Supplemental digital content

Methods

Preparation of recombinant HGN194 forms

Human J chain precursor (accession number NP_653247), human IgA1 (allele IGHA1*01, accession number J00220), and human IgA2 (allele IGHA2*01, accession number J00221) constant region nucleotide sequences were codon optimized for expression in human cells, synthesized by Genscript, and their accuracies were confirmed by sequencing. Appropriate restriction sites were added to 5' and 3' termini during gene synthesis for cloning into the expression vectors. Next, constant regions were cloned into a mammalian expression vector that was then used for the subcloning of the HGN194 VH region. The HGN194 VH and VL chain were also codon optimized and synthesized by Genscript and then cloned into an IgG1 and Ig-lambda expression vector (kindly provided by Dr. Michel Nussenzweig, Rockefeller University). MAbs HGN194 dIgA1, dIgA2, and IgG1 were produced by transient transfection of suspension cultured 293 freestyle cells with polyethylenimine and expression plasmids encoding corresponding heavy and light chains (in the case of dIgA1 and dIgA2 the J chain expression plasmid was also included). Supernatants from transfected cells were collected after 7 to 10 days of culture. Recombinant HGN194 dIgA1, dIgA2, and IgG1 were affinity purified with Peptide M (dIgA1 and dIgA2) (Invivogen) or Protein A (IgG1) (GE Healthcare) chromatography according to the manufacturer's instructions, and finally desalted against phosphate-buffered saline (PBS) using a HiTrap FastDesalting column. Purified Abs were quantified by ELISA using dIgA1 and dIgA2 or IgG1-specific Abs (Southern Biotech) and the Certified Reference Material 470 (ERMs-DA470, Institute for Reference Materials and Measurements) [1] used as standard material for Ig subclass quantification. Purity and polymeric state of dIgA1 and dIgA2 were confirmed by native-PAGE analysis and gel filtration chromatography (S200 column). The presence of dIgA1 and dIgA2 associated J-chain was confirmed by Western blot from both native and SDS-PAGE gels.

Animal care

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the U.S. Public Health Services/National Institutes of Health, as well as according to the recommendations in the Weatherall report on "The Use of Non-human Primates in Research" (http://www.acmedsci.ac.uk/images/project/nhpdownl.pdf). The protocol was approved by the Committee on the Ethics of Animal Experiments of Emory University (IACUC ID: YER-2000618; Emory University Animal Welfare Assurance Number A3180-01). The RMs were housed at the Yerkes National Primate Research Center (YNPRC, Emory University, Atlanta, GA). YNPRC facilities are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. Animal experiments were approved by the Institutional Animal Care and Use Committees at Emory and the Dana-Farber Cancer Institute via a Collaborating Institution Animal Use Agreement. Because the experiments described here involved a virus that may cause an incurable disease, such as AIDS, discomfort, stress and pain may occur. Animals were closely monitored and observed for development of disease at least twice daily. If the animals are determined to be under stress or in discomfort, appropriate anesthetics and/or analgesics are administered as directed by the clinical veterinary staff. Euthanasia is also an option should treatment not alleviate stress. In the current study, no untoward clinical problems were noted, and none of the virus-infected monkeys progressed to AIDS.

TRIM5α genotyping

Genomic DNA was isolated from whole blood with the DNeasy Blood & Tissue Kit (Qiagen). A portion of the TRIM5α gene was then PCR amplified with primers TRIM5αF (CATGACCTTGAAGAAGCC) and TRIM5αR (GCTTCCCTGATGTGATAC) with GoTaq DNA polymerase (Promega) to yield an 872 bp band. The PCR product was purified and 10 µl digested with *NsiI*. Digested samples were separated through a 1.5% agarose gel, and the presence of CypA alleles identified a doublet of bands around 450 bp generated by *NsiI*

digestion at a site unique to the CypA allele. All samples were determined to not be homozygous for the CypA allele (as indicated by the presence of the 872 bp band post digestion) were then sequenced directly using the primer TRIM5αSeq (CAGTGCTGACTCCTTTGCTTG). The genotypes were then determined by analyzing sequences using the JustBio Translator (http://www.justbio.com/index.php?page=translator).

Surface-plasmon resonance (SPR) analysis of HGN194 dIgA1, dIgA2, and IgG1 binding to soluble monomeric gp120$_{1157ip}$

SPR (Biacore 3000, GE Healthcare) was used to assess relative binding affinity of HGN194 nmAbs to soluble gp120$_{1157ip}$. A Biacore CM5 (GE Healthcare) chip was amine-coupled with either gp120$_{1157ip}$ or nmAb dIgA1 and dIgA2, respectively, to high surface densities of 5,000-10,000 response units. Different concentrations of analytes were then injected in randomized duplicate runs (association/dissociation phases of up to 3 min - flow rate of 30 µl/min). In all cases, Biacore HBSEP buffer was used (10 mM Hepes, pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.1% P-20) at 25°C. Optimal regeneration was achieved by injection of 25 mM glycine (pH 1.5) (flow rate of 100 µl/min for 6 s). The signal from the reference flow cell was subtracted from the analyte binding response obtained from flow cells with immobilized ligands.

Avidity assay

Analysis of antibody avidity was performed essentially as described [2]. Briefly, ELISA plates were coated with concanavalin A (Sigma) at 50 µg/ml in PBS for 1 h at room temperature, washed 3 times with H_2O and incubated ON with gp120$_{1157ip}$ at 100 µg/ml in PBS at 4°C. Then, plates were blocked with 3% NZ-Caze (Sigma), 0.5% Tween 20 (Sigma) in PBS for 2 h. After washing, plates were incubated with antibodies at 0.5 µg/ml for 1 h. To determine the avidity index, identical samples were incubated 3 times for 5 min with PBS or PBS with 8M urea (Sigma), respectively. After that, plates were washed 5 times with PBS and incubated with

secondary antibodies labeled with horseradish peroxidase. Finally, color reaction was developed with TMB (Invitrogen). Avidity index (AI) was calculated as follows:

$$AI(\%) = \left(\frac{OD_{450nm} \text{ of sample washed with PBS}}{OD_{450nm} \text{ of sample washed with 8 M Urea}} \right) \times 100$$

HGN194 dIgA1 and dIgA2 binding to cell-expressed rhesus macaque CD89

Binding of HGN194 dIgA1 and dIgA2 to CD89 was assessed by flow cytometry. HGN194 dIgA1, dIgA2, and IgG1 were added to 0.5 x 10^6 cells at 20 µg/ml for 1 h at 4°C. After washing, cells were stained with 5 µl of phycoerythrin (PE)-conjugated anti-human F(ab')2 (Jackson Immunoresearch) for 30 min at 4°C. Cells were washed, fixed with 1% paraformaldehyde, and analyzed by flow cytometry (FACSDiva).

References

1. Schauer U, Stemberg F, Rieger CH, Borte M, Schubert S, Riedel F*, et al.* **Establishment of age-dependent reference values for IgA subclasses**. *Clin. Chim. Acta.* 2003,**328**:129-133.

2. Cole KS, Murphey-Corb M, Narayan O, Joag SV, Shaw GM, Montelaro RC. **Common themes of antibody maturation to simian immunodeficiency virus, simian-human immunodeficiency virus, and human immunodeficiency virus type 1 infections**. *J. Virol.* 1998,**72**:7852-7859.

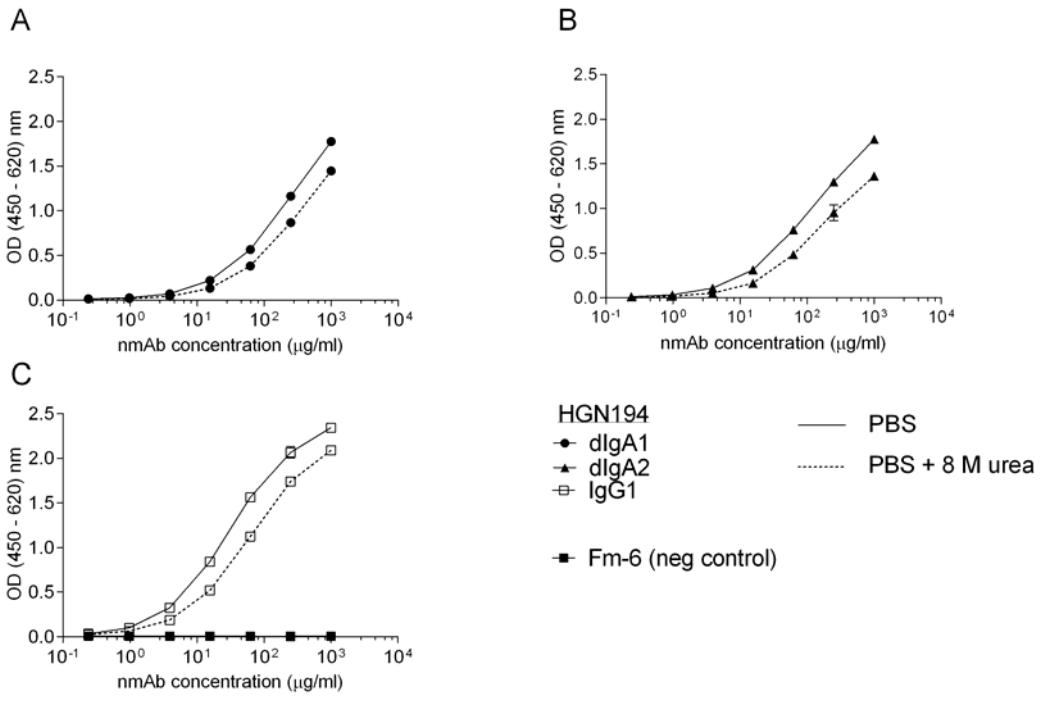

Fig. S1. HGN194 dIgA1, dIgA2, and IgG1 do not have any significant difference in avidity index. Plates were coated with concanavalin A and $gp120_{1157ip}$ was added. To determine the avidity index, identical samples were incubated 3 times for 5 min with PBS (solid line) or PBS with 8 M urea (dashed line), respectively.

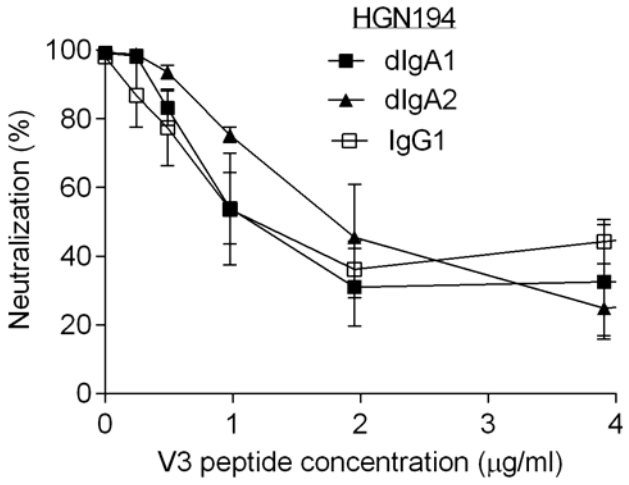

Fig. S2. V3 peptide inhibits equally the HGN194 dIgA1, dIgA2, and IgG1-mediated neutralization of SHIV-1157ipEL-p. Each HGN194 isotype was maintained at the IC_{90} throughout the experiment while the V3 peptide concentration varied from 0 to 3.7 μg/ml. The data shown are representative results obtained from two independent experiments.

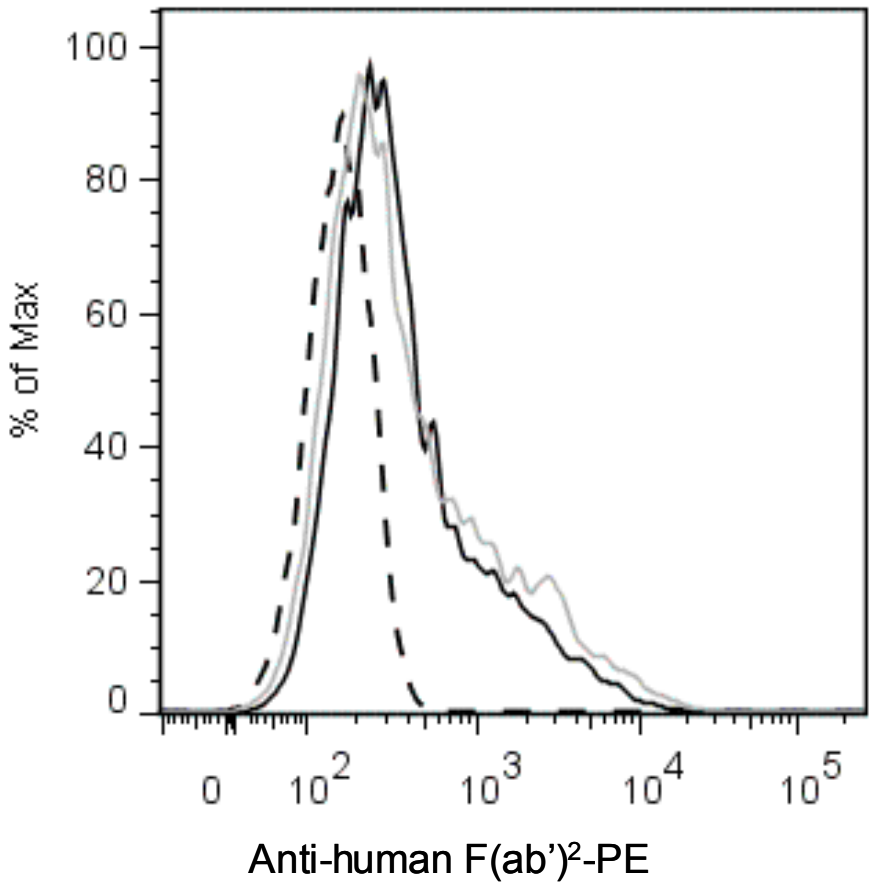

Fig. S3. **HGN194 dIgA1 and dIgA2 bind similarly to the rhesus macaque IgA Fc receptor, CD89.** Binding of HGN194 dIgA1 and dIgA2 to RM CD89 was assessed by flow cytometry. Human HGN194 dIgA1 (black line) and dIgA2 (grey line) were tested at 20 µg/ml. A dashed line represents the negative control (no mAb + anti-human F(ab')2-PE). The data shown are representative results obtained from two independent experiments.

Table S1. Pharmacokinetic study: HGN194 isotype concentrations (μg/ml) in adult rhesus monkey rectal fluids.

HGN194 isotypes	RM names	Time post-antibody delivery (h)			
		0.5	3	6	24
dIgA1	RVe-7	102.5	111.5	<0.001	<0.001
dIgA1	RHg-7	326.9	73.5	8.5	<0.001
dIgA1	RBv-10	1053.8	53.7	<0.001	<0.001
dIgA1	RRv-10	580.2	39.7	21.7	<0.001
dIgA2	RVe-7	123.1	20.3	2.6	<0.001
dIgA2	RHg-7	156.6	18.1	12.4	<0.001
dIgA2	RBv-10	88.3	5.7	5.1	<0.001
dIgA2	RRv-10	558.5	3.4	9	<0.001
IgG1	RVe-7	119.5	7.4	1.8	0.2
IgG1	RHg-7	225.6	130.7	21.1	5.5
IgG1	RBv-10	237.2	49	1.2	0.1
IgG1	RRv-10	48.9	0.2	0.1	<0.0002

Table S2. Genetic characteristics of infant rhesus monkeys enrolled in this study

RM names	Mamu MHC			MamuCD16			TRIM5α
	A01	B08	B17	3A-1	3A-2	3A-3	
Group 1 (dIgA1)							
RAy-14	-	-	-	X	X		CypA / Q
RLz-14	-	-	-		XX		TFP / TFP**
RNv-14	+	-	-	XX			Q / TFP
ROw-14	-	-	-	X		X	Cyp A / Q
RTu-14	-	-	-	XX			Cyp A / Q
RWw-14	-	-	-	X		X	Cyp A / TFP**
Group 2 (dIgA2)							
RAk-14	-	-	-	X	X		Cyp A / Q
RGu-14	-	-	-		XX		TFP / TFP
RGz-14	+	-	-		XX		Cyp A / TFP**
RIl-14	-	-	-	X		X	TFP / TFP
RYu-14	-	-	-	XX			Cyp A / Q
RYv-14	-	-	-	XX			TFP / TFP**
Group 3 (IgG1)							
RCu-14	-	-	-		X	X	TFP / TFP**
RCw-14	-	-	-	X	X		Cyp A / Q
RJk-14	+	-	-		XX		Q / TFP
RKv-14	-	-	-		XX		TFP / TFP**
RMy-14	+	-	-	XX			CypA / TFP**
RRs-14	-	-	-		XX		CypA / TFP**
Group 4 (controls)							
RCl-14	-	-	-	X	X		Q / TFP
RCq-14	+	-	-	XX			Cyp A / Q
RDp-14	+	-	-	X	X		TFP / TFP**
RLp-14	-	-	-				CypA / TFP**
RMq-14	-	-	-	XX			CypA / TFP**
RRn-14	-	-	-	X	X		CypA / TFP**
RRp-14	-	-	-	X	X		CypA / TFP**
RUv-14	+	-	-	X	X		CypA / Q
RVk-14	+	-	-	X	X		CypA / Q
RVn-14	-	-	-		XX		CypA / Q
RZq-14	-	-	-		XX		CypA / TFP**

** indicates that the animals have a higher susceptibility to infection, based on their TRIM5α genotype.