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Nonhuman Primate IgA: Genetic Heterogeneity and Interactions with CD89¹,²

Kenneth A. Rogers,* Lakshmi Jayashankar,* Franco Scinicariello,† and Roberta Attanasio³*

Nonhuman primates are extremely valuable animal models for a variety of human diseases. However, it is now becoming evident that these models, although widely used, are still uncharacterized. The major role that nonhuman primate species play in AIDS research as well as in the testing of Ab-based therapeutics requires the full characterization of structure and function of their Ab molecules. IgA is the Ab class mostly involved in protection at mucosal surfaces. By binding to its specific Fc receptor CD89, IgA plays additional and poorly understood roles in immunity. Therefore, Ig heavy α (IGHA) constant (C) genes were cloned and sequenced in four different species (rhesus macaques, pig-tailed macaques, baboons, and sooty mangabeys). Sequence analysis confirmed the high degree of intraspecies polymorphism present in nonhuman primates. Individual animals were either homozygous or heterozygous for IGHCA genes. Highly variable hinge regions were shared by animals of different geographic origins and were present in different combinations in heterozygous animals. Therefore, it appears that although highly heterogeneous, hinge sequences are present only in limited numbers in various nonhuman primate populations. A macaque recombinant IgA molecule was generated and used to assess its interaction with a recombinant macaque CD89. Macaque CD89 was able to bind its native ligand as well as human IgA1 and IgA2. Presence of Ag enhanced macaque IgA binding and blocking of macaque CD89 N-glycosylation reduced CD89 expression. Together, our results suggest that, despite the presence of IgA polymorphism, nonhuman primates appear suitable for studies that involve the IgA/CD89 system. The Journal of Immunology, 2008, 180: 4816–4824.

A variety of nonhuman primate (NHP)⁴ species are routinely used in biomedical research for studies related to the pathogenesis and vaccine prevention of infectious diseases, especially HIV infection and AIDS (1). In addition, NHP species are being increasingly used for testing Ab-based therapeutics (2–7). Because Abs are one of the most important effector molecules generated during the immune response, it is essential to fully characterize their structure/function relationships as well as their interactions with the corresponding Fc receptors for all NHP species used in the above mentioned studies. Indeed, it is widely recognized that the characterization of NHP Ab molecules is still incomplete. Results from recent studies (8) indicate that, although NHP and human Ab molecules are very similar to each other, there are several differences that might lead to the incorrect interpretation of results from studies performed in these species.

IgA is the most abundant class of Ab secreted into the mucosal epithelial linings. As the mucosal immune system is the initial barrier against most pathogens, secretory IgA provides a first line of defense (9, 10). Secretory IgA is primarily polymeric. In human serum, IgA constitutes 15–20% of total immunoglobulins and is mostly monomeric (11). IgA mechanisms of defense include immune exclusion, intracellular neutralization, and virus excretion (12–16). Serum IgA interacts with several poorly characterized receptors specific for its Fc portion. The best characterized of these receptors is FcαRI, or CD89, expressed on the surface of eosinophils, neutrophils, dendritic cells, monocytes, and macrophages (17). The mechanisms mediated by immune complexes of IgA cross-linked to CD89 include Ab-dependent cell-mediated cytotoxicity, phagocytosis, and respiratory burst, as well as release of cytokines and inflammatory mediators (14, 17).

The C region of the IgA H chain is encoded by Ig H chain α (IGHA) genes. In humans, IgA exists as two subclasses, IgA1 and IgA2 (encoded, for the C region of the α-chain, by IGHAI and IGHAI2 genes, respectively) (18, 19). Old World monkeys possess only one IgA gene (20, 21). An IgA coding region consists of a constant domain (CH1) and a hinge region followed by two other constant domains (CH2 and CH3). Each CH domain is encoded by separate exons, with the hinge uniquely encoded by the 5’ end of the CH2 exon (18).

Interestingly, in NHPs, IGHAI genes exhibit intraspecies sequence polymorphism. In particular, the hinge region of Old World monkeys is characterized by a partially reiterated structure with high sequence variability (22–24). This region accumulates mutations very rapidly, probably as a result of adaptive evolution due to selective pressure from bacterial proteases (22, 23).

Although partial IGHAI sequences have been described for several NHP species, sequences of the complete IGHAI coding regions are available only for rhesus macaques of Indian origin (25) and sooty mangabeys (26). Analysis of the complete Indian rhesus macaque sequences shows that the IGHAI CH1 and CH2 domains are

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4 Abbreviations used in this paper: NHP, nonhuman primate; NIP, 5-iodo-4-hydroxy-3-nitrophenacetyl; MFI, mean fluorescence intensity.

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also polymorphic. The high levels of polymorphism found in the rhesus macaques have not been described in humans, although it appears that IgA may be polymorphic in mice, pigs, and other primates (22, 23, 27, 28).

We recently demonstrated that the IgG/CD16 system differs between NHPs and humans. Recombinant NHP CD16 binds to human IgG subclasses different from those bound by human CD16, despite a nearly complete conservation of residues known to be involved in ligand binding (8). Because of its major role in mucosal and systemic immunity, and because of the possible therapeutic potential in modulating the IgA/CD89 interactions (29), IgA is an Ab class that requires full characterization in NHP species. Humanized IgA-based therapeutics should be tested in these species only if their ability to interact with NHP CD89 is ascertained.

In this study, we report the cloning and complete sequence characterization of IGHA genes from either Asian or African NHP species, i.e., rhesus macaques (Macaca mulatta) of Chinese origin, pig-tailed macaques (Macaca nemestrina), baboons (Papio hamadryas anubis), and sooty mangabeys (Cercocebus torquatus hamadryas anubis). IGHA genes from Indian rhesus macaques as well as two IGHA genes from sooty mangabeys have been previously described (25, 26). Because it is logical to speculate that this high sequence variability may result in different functional properties, we have developed a system for the generation of macaque recombinant IgA molecules and used a macaque recombinant IgA molecule to assess the IgA/CD89 interaction in this NHP species.

**Materials and Methods**

**Blood samples and RNA Extraction**

Blood samples from rhesus macaques of Chinese origin, pig-tailed macaques, baboons, and sooty mangabeys were obtained from the Yerkes National Primate Research Center (Emory University, Atlanta, GA) and the Southwest Foundation for Biomedical Research (San Antonio, TX). Total RNA was extracted from whole blood using the QiAmp RNA blood mini kit (Qiagen). The animals were designated according to the standardized abbreviation rules of IMGT (the international ImMunoGeneTics information system (30) as Papyanu (baboons), Macnem (rhesus macaques), Macnem (pig-tailed macaques), and Certoraty (sooty mangabeys). Animal blood was collected under approval of the appropriate institutional review committees.

**Amplification of IGHA constant region from total RNA**

After reverse transcription of total RNA into cDNA using oligo(dT) primers followed by primer extension with the AMV reverse transcriptase (Roche Diagnostics Corp), the full length IGHA cDNA was amplified by PCR using the forward primer IgA10 (5′-ATGGATCCATGAGCAGAATTAATTCGCGTT). After digestion with HpaII restriction enzyme site. The reaction was run for 40 cycles, each cycle consisting of 94°C for 1 min, 58°C for 1 min, and 72°C for 2 min.

**Cloning of amplified sequences**

Cloning, screening, sequencing, and sequence analysis of amplified gene sequences was performed as previously described (32), except that DNA sequences were determined using an ABI model 3100 automated sequencer (PerkinElmer). All sequences were obtained twice from two independent RT-PCR. IGHA sequences were aligned with the published human and Indian rhesus macaques IGHA genes using the CLUSTAL format of the Megalign part of the Lasergene software package (DNASTAR Inc). The IMGT standardized nomenclature and numbering have been used to show and discuss IGHA data (31).

**Production of recombinant IgA with a rhesus IGHA**

Recombinant IgA was generated by transfection of an IgA IgH expression vector containing a variable heavy domain with affinity for hapten 5-hydroxy-3-nitrophenacetyl (VHNIP) and IGHA cDNA of a rhesus macaque of Chinese origin (GEDH accession number AY294614). The Ig H chain expression vector was engineered from pLNOH2 (provided by Drs. Lars Norderhaug and Inger Sandlie, University of Oslo and Ab Design AS, Nesoddtangen, Norway). pLNOH2, derived from pcDNA3 (Invitrogen), encodes a human IGHG3 constant region (CH) and a variable heavy domain (VHNIP) (33).

To construct the IgA H chain expression vector, both VHNIP and human IGHG3 genes were removed from pLNOH2 by digestion at BsoI and BanHI restriction sites and replaced by ligation with a similarly digested cassette containing the VHNIP cDNA and IGHA cDNA. Production of the cassette was done by separate PCR amplification of the VHNIP and IGHA cDNAs followed by the joining of the two overlapping products by a third PCR amplification. The VHNIP gene was amplified from pLNOH2 using primers H1H (5′-GGATCCACATGAGCAGAATTAATTCGCGTT) and HIIR2 (5′-GAGACTGTGAGAGTGG). PCR conditions for all the reactions were the same as described above except that the annealing temperature was 56°C. The complete expression vector was cloned into Escherichia coli. After verifying the vector sequence, a clone was expanded and vector prepared with an EndoFlEa plasmid maxi kit (Qiagen).

**Detection of Ig λ (IGL) and complete recombinant IgA from J558L cell supernatants**

Production of Ig λ (IGL) L chain and complete recombinant IgA from J558L cell supernatants was determined by capture ELISAs. Microtitre plates were coated with goat anti-mouse Ig λ Ab (Invitrogen) at 4 and 8 μg/ml or with NIP-BSA at 15 μg/ml in 1% coating buffer (KPL). After incubation at 4°C overnight, the plate was blocked with 5% FCS in PBS at 37°C for 30 min. After washing, samples were added. For Ig λ assays, tissue culture supernatant from untransfected J558L cells, purified mouse myeloma IgG1 with a λ chain (Sigma-Aldrich) (positive control), or 5% FCS in either culture media, or PBS (negative control) was added and incubated at 37°C for 1 h. For the IgA assays, supernatants from the transfected and untransfected J558L cells were added. After washing, HRP-labeled secondary Abs were added. Anti-mouse Ig λ (Invitrogen) was used to detect Ig λ and IgA. In addition, for IgA assays, anti-rhesus Ig α was added (KPL). Plates were incubated at 1 h at 37°C washed and developed by addition of ABTS/H2O2 followed by addition of stop solution. Absorbance was measured at 405 nm using an automated Benchmark microplate reader (Bio-Rad).

**Generation of HeLa cells expressing recombinant rhesus macaque CD89**

Clones of HeLa cells expressing rhesus macaque CD89 were generated. First, rhesus macaque CD89 cDNA was inserted into pcDNA3.1 (Invitrogen) by methods previously described (35). From the resulting vector a product containing the CDNA of rhesus macaque CD89, a cytomegalovirus promoter and a bovine growth hormone polyadenylation signal was amplified using PCR primers pHp (5′-CTCGTGTATACACGTAGGGT TACGCTTGGTGC) and Psα (5′-ACTTTTCGCGCGCTTCACCGGC CATCGACACAGAATAATCCGGTC). After digestion with HpaI and SalI, the resulting fragment was ligated into HpaI and SalI digested pLSXN (Clontech Laboratories) to create an expression vector...
with long-terminal repeats that allow for stable integration into host chromosomes. Twenty micrograms of vector was electroporated into 250 μl of HeLa cells suspended in DMEM at 14 × 10⁶ cells/ml by the methods described above. Cells were diluted serially into 96-well microtiter plates and grown in 100 μl of DMEM (containing 10% FCS and consisting of 50% fresh medium and 50% 0.2 μm filtered HeLa cell-conditioned media) with 400 μg/ml G418. Wells with single cells were identified by microscopy and the clones subsequently expanded. Flow cytometric analysis was used to identify clones expressing CD89. In brief, adherent cells were removed from flasks with 0.25% trypsin/EDTA. Aliquots of 0.5 × 10⁶ cells were stained with 5 μl of anti-human CD89 PE (clone A59) or a mouse isotype control (BD Biosciences) for 30 min at 4°C, washed three times with PBS to remove unbound Ab, and suspended in 1% paraformaldehyde. Fluorescent cells were counted on a FACS Calibur machine and analyzed with CellQuest software (BD Biosciences).

**Determination of recombinant IgA binding to CD89**

Binding of recombinant IgA to CD89 was assessed by flow cytometry using Abs that were heat aggregated at 63°C for 1 h. IgA1, IgA2, IgG, and IgM (human myeloma proteins; Binding Site Ltd.) with Igκ light chains were added to 0.5 × 10⁶ cells at 20 μg/ml and incubated for 1 h at 4°C. PBS washed cells were then stained with 5 μl of either FITC-conjugated mouse anti-human Igκ (Invitrogen), FITC-conjugated mouse anti-human IgG, rat anti-human IgA, or mouse IgG1 and the clones subsequently expanded. Flow cytometric analysis was used to identify clones expressing CD89. In brief, adherent cells were removed from flasks with 0.25% trypsin/EDTA. Aliquots of 0.5 × 10⁶ cells were stained with 5 μl of anti-human CD89 PE (clone A59) or a mouse isotype control (BD Biosciences) for 30 min at 4°C. For some experiments, mouse-human CD89 PE was also added. Cells were washed and analyzed by flow cytometry as described above. For N-glycosylation blocking experiments, tunicamycin was added to half of the cell cultures at 1 μg/ml 30 h before harvesting cells. Experiments to determine binding of recombinant rhesus macaque IgA were performed as variations of the above protocol. Supernatants from cultures secreting recombinant IgA were performed as variations of the above protocol. Supernatants from cultures secreting recombinant IgA were performed as variations of the above protocol.

**Results**

**Sequencing of NHP IGHA**

IGHA sequences were obtained from three pig-tailed macaques, four baboons, and three sooty mangabeys. The alignment of these sequences with the three human IgA sequences, as well as previously described NHP sequences (25, 26) are shown in Fig. 1. Animals were presumed homozygous for IGHA when all ten or more clones were determined to have the same sequence. Four unique IGHA gene sequences were identified in the pig-tailed macaques, with one monkey homozygous and two heterozygous. The sequence from the homozygous pig-tailed macaque was also found in one of the heterozygous pig-tailed macaques. Four sequences were present within the four baboons. Two baboons were homozygous and two heterozygous, each having one unique sequence and one in common with a homozygous baboon. Five different gene sequences were present in the three sooty mangabeys. One of these sequences was identical with the one previously reported by us (26).

The baboon IGHA allelic sequences exhibit 83% amino acid identity to the corresponding human IGHA1 sequence (18). They share 91.2–94.8% identity with the corresponding rhesus macaque IGHA allelic sequences, 91.8–96.0% with the corresponding pig-tailed macaques IGHA alleles and 90.6–95.4% with the mangabey counterparts. Among the rhesus macaque species, IGHA alleles exhibit highly conserved sequences with the three human IgA sequences, as well as previous NHP included in this study. Clearly, a high degree of heterogeneity characterizes the hinge region of these Ab molecules. Two different IGHA hinge sequences were identified in several animals, whereas some others exhibited only one hinge sequence. Interestingly, hinge region sequences present in some NHPs were also present in others, although in combination with different alleles. Hinge sequences from 3 baboon IGHA alleles (Papcynanu A.I, C.II, and D.I) were also observed in two mangabey IGHA alleles. Similarly, Chinese and Indian rhesus macaque sequences share some hinge sequences with each other and also with pig-tailed macaques. In humans, IgA1 protease cleave proline-serine and proline-threonine bonds in the hinge region, but not at all positions (36). Only two or three of these bonds can be identified in the NHP hinge sequences, specifically one in a sequence from a rhesus macaque and potentially two in a sequence from a pig-tailed macaque (Fig. 2). Similar bonds are present in two other hinge sequences (that shared by a mangabey and a baboon as well as that shared by a rhesus macaque and a pig-tailed macaque). These bonds align with a similar bond found in human IgA1, which is not cut. The arginine-glycine (RG) bond found in the hinge sequence shared between a baboon and a mangabey is another possible cleavage site. This corresponds to a proline-valine bond found in humans and cut by a *Clostridium ramosum* IgA protease. The same substitutions (RG) are also present in IgA1 from chimpanzee, which is cut (36). Possibly the position itself could be important, but a human IgA2m (2) is also cut here despite a truncated hinge (37). IgA cleavage sites must be presented to bacterial proteases in the context of the larger IgA molecule for efficient digestion. No cleavage is observed when the human IgA1 hinge is replaced by that of IgG2. By contrast, cleavage is observed when the IgA1 has its hinge replaced with that of IgA2 (37). Therefore, the various NHP IgA molecules should be individually examined for resistance/susceptibility to bacterial proteases.

In humans, conserved cysteines are located in IgA CH2 at positions 1.2 and 1.1 in the IMGT unique numbering for C-DOMAIN (31). At least one of these cysteines is conserved at the same position in all the NHP hinge regions, although in more than half of the sequence, one of these is substituted. Specifically, the cysteines were replaced with serine at position 1.2 in three mangabey alleles (Cerorotay A.II, B.II, and C.I) and by aspartate at position 1.1 in baboons (Papcynanu B.I and C.II), rhesus macaques (Macnem A.I, A.II, B.II, C.II, D.I, D.II, F.I, and G.I), pig-tailed macaques (Macnem A.I and C.II) and mangabeys (Cerorotay A.I, B.I, C.II, and D.I).

The human CH3 domain consists of 131 aa. Very high similarities exist between human, baboon, rhesus macaques, pig-tailed...
macaques, and mangabey CH3 exons (Fig. 1). The length of the NHP CH3 domain was not confirmed by our sequences, which lack the last ten amino acid residues. However, through analysis of the rhesus macaque genome, a full-length \textit{IGHA} gene was identified within a contig (accession number NW0011220011). The encoded gene product (Fig. 1, Macmul F.I) has a CH3 domain consisting of 131 aa.

Sequence analysis of all the NHP \textit{IGHA} allelic variants shows that the residues in the CH2 and CH3 domains involved in FcR1 interactions (15) are conserved with the exception of a serine at position 46 and a glutamate at position 48 (positions 45 and 45.2 in the IMGT unique numbering, respectively) in CH3, which are substituted by threonine and glutamine, respectively.
Generation of recombinant IgA with a rhesus macaque Fc region

To evaluate expression and function of NHP IgA, a chimeric Ab with specificity for the hapten NIP and with rhesus macaque IGHA region was generated. This was done by constructing a vector for expression of a rhesus macaque IgA/H9251-chain with a murine NIP-specific variable domain. Transfection of this vector into J558L cells, which produce a murine NIP-specific IgA/H9261-L chain, resulted in the production of IgA. Expression of the fully formed chimeric IgA molecules was determined from cell supernatants by ELISA using NIP coupled to BSA-coated microtiter plates and either anti-rhesus IgA/H9251 or anti-murine IgA/H9261 secondary Ab (Fig. 3).

Rhesus macaque IgA binds to recombinant rhesus CD89 expressed on HeLa cells

In humans, IgA/Ag complexes can initiate a variety of cellular immune responses by binding and cross-linking CD89 expressed on cells of the myeloid lineage. We have previously identified CD89 in cynomolgus and rhesus macaques (34). To assess rhesus macaque IgA interactions with CD89, stable HeLa cell clones expressing high levels of recombinant rhesus macaque IgA were generated. CD89 presence on these clones was verified by positive staining with anti-human CD89 (data not shown). Expression was specific, because neither clones incubated with an isotype control nor untransfected HeLa cells incubated with anti-human CD89 stained positive. Recombinant rhesus macaque IgA was able to bind CD89 in the absence of Ag.

FIGURE 3. Production of recombinant Abs with macaque Fc as detected by binding to NIP-BSA using ELISA. Supernatants from J588L cells transfected with an expression vector for rhesus IGHA, untransfected, or transfected with vector pLNOH2 encoding human IgG H chain specific for NIP were incubated on NIP-BSA coated plates to capture Abs. Bound Abs were detected with anti-mouse Ig A. Additionally, detection was done with anti-mouse Ig G for the pLNOH2 positive control.
(27.6 MFI versus control supernatant 22.4 ± 1.8), although this binding was enhanced by the presence of Ag (31.6 and 31.3 MFI for Ag at 1.88 and 7.5 μg/ml, respectively, it was reduced in the presence of excess Ag (26.6 MFI for 15 μg/ml Ag). Macaque CD89 was also capable of binding to heat-aggregated human IgA1 and IgA2, but not to human IgM (Fig. 5) of IgG. As shown for rhesus macaque IgA, human IgA bound in proportion to the amount of CD89 expressed on cells.

Fc receptor glycans have been shown to be important for receptor expression and Ig Fc binding (38). The effects of blocking N-glycosylation with tunicamycin on rhesus CD89 expression and binding properties were examined, because rhesus macaque CD89 has N-glycosylation motifs conserved with sites in human CD89 occupied by N-glycans (Fig. 5). Expression of CD89 was dramatically reduced in cells treated with tunicamycin (57–73% reduction) with a disproportionately greater decrease in ability to bind to human IgA1 and IgA2 (84–95%).

Discussion
Despite the wide use of NHP species as animal models for a variety of human diseases as well as for vaccine development and Ab-based therapeutics, NHP Ab molecules and corresponding receptors are still uncharacterized. NHP species are especially used in AIDS research, which includes studies that require the assessment of IgA responses because of the role that the mucosal immune system plays in HIV entry and establishment of infection and because of the potential development of HIV mucosal vaccines (39). Exceptionally high levels of IGH A hinge region polymorphism are known to be present in many NHP species. Previously, we have demonstrated that, in addition to the hinge region, the entire IGH A genes of rhesus macaques of Indian origin are highly polymorphic. Therefore, we expanded our previous studies to include all exons from IGH A genes from two Asian species.

FIGURE 4. Rhesus IgA binds to rhesus macaque CD89. HeLa cells expressing recombinant rhesus macaque CD89 were incubated with supernatant of J588L cells expressing recombinant rhesus IgA (containing a mouse Ig lambda) or control supernatant and stained with anti-mouse Ig lambda FITC and anti-human CD89 PE. A. Histogram plot of HeLa cells expressing CD89 incubated with rhesus macaque IgA (filled), or control supernatant (dashed line), and untransfected control HeLa cells incubated with IgA (thick line). Two-color dot plots (C) HeLa cells with IgA supernatant, (B) and (D) HeLa cells expressing CD89 with control supernatant and IgA supernatant, respectively.

FIGURE 5. Recombinant rhesus macaque CD89 binds to heat-aggregated human myeloma IgA1 and IgA2; however, blocking N-glycosylation reduces expression of recombinant rhesus macaque CD89 and decreases in binding of human IgA1 and IgA2. Cells were stained with anti-human CD89 PE and anti-human Ig lambda FITC. Some HeLa cells were treated with tunicamycin to inhibit the N-glycosylation pathway; 10,000 cells were counted for each assay. Transfected with gene for rhesus CD89 HeLa cells; transfected HeLa cells with N-glycosylation blocked; control untransfected HeLa cells.
pressed on systemic myeloid lineage cells and subsequent release by causing immune activation through cross-linking of CD89 or alternatively, following breaching of the gut barrier, IgA-opsonized NHP species could result in presence of different cleavage sites, thus minimize immune activation. The high extent of hinge variability in IgA (47). Thus, in mangabeys, presence of intact IgA would involve the action of gut microbes, which are controlled by intact inflammation, which is minimal in mangabeys. Immune activation may influence the flexibility of NHP IgA molecules with respect to mucosal sites in this species, such viral replication is not accompanied by extensive immune activation. Considering the emerging view that HIV immunopathogenesis at the mucosa during acute and chronic infection may be a decisive factor in disease progression (46) and the immunomodulatory potential of IgA, it is prudent to evaluate the variability of genes involved in mucosal immune responses.

Our results clearly show the high degree of sequence hypervariability of the hinge regions, which contributes to segmental flexibility as well as to intermolecular covalent assembly. These results confirm the evolutionary instability of this region. We identified two different IGH A hinge sequences in several animals, whereas some others showed only one hinge sequence. Interestingly, IGH A hinge region sequences present in some NHPs were also present in others, although in combination with different alleles, as observed between African and Asian species studied. Therefore, it appears that there is only a limited number of hinge sequences present in the various NHP populations. These sequences are shared by animals of different geographic origins and are present in different combinations in heterozygous animals. The hinge sequences differed in the number of proline residues, which may influence the flexibility of NHP IgA molecules with respect to immune complex formation. In addition, because different hinge sequences are responsible for the differential susceptibility of Ab molecules to proteolysis, NHP and human IgAs may differ with respect to sensitivity to bacterial proteases.

As mentioned above, SIV pathogenesis differs greatly between macaques and mangabeys, including the extent of immune activation, which is minimal in mangabeys. Immune activation may involve the action of gut microbes, which are controlled by intact IgA (47). Thus, in mangabeys, presence of intact IgA would minimize immune activation. The high extent of hinge variability in NHP species could result in presence of different cleavage sites, different susceptibility to the action of proteases and different levels of intact IgA. Intact IgA can potentially control gut microbes, or alternatively, following breaching of the gut barrier, IgA-opsonized bacteria might modulate the immune responses for example by causing immune activation through cross-linking of CD89 expressed on systemic myeloid lineage cells and subsequent release of cytokines. On the basis of hinge sequences, it is reasonable to assume that the four NHP species examined in this study express protease-resistant IgA molecules. However, specific alleles identified in individual animals from each species might encode molecules susceptible to cleavage. Therefore, although the variability in IgA hinge sequences might result in SIV pathogenesis differences at the individual level, it is not likely involved in differences at the species level. Clearly, NHP IgA molecules with different sequences should be evaluated for their protease resistance/susceptibility.

The IGH A CH2 domain contains a variable number of cysteines, which form the interchain disulfide bonds by connecting two parallel polyproline double helices. In humans, conserved cysteines are located at CH2 positions 1.2 and 1.1 in the IMGT unique numbering (31), right after the hinge (48). As expected, these hydrophobic cysteines are present at the same position in NHP; however, although one of two cysteines is always present, there is a change to serine at position 1.1 (C1.1>S) in some alleles of Ceroraty and there is a frequent change to aspartate at position 1.2 (C1.2>D). It remains to be seen whether the substitution of cysteines affects the flexibility or alters the interactions between the H chains.

Very high intra- and inter-species heterogeneity is present in all the CH domains for the NHPs studied. Specifically, sequence variability was observed in clusters in CH1, CH2, and CH3 domains. The amino acid substitutions present between IGH A allelic variants of one species differs not only from each other but also from other NHP IGH A sequences in the hinge region and in the CH1 domain. The other amino acid substitutions present between the IGH A allelic variants are clustered toward the end of the CH2 and in the middle of the CH3. These substitutions, which are present over a stretch of amino acids, include the majority of differences within human, macaque, baboon, and mangabey IGH A sequences and may contain mutation hot spots. Such regions may have evolved by slowly accumulating point mutations. IGH A hypervariability may potentially result in molecules with new functional properties. Different IGH A alleles may provide different degrees of protection against different pathogens.

As mentioned, many effector functions mediated by IgA molecules are triggered by interaction of their Fc region with Fc α receptors (FcαR1). Fc α is a two-fold symmetric dimer of CH2 and CH3 of IgA H chains (37). Critical IgA residues in FcαR1 (CD89) interactions, although located in CH2 and CH3 domains, lie close enough for the FcαR1 domain to readily interact with both (15). In human IgA the binding remains relatively unaffected by amino acid substitutions of the hinge region (15), probably because CD89 binds to the CH2/CH3 interface.

Sequence analysis of all the NHP IGH A allelic variants showed that the residues involved in the IgA/CD89 interactions are conserved with the exception of serine at position 45 and glutamate at position 45.2 in CH3 (31). Specifically, S45 and E45.2 in all the NHPs studied were substituted by threonine and glutamine, respectively. Although these amino acid changes result in no major change in the hydrophilic/hydrophobic profile, the charge nature of the residue at position 45.2 is changed. The human IgA/CD89 interface is composed of a central hydrophobic core flanked by charged residues, one being glutamate at position 45.2 (48). Interestingly, mutational studies involving removal of the charged nature of glutamate at position 12 and aspartate at position 13 in CH2 (adjacent to the AB/helix loop) and glutamate at position 109 in CH3 (in the FG loop) appear to have little effect on the ability of the Abs to interact with CD89 (49). Similarly, the substitution of charged glutamate to neutral glutamine at position 45.2 in NHPs does not appear to disrupt this
interaction, because recombinant macaque CD89 binds to macaque IgA as well as to both human IgA isotypes.

Previously we have shown that macaque CD89 is highly homologous to its human counterpart, and that macaque and human CD89 share a common expression pattern on different cell types (35). Here, we show that macaque CD89 is able to bind to its native ligand as well as to both human IgA1 and IgA2. Furthermore, we show that macaque IgA binding is enhanced in the presence of the Ag (NIP (5) BSA). Interestingly, blocking of macaque CD89 N-glycosylation reduced CD89 expression and possibly the ability of the residual CD89 to bind IgA. It will be interesting to determine whether or not similar effects characterize the IgA/CD89 interaction in humans. Indeed, NHPs might represent the only valid model to study this interaction, as mice, the preferred immunological model, lack CD89 (50). In addition, studies focusing on modeling the role of CD89 in IgA nephropathy, including those using mice transgenic for human CD89, have given conflicting results (51–53).

Clearly, to select a specific NHP model for each experimental protocol, it is necessary to confirm and fully understand the extent of the variations existing between them. Selecting or breeding animals that are well characterized and as homogenous as possible in immune response genes will improve these models. Indeed, rhesus macaques are characterized by an extremely high polymorphism of MHC genes (54, 55). The high degree of IGHA gene polymorphism in NHP species represents the first and, for the moment, only description of high levels of intraspecies IGHA heterogeneity. Despite the presence of this polymorphism, the similarity of the IgA/CD89 interaction between macaques and humans suggests that these models appear suitable for the testing of IgA-based therapeutics. Clearly, the system described in this study for the generation of recombinant NHP IgA molecules will provide an invaluable tool for future studies designed to characterize the functional properties of the IgA corresponding to the various identified genes and represents a first step for the generation of NHP secretory IgA.

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Disclosures
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