but subsequently bound protein G. No IgG3 was detectable in macaque sera by any of these selective purification procedures. These data indicate that baboons, like humans, exhibit four IgG subclasses, while macaques appear to lack the IgG3 human subclass analog.

Evaluation of the baboon antibody response to HBsAg immunization. Specifically, we analyzed the anti-HBs IgG subclass response. Previous studies have demonstrated that the predominant IgG subclass observed in humans vaccinated with HBsAg was IgG1 (33, 34, 40). Sera from baboons were diluted 1:50 and tested for IgG subtype distribution with an indirect ELISA. In Table 4, sera obtained from a pool of vaccinated humans again demonstrated that IgG1 predominates in the anti-HBs response. Pooled sera from HBsAg-immunized baboons also showed that IgG1 had the predominant response. Next we evaluated the anti-HBs IgG subclass distribution in individually immunized baboons. Baboons exhibited a predominantly IgG1 and IgG2 anti-HBs response to HBsAg vaccination (OD_{410} values of 0.231 to 0.407 and 0.210 to 0.382, respectively). HBsAg-immunized baboons also produced smaller amounts of IgG4 anti-HBs, with little or no IgG3 being detected. These data indicate that in baboons, IgG1 has predominance in response to HBsAg immunization, similar to that in humans.

TABLE 3. Reactivity of anti-human IgG subtype-specific probes with protein A and protein G cross-purified human, baboon, and macaque IgG[a]

Method	IgG coat	OD_{410} with HRP-labeled probe[b]			
		Anti-IgG1	Anti-IgG2	Anti-IgG3	Anti-IgG4
Protein G	Human	0.122 (0.006)	0.044 (0.002)	1.066 (0.053)	0.063 (0.003)
	Baboon	0.365 (0.018)	0.171 (0.009)	1.142 (0.057)	0.153 (0.008)
	Macaque	0.015 (0.001)	0.022 (0.001)	0.025 (0.001)	0.019 (0.001)
Protein A	Human	0.536 (0.027)	0.361 (0.018)	0.374 (0.019)	0.590 (0.030)
	Baboon	0.222 (0.011)	0.163 (0.008)	0.071 (0.004)	0.162 (0.008)
	Macaque	0.277 (0.014)	0.346 (0.017)	0.054 (0.003)	0.184 (0.009)

[a] The unbound serum from protein A was passed over and bound to protein G, and the unbound serum from protein G was passed over and bound to protein A.
[b] Values represent the means of triplicate determinations at OD_{410} minus background. Numbers in parentheses represent the standard errors of the means.

DISCUSSION

Each IgG subclass can exhibit its own set of functional properties. For example, the IgG1 and IgG3 subtypes represent the predominant antibodies that react against protein antigens, such as the toxins produced by pathogenic bacteria, and against a number of viral agents. IgG2 responses have been shown to represent the predominant response to the polysaccharide (Ps) capsule of certain disease-producing bacteria, like *Haemophilus influenzae* type b. Some of the IgG subclasses are capable of efficiently crossing the placenta and entering the fetal bloodstream, while others are less efficient (35). IgG1 and IgG3 readily activate complement, while IgG2 is a weak activator and IgG4 fails to activate the complement cascade. IgG1 and IgG3 are cytophilic antibodies by virtue of their ability to bind Fc receptors. These antibodies mediate opsonization and can mediate killing by natural killer cells. The IgG2 and IgG4 subclasses either fail to exhibit these activities or do so less efficiently than IgG1 and IgG3. Thus, the lack of a particular IgG subclass may have an overall deleterious effect, and an individual may be more susceptible to certain kinds of infections. This makes knowledge of the IgG subtype distribution important for our understanding of the specific immune responses to infection and vaccination. Human IgG subclass distribution has also been utilized in an attempt to characterize a particular immune response as being Th-1 or Th-2 like (31, 48).

Investigations have utilized the baboon as a nonhuman primate model for assessing the safety and immunogenicity of candidate vaccines in adults, pregnant females, and their infants (2, 5, 13, 38, 44, 52, 54, 56). The baboon was selected because of similarities to humans in ontogeny, immunology, reproductive physiology, placentation, and maternal-fetal transfer (7, 8, 16, 17, 39, 44, 49). The advantages of the baboon over other commonly used simian primates, such as macaques, include the ease of timed pregnancies due to the estrogen-sensitive sex skin in cycling females, the comparative availability because baboons breed year-round, the lack of susceptibility to herpes B virus, the relative ease of handling, and lower associated costs (reviewed in reference 23).

Reports decades old that used relatively insensitive immunologic techniques suggested that baboons, like humans, exhibit four IgG subclasses (7, 8). Other investigations suggested that macaque species exhibit only three IgG subclasses (32). Our data confirm more recent studies by other investigators that baboons and macaques exhibit four and three IgG subclasses, respectively (8, 32). We extend these findings and demonstrate that the macaque appears to lack the IgG3 subclass. The full complement of IgG subclasses in baboons becomes important when selecting a nonhuman primate model for immunologic investigations. The IgG3 subclass in humans represents a cytophilic antibody that can activate complement, and mediate opsonization and killing by natural killer cells. This subclass of antibody can exhibit antimicrobial activities based on the ability to mediate antibody-dependent cell-mediated cytotoxicity. Investigations in humans have also reported that IgG3 can predominate and may be associated with clinical immunity following infection with *Plasmodium falciparum* (42, 50). Thus, the IgG3 subclass may play an important role in immunity to infectious agents.

The reasons we were unable to detect IgG3 in macaque sera remain to be determined. An extensive search of the literature revealed only one study in which a macaque IgG3 was detected (11). In this study, macaques were immunized with intact oral microorganisms, and the serum antibody response was evaluated by using anti-human IgG subclass-specific probes. IgG3-specific responses were reported. The possibility exists that macaques contain IgG3, and it is present at low levels that were

TABLE 4. Anti-HBs antibody subtype distribution in HBsAg-immunized baboons

Sample	OD_{410}[a]			
	Anti-IgG1	Anti-IgG2	Anti-IgG3	Anti-IgG4
Background	0.035 (0.002)	0.077 (0.004)	0.000 (0.000)	0.000 (0.000)
Human (−)	0.000 (0.000)	0.000 (0.000)	0.014 (0.001)	0.018 (0.001)
Human (+)	0.359 (0.018)	0.059 (0.003)	0.145 (0.007)	0.023 (0.001)
Baboon (−)	0.040 (0.002)	0.080 (0.004)	0.000 (0.000)	0.000 (0.000)
Baboon 1	0.231 (0.012)	0.210 (0.011)	0.025 (0.001)	0.160 (0.008)
Baboon 2	0.252 (0.013)	0.219 (0.011)	0.027 (0.001)	0.150 (0.008)
Baboon 3	0.318 (0.016)	0.267 (0.013)	0.032 (0.002)	0.184 (0.009)
Baboon 4	0.288 (0.014)	0.307 (0.015)	0.039 (0.002)	0.197 (0.010)
Baboon 5	0.407 (0.020)	0.382 (0.019)	0.072 (0.004)	0.292 (0.015)

[a] Values represent the mean OD_{410} of triplicate determinations minus background. Numbers in parentheses represent the standard errors of the means.

undetectable in our assays. Alternatively, the anti-human IgG reagents employed may fail to cross-react with macaque IgG3 preparations. It is also possible that the anti-human IgG3 subtype reagents employed by other investigators cross-reacted with non-IgG3 subclasses in the macaque. In our evaluation of anti-human IgG subclass reagents, we observed some cross-reactivity among different IgG myeloma proteins with a particular reagent (unpublished observation) (Table 1). The fact that we could detect IgG3 in baboons by similar methods suggests that macaques may lack this particular subclass.

The present report describes a comparison of IgG subclasses among humans, baboons, and macaques by using anti-human IgG subclass reagents. These reagents were also used to evaluate IgG subclass distribution in response to HBsAg immunization in baboons and humans. Together these data further support the advantages of the use of certain nonhuman primate species to model human immunologic situations.

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REFERENCES

1. **Alford, P. L., D. R. Lee, A. A. Binhazim, G. B. Hubbard, and C. M. Matherne.** 1996. Naturally acquired leprosy in two wild born chimpanzees. Lab. Anim. Sci. **46:**341–346.
2. **Attanasio, R., J. S. Allan, S. A. Anderson, T. C. Chanh, and R. C. Kennedy.** 1991. Anti-idiotypic antibody response to monoclonal anti-CD4 preparations in nonhuman primate species. J. Immunol. **146:**507–514.
3. **Barnett, S. W., K. K. Murthy, B. G. Hendier, and J. A. Levy.** 1994. An AIDS-like condition induced in baboons by HIV-2. Science **266:**642–646.
4. **Belshe, R. B., L. S. Richardson, W. T. London, D. S. Sly, J. H. Lorfeld, E. Camargo, D. A. Prevar, and R. M. Chanock.** 1977. Experimental respiratory syncytial virus infection of four species of primates. J. Med. Virol. **1:**157–162.
5. **Boulanger, D., G. D. Reid, R. F. Sturrock, I. Wolowczuk, J. M. Balloul, D. Grezel, R. J. Pierce, M. F. Otieno, S. Guerret, and J. A. Grimaud.** 1991. Immunization of mice and baboons with the recombinant Sm28GST affects both worm viability and fecundity after experimental infection with *Schistosoma mansoni*. Parasite Immunol. **13:**437–490.
6. **Chang, S. P., S. E. Case, W. L. Gosnell, A. Hashimoto, K. J. Kramer, L. Q. Tam, C. Q. Hashiro, C. M. Nikaido, H. L. Gibson, C. T. Lee-Ng, P. J. Barr, B. T. Yokota, and G. S. N. Hui.** 1996. A recombinant baculovirus 42-kilodalton C-terminal fragment of *Plasmodium falciparum* merozoite surface protein 1 protects *Aotus* monkeys against malaria. Infect. Immun. **64:**253–261.
7. **Damian, R. T., N. D. Greene, and S. S. Kalter.** 1971. IgG subclasses in the baboon (*Papio cynocephalus*). J. Immunol. **106:**246–257.
8. **Del Portillo, H. A., G. W. Schmidt, and R. T. Damian.** 1987. Immunochemical analysis of baboon *Papio cynocephalus* IgG subclass. Vet. Immunol. Immunopathol. **16:**201–214.
9. **Desrosiers, R. C.** 1995. Non-human primate models for AIDS vaccines. AIDS **9:**5137–5145.
10. **Desrosiers, R. C., M. D. Daniel, and Y. Li.** 1989. HIV-related lentiviruses of nonhuman primates. AIDS Res. Hum. Retroviruses **5:**465–473.
11. **Ebersole, J. L., G. R. Bauman, S. E. Cox-O'Dell, and A. Giardino.** 1997. Evidence for serum immunoglobulin G (IgG) antibody responses in *Macaca fascicularis* identified by monoclonal antibodies to human IgG subclasses. Oral Microbiol. Immunol. **12:**193–203.
12. **Fritz, D. L., N. K. Jaax, W. B. Lawrence, K. J. Davis, M. L. Pitt, J. W. Ezzell, and A. M. Friedlander.** 1995. Pathology of experimental inhalation anthrax in the rhesus monkey. Lab. Investig. **73:**691–702.
13. **Fusco, P. C., F. Michon, J. Y. Tai, and M. A. Blake.** 1997. Preclinical evaluation of a novel group B meningococcal conjugate vaccine that elicits bactericidal activity in both mice and nonhuman primates. J. Infect. Dis. **175:**364–372.
14. **Geiman, Q. M., and M. H. Meagher.** 1967. Susceptibility of a New World monkey to *Plasmodium falciparum* from man. Nature **215:**437–439.
15. **Handt, L. K., H. J. Klein, H. Rozmiarek, J. G. Fox, W. J. Pouch, and S. L. Motzel.** 1995. Evaluation of two commercial serologic tests for the diagnosis of Helicobacter pylori infection in the rhesus monkey. Lab. Anim. Sci. **45:**613–617.
16. **Hankins, G. D., C. L. Lowery, R. T. Scott, W. R. Morrow, K. D. Carey, M. M. Leland, and E. V. Colvin.** 1990. Transplacental transfer of zidovidine in the near term pregnant baboon. Am. J. Obstet. Gynecol. **163:**728–732.
17. **Hendrickx, A. G.** 1971. Embryology of the baboon, p. 3–30. University of Chicago Press, Chicago, Ill.
18. **Hicks, J. T., J. L. Sullivan, and P. Albrecht.** 1977. Immune responses during measles infection in immunosuppressed rhesus monkeys. J. Immunol. **119:**1452–1456.
19. **Huang, C., P. M. Chen, J. K. Kno, W. H. Chin, S. T. Lin, and Y. C. Lin.** 1962. Experimental whooping cough. N. Engl. J. Med. **266:**105–111.
20. **Hubbard, G. B., D. R. Lee, J. W. Eichberg, B. J. Gormus, K. Xu, and W. M. Meyers.** 1991. Spontaneous leprosy in a chimpanzee (*Pan troglodytes*). Vet. Pathol. **28:**546–548.
21. **Jaax, N. K., K. J. Davis, T. J. Geisbert, P. Vogel, G. P. Jaax, M. Topper, and P. B. Jahrling.** 1996. Lethal experimental infection of rhesus monkeys with Ebola-Zaire (Mayinga) virus by the oral and conjunctival route of exposure. Arch. Pathol. Lab. **120:**140–155.
22. **Kennedy, R. C., J. W. Eichberg, R. E. Lanford, and G. R. Dreesman.** 1986. Anti-idiotypic antibody for type B viral hepatitis in chimpanzees. Science **232:**220–223.
23. **Kennedy, R. C., M. H. Shearer, and W. H. Hildebrand.** 1997. Nonhuman primate models to evaluate vaccine safety and immunogenicity. Vaccine **15:**903–908.
24. **Kennedy, R. C., M. H. Shearer, W. H. Hildebrand, and R. S. Simmonds.** 1997. Nonhuman primates and their potential use in immunologically based investigations. Immunologist **5:**5–10.
25. **Krawczynski, K., and D. W. Bradley.** 1989. Enterically transmitted non-A, non-B hepatitis: identification of virus-associated antigen in experimentally infected cynomolgus macaques. J. Infect. Dis. **159:**1042–1049.
26. **Laemmli, U. K.** 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature **227:**680–685.
27. **Lankas, G. R., and R. D. Jensen.** 1987. Evidence of hepatitis A infection in immature rhesus monkeys. Vet. Pathol. **24:**340–344.
28. **Larsen, J. W., W. F. London, A. E. Palmer, J. W. Tossell, R. A. Bronsteen, M. Daniels, B. L. Curfman, and J. L. Sever.** 1978. Experimental group B streptococcal infection in the rhesus monkey. I. Disease production in the neonate. Am. J. Obstet. Gynecol. **132:**686–690.
29. **LeDuc, J. W., S. M. Lemon, C. M. Keenan, R. R. Graham, R. Marchwicki, and L. N. Binn.** 1983. Experimental infection of the New World owl monkey (*Aotus trivirgatus*) with hepatitis A virus. Infect. Immun. **40:**766–772.
30. **Lemon, S. M., L. N. Binn, R. Marchwicki, P. C. Murphy, L. H. Ping, R. W. Jansen, L. V. Asher, J. T. Stapleton, D. G. Taylor, and J. W. LeDuc.** 1990. In vivo replication and reversion to wild type of a neutralization-resistant antigenic variant of hepatitis A virus. J. Infect. Dis. **161:**7–13.
31. **Lkyllouriotis, P., M. Lkyllouriotis-Lazarou, S. Natter, R. Steiner, S. Spitzauer, S. Kapiotis, P. Valent, A. M. Hirschl, S. E. Guber, G. Laufer, G. Wollenek, E. Wolner, M. Wimmer, and R. Valenta.** 1999. IgG subclass reactivity to human cardiac myosin in cardiomyopathy patients is indicative of a Th1-like autoimmune disease. Clin. Exp. Immunol. **115:**236–247.
32. **Martin, L. N.** 1982. Chromatographic fractionation of rhesus monkey (Macaca mulatta) IgG subclasses using DEAE cellulose and protein A-sepharose. J. Immunol. Methods **50:**319–329.
33. **Mattila, P. S., J. Pelkonen, and O. Makela.** 1986. Proportions of IgG subclasses in antibodies formed during vaccination with hepatitis B surface antigen. Scand. J. Immunol. **24:**699–704.
34. **Morell, A., B. Roth-Wicky, and F. Skvaril.** 1983. Immunoglobulin G subclass restriction of antibodies against hepatitis B surface antigen. Infect. Immun. **39:**565–568.
35. **Nagao, A. T., C. C. Martinez, V. S. Vieira, O. A. Takano, B. T. Costa-Carvalho, and M. M. Carneiro-Sampaio.** 1998. Placental transfer of IgG and IgG subclass antibodies to anti-purified Escherichia coli LPS O16, O6, and O111. Scand. J. Immunol. **47:**609–614.
36. **Novembre, F. J., M. Saucier, D. C. Anderson, S. A. Klumpp, S. P. O'Neil, C. R. Brown II, C. E. Hart, P. C. Guenthner, R. B. Swenson, and H. M. McClure.** 1997. Development of AIDS in a chimpanzee infected with human immunodeficiency virus type 1. J. Virol. **71:**4086–4091.
37. **Onderdonk, A. B., and R. C. Kennedy.** 1998. Use of animal models for vaccine development, p. 19–42. *In* L. C. Paoletti and P. McInnes (ed.), Vaccines: from concept to clinic. A guide to the development and testing of vaccines for human use. Harvard Medical Press, Cambridge, Mass.
38. **Paoletti, L. C., R. C. Kennedy, T. C. Chanh, and D. L. Kaspar.** 1996. Immunogenicity of group B *Streptococcus* type III polysaccharide-tetanus toxoid vaccine in baboons. Infect. Immun. **64:**677–679.
39. **Pauerstein, C. J., C. A. Eddy, H. D. Croxatto, R. Hess, T. M. Siler-Khodr, and H. B. Croxatto.** 1978. Temporal relationships of estrogen, progesterone and lutenizing hormone levels to ovulation in women and infrahuman primates. Am. J. Obstet. Gynecol. **130:**876–886.
40. **Rossi, M. E., C. Azzari, M. Resti, C. Appendino, P. Pezzati, and A. Vierucci.** Selectivity in IgG subclass response to hepatitis B vaccine in infants born to HBsAg-positive mothers. Clin. Exp. Immunol. **72:**196–200.
41. **Rubens, C. E., H. V. Raff, J. C. Jackson, E. Y. Chi, J. T. Bielitzki, and R. L. Hillier.** 1991. Pathophysiology and histopathology of group B streptococcal sepsis in *Macaca nemestrina* primates induced after intraamniotic inoculation: evidence for bacterial cellular invasion. J. Infect. Dis. **164:**320–330.
42. **Rzepczyk, C. M., K. Hale, N. Woodroffe, A. Bobogare, P. Csurhes, A. Ishii,**

and A. Ferrante. 1997. Humoral immune responses of Solomon Islanders to the merozoite surface antigen 2 of *Plasmodium falciparum* show pronounced skewing towards antibodies of the immunoglobulin G3 subclass. Infect. Immun. **65:**1098–1100.
43. **Sauer, L. W., and L. Hambrecht.** 1929. Experimental whooping cough. Am. J. Dis. Child. **37:**732–744.
44. **Shearer, M. H., A. H. Lucas, P. W. Anderson, K. D. Carey, H. B. Jenson, T. C. Chanh, J. R. Stanley, and R. C. Kennedy.** 1997. The baboon as a nonhuman primate model for assessing the effects of maternal vaccination with *Haemophilus influenzae* type b polysaccharide vaccines. Infect. Immun. **65:**3267–3270.
45. **Shearer, M. H., F. J. Stevens, F. A. Westholm, H. B. Jenson, T. C. Chanh, K. D. Carey, G. L. White, A. Solomon, and R. C. Kennedy.** 1995. Serologic crossreactions among primate immunoglobulins. Dev. Comp. Immunol. **19:** 547–557.
46. **Shevtsova, Z. V., B. A. Lapin, N. V. Doroshenko, R. I. Krilova, L. I. Korzaja, I. B. Lomovskaya, Z. N. Dzhelieva, G. K. Zairov, V. M. Stakhanova, E. G. Belova, and L. A. Sazhchenko.** 1988. Spontaneous and experimental hepatitis A in Old World monkeys. J. Med. Primatol. **17:**177–194.
47. **Shope, T., D. Dechairo, and G. Miller.** 1973. Malignant lymphoma in cottontop marmosets after inoculation with Epstein-Barr virus. Proc. Natl. Acad. Sci. USA **70:**2487–2491.
48. **Sousa, A. O., S. Henry, F. M. Maroja, F. K. Lee, L. Brum, M. Singh, P. H. Lagrange, and P. Aucouturier.** 1998. IgG subclass distribution of antibody responses to protein and polysaccharide mycobacterial antigens in leprosy and tuberculosis patients. Clin. Exp. Immunol. **111:**48–55.
49. **Stevens, V. C., S. J. Sparks, and J. E. Powell.** 1990. Levels of estrogens, progestogens and luteinizing hormone during the menstrual cycle of the baboon. Endocrinology **87:**658–666.
50. **Taylor, R. R., S. J. Allen, B. M. Greenwood, and E. M. Riley.** 1998. IgG3 antibodies to *Plasmodium falciparum* merozoite surface protein 2 (MSP2): increasing prevalence with age and association with clinical immunity to malaria. Am. J. Trop. Med. Hyg. **58:**406–413.
51. **Ticehurst, J., L. L. Rhodes, K. Krawczynski, L. V. Asher, W. F. Engler, T. L. Mensing, J. D. Caudill, M. H. Sjogren, C. H. Hoke, J. W. LeDuc, J. W. Bradley, and L. N. Binn.** 1992. Infection of Owl monkeys (*Aotus trivirgatus*) and cynomolgus monkeys (*Macaca fascicularis*) with hepatitis E virus from Mexico. J. Infect. Dis. **165:**835–845.
52. **Vaslin, B., J. M. Claverie, O. Benveniste, F. C. Baree-Sinoussi, and D. Dormont.** 1994. Nef and gag synthetic peptide priming of antibody responses to HIV type antigens in mice and primates. AIDS Res. Hum. Retroviruses **10:**1241–1250.
53. **Warren, J. T., and M. A. Levinson.** 1995. Fourth annual survey of worldwide HIV, SIV, and SHIV challenge studies in vaccinated nonhuman primates. J. Med. Primatol. **24:**150–168.
54. **Watts, A. M., J. R. Stanley, M. H. Shearer, P. S. Hefty, and R. C. Kennedy.** 1999. Fetal immunization of baboons induces a fetal specific antibody response. Nat. Med. **5:**427–430.
55. **Watts, A. M., J. R. Stanley, M. H. Shearer, and R. C. Kennedy.** 1998. Maternal and fetal vaccination strategies for hepatitis B surface antigen in nonhuman primate models: evaluation of therapeutic modalities during pregnancy p. 185–193. *In* R. F. Schinaze, J. P. Sommadossi, and H. C. Thomas (ed.), Therapies for viral hepatitis. International Medical Press, London, United Kingdom.
56. **Yole, D. S., R. Pemberton, G. D. Reid, and R. A. Wilson.** 1996. Protective immunity to *Schistosoma mansoni* induced in the olive baboon Papio anubis by the irradiated cercaria vaccine. Parasitology **112:**37–46.
57. **Young, L. S., S. Finerty, L. Brooks, F. Scullion, A. B. Rickinson, and A. J. Morgan.** 1989. Epstein-Barr virus expression in malignant lymphomas induced by experimental virus infection of cottontop tamarins. J. Virol. **63:** 1967–1974.
58. **Young, M. D., J. A. Porter, and C. P. Johnson.** 1966. *Plasmodium vivax* transmitted from monkey to man. Science **153:**1006–1007.