Vaccination with Cancer- and HIV Infection-Associated Endogenous Retrotransposable Elements Is Safe and Immunogenic


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Vaccination with Cancer- and HIV Infection-Associated Endogenous Retrotransposable Elements Is Safe and Immunogenic


The expression of endogenous retrotransposable elements, including long interspersed nuclear element 1 (LINE-1 or L1) and human endogenous retrovirus, accompanies neoplastic transformation and infection with viruses such as HIV. The ability to engender immunity safely against such self-antigens would facilitate the development of novel vaccines and immunotherapies. In this article, we address the safety and immunogenicity of vaccination with these elements. We used immunohistochemical analysis and literature precedent to identify potential off-target tissues in humans and establish their translatability in preclinical species to guide safety assessments. Immunization of mice with murine L1 open reading frame 2 induced strong CD8 T cell responses without detectable tissue damage. Similarly, immunization of rhesus macaques with human LINE-1 open reading frame 2 (96% identity with macaque), as well as simian endogenous retrovirus-K Gag and Env, induced polyfunctional T cell responses to all Ags, and Ab responses to simian endogenous retrovirus-K Env. There were no adverse safety or pathological findings related to vaccination. These studies provide the first evidence, to our knowledge, that immune responses can be induced safely against this class of self-antigens and pave the way for investigation of them as HIV- or tumor-associated targets. The Journal of Immunology, 2012, 189: 000–000.

Both tumors and tumor cell lines exhibit aberrant expression of long interspersed nuclear element 1 (L1) (1–5) and human endogenous retrovirus (HERV)-K (6–17). In studies of breast cancer, ovarian clear cell carcinoma, and non-small cell lung cancer, L1 or HERV activity is associated with a poor prognosis and tumor progression to a more invasive phenotype (3, 11, 15, 16, 18). Such tumor-associated expression of endogenous retrotransposable element (ERE) Ags makes them potential targets for vaccines and immunotherapies. Naturally arising T and B cell responses to HERV-K Env in breast cancer patients suggest that immune tolerance, if any, of this self-antigen can be overcome even without therapeutic intervention (16). Further, HERV-E–specific T cells mediated tumor regression of a metastatic renal cell carcinoma following an allogeneic stem cell transplant (19). These findings suggest that active or passive immunization against ERE Ags could potentially benefit cancer patients whose tumors express such target Ags.

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ERE expression is also associated with certain viral infections. EBV infection transactivates expression of HERV-K18 Env, a T cell superantigen, which enables the virus to establish long-term infection of its host (20). HIV-1 infection triggers HERV-K RNA and protein expression because Ab responses to HERV-K are more commonly found in HIV-1-infected persons (21–24). Further, T cell responses have been observed against a variety of HERV peptides in HIV-1-infected patients, with the magnitude and breadth of these responses correlating negatively with viral load (24–26). Indeed, the HERV-K family human mouse mammary tumor virus-like (HML)-2 Gag and Env proteins are detectable in HIV-1-infected cells in vitro (R.B. Jones, K.E. Garrison, S. Mujib, V. Mihajlovic, N. Aidarus, D.V. Hunter, E. Martin, M.J. Vivek, W. Zhan, N.F. Faruk, et al., submitted for publication). T cell lines targeting these Ags are capable of killing autologous HIV-1-infected, but not uninfected cells, independently of the HIV-1 clade (R.B. Jones et al., submitted for publication). These studies open the possibility that ERE Ags might be useful surrogate targets for vaccination against HIV-1, which ordinarily escapes immune control by mutation.

The utility of EREs as tumor- or viral infection-associated Ags will be critically dependent on the level of expression in healthy cells and upon their immunogenicity. Because EREs constitute some 41.3% of the human genome (27, 28), selecting the correct elements will be challenging, especially in light of T cell responses to degraded open reading frames (ORFs) of HERV-E, H, and L (19, 24–26). Indeed, the majority of EREs are inactive after germ-line invasion as a result of inactivating mutations. Only ~100 of the half-million genomic copies of L1 are known to be active in modern humans (2, 29–31). Likewise, no loci of the most recent of the HERV-K subfamily to enter the human genome (HML-2) have proved to be replication competent (32–36).

To define the safety of vaccines and therapeutics targeting EREs, it is critical to first define the expression of L1 and HERV-K (HML-2) in healthy tissues. The reported findings of L1 (37–43) and HERV-K (44) expression underscore the potential for these Ags to be expressed in normal healthy cells in situ. This potential raises the safety concerns of inducing autoimmune disorders or immune complex disease by ERE-based vaccines and immunotherapies. We sought to investigate the preclinical safety and immunogenicity of vaccines based on LINE-1 open reading frame 2 (L1O2) and consensus sequences of simian endogenous retrovirus-K (SERV-K) Gag and Env in relevant animal models. In this study, we probed mouse, human, and nonhuman primate healthy adult tissues with novel or commercially available Abs to L1O2 and HERV/SERV-K Gag and Env, to identify potential target tissues, which, together with literature precedent (39, 42, 45), became the focus of our preclinical safety studies. Next we demonstrated that immunization of mice with murine L1O2 (mL1O2) is safe and immunogenic, inducing a CD8 T cell response without associated tissue damage, allowing us to proceed to rhesus macaque vaccination studies. Vaccination with human L1O2 (hL1O2) and SERV-K Gag and Env induced T cell responses to all three Ags and Ab responses to SERV-K Env in Indian rhesus macaques without vaccine-related pathology. The safety and immunogenicity findings reported in this article support the evaluation of ERE-targeting vaccines and immunotherapeutics in relevant disease models.

**Materials and Methods**

**L1O2 Ag constructs**

LINE-1.3 (L19088) (46) was used for human constructs (hL1O2). A L1O2 consensus sequence of eight hot L1 elements identified by similarity to L1.3 (47) was used to probe the mouse genome for similar intact L1O2 genes (O88913, O88914, O88915, Q7929, Q91288, Q91289, Q9QU12, Q9QWY0, Q9QWY2, and Q9QWY3), which were used to produce an mL1O2 consensus sequence. Amino acid substitutions D205A in the endonuclease domain and D702A in the reverse transcriptase (RT) domain were made in both hL1O2 and mL1O2 by site-directed mutagenesis to prevent enzymatic functions. Full-length (FL) L1O2—as well as L1O2 fragment 1 (Fr1) covering aa 1–400, including the endonuclease domain; Fr2 covering aa 401–800, including the RT domain; and Fr3 covering aa 801–1275—was synthesized for both hL1O2 and mL1O2. Alignments of L1O2 were made with the draft rhesus genome sequences. Three consensus sequences of L1O2 were found in the RT domain, and the endonuclease domain was missing. Investigation of the translated nucleotide database for rhesus macaques using tBLASTn identified 47 nucleotide sequences for rhesus L1O2. A consensus sequence of these had a predicted amino acid sequence identity of 92% (96% positivity according to the NCBI BLOSUM62 scoring matrix) with hL1O2. We opted to use the mL1O2 sequence in macaque vaccination studies on the grounds of its known provenance and high conservation with the expected rhesus form.

**SERV-K Ag constructs**

tBLASTn and a library of HERV-K(HML-2) RT peptide sequences were used to screen build 36.3 of the Celera genome assembly. PERL scripts were used to extract, defragment, and align matches corresponding to proviral insertions containing both long terminal repeats (LTRs). The alignments constructed were edited manually in Se-Al (http://tree.bio.ed.ac.uk/software/seqal/) to construct conserved ORFs. A second round of screening was then performed, in which the consensus gag, pol, and env ORFs were used to BLAST search the human genome. Reading frames that approximated the expected size were considered potentially intact and were manually inspected in Se-Al. Phylogenetic screening of the macaque genome identified anERV family closely related to HML-2 (48). Five intact (or nearly intact) SERV-K (HML-2) gag and env gene sequences (NC_007858.1, NC_007862.1, NC_007868.1, NC_007875.1, and NC_007876.1) and four intact (or nearly intact) env genes (NC_007868, NC_007858, NC_007862.1, and NC_007876.1) were identified in the macaque genome, and used to construct consensus SERV-K (HML-2) gag and env sequences.

**Vaccines**

Genes were synthesized in their native (L1O2) or codon-optimized form (ERV-K), at GeneArt, and cloned into pPV7563, as described previously (49). DNA vaccines were precipitated onto gold beads, as described (50). The control vaccine plasmid was vector backbone only. For rhesus macaque experiments, the Ag plasmids were coprecipitated onto gold beads at a 9:1 ratio, together with pPV7563-encoded rhesus GM-CSF, as described (49). For recombinant adenovirus serotype 5 (rAd5) production, the genes were cloned into pShuttle-CMV and recombined with the Ad5 genome, using the AdEasy System (Q-Biogene, Carlsbad, CA). Control rAd5 vectors encoded enhanced GFP (eGFP). Final production and purification of rAd5 vaccines were performed by ViraQuest (North Liberty, IA).

**Primary Abs**

Anti–HERV-K Env mouse mAb HERM-1811-5 was obtained from Austral Biologics (San Ramon, CA). Abs capable of binding human, macaque, and mouse L1O2, or both HERV- and SERV-K Gag, were derived by affinity-purification from peptide-KLH hyperimmunized New Zealand White rabbit serum by Lampire (Pipersville, PA) for hL1O2, and Cambridge Research Biochemicals (Bilkingham, U.K.) for HERV-K Gag. Surface-accessible, immunogen peptides were selected using Protein software from DNASTAR-Lasergene 6. For mL1O2, an anti-RT 781–800 FKENCYKPLLKEIKEETNKWK peptide (90% conserved with the predicted macaque sequence) was selected. For HERV-K Gag, peptides were selected from p15: 229–250 ENKTQPPV AYQYWPPAELQYR and p16: 355–375 GSSSTKLLlDKlKEGIVQYGNPS (96% conserved with the SERV-K CA). Peptide synthesis and conjugations for hL1O2 were performed by New England Peptide, and for HERV-K Gag by Cambridge Research Biochemicals. Abs were validated for Ag specificity and cross-species reactivity by ELISA, Western blot, and immunoprecipitation, followed by in-gel digestion and MALDI-TOF mass spectrometry to demonstrate Ag specific pull-down from transfected cell lysates.

**In-gel digestion and MALDI-TOF mass spectrometry**

Bands of interest in Bis-Tris gels were excised and cut into small pieces; then the proteins were reduced, alkylated, and digested with trypsin (Promega, Madison, WI) overnight at 37°C. The supernatant was removed, and two extractions with 60% acetonitrile/0.1% trifluoroacetic acid (TFA) were performed. All three fractions were combined, and their volume re-
duced to 5 μl in a speed-vac. Fifteen microliters of 0.1% TFA was added to each sample, and the peptides were purified using C18 ZipTips (Millipore, Bedford, MA) according to the manufacturer’s instructions. One microliter of the eluted peptide solution was mixed with 1 μl saturated solution of α-cyan-4-hydroxycinnamic acid (50% acetonitrile/0.1% TFA) and spotted onto a stainless steel MALDI plate. Spectra were acquired on a Bruker Ultraflex II MALDI mass spectrometer in reflector mode. After internal calibration using two trypsin autolysis peaks (mass-to-charge ratio 842.50 and 2211.00 Da), experimental peptide masses were matched against mass lists generated by in-silico digestion of the Ag sequences.

### Tissue arrays

Tissue arrays were created for humans, mice, and rhesus and cynomolgus macaques. One or more representative sections from each pivotal organ (with a minimum of one section from each paired organ) were collected and processed from healthy individuals. The tissue list comprised the following: gastrointestinal tract (tongue, salivary glands, stomach, duodenum, jejunum, ileum, cecum, and colon); endocrine organs (thyroid, pancreas, adren al, and thyroid); skeletal muscle (gastrocnemius in mice and quadriceps in macaques); cardiovascular system (heart, aorta, and mesenteric arteries); skin; lymphoid organs (tonsil, spleen, lymph node, and thymus); bone marrow; central (cerebrum, including hippocampus and hypothalamus); cerebellum; medulla; pituitary; and spinal cord) and peripheral (sciatic nerve) nerve tissue. All tissue samples were pretreated with a Tris/EDTA, pH 8.0, Ag retrieval system (Ventana MultichannelSlides, Pittsburgh, PA). Immunohistochemistry was performed by loading onto the Ventana XT Autostainer (Roche Diagnostics, Indianapolis, IN). The system dewaxes the slides; pretreats them with a Tris/EDTA, pH 8.0, Ag retrieval system (Ventana mCC1) for 16 min; blocks endogenous peroxidase; and then stains with a primary Ab for 1 h at room temperature, and subsequently a biotinylated secondary Ab (Merck KGaA, Darmstadt, Germany) was applied according to the manufacturer’s instructions. After removal of the slides from the Ventana system, they were dehydrated, treated with xylene to clear, and mounted using DPX. Primary Abs were validated on fixed eGFp- or Ag-transfected HEK 293 cells. Each cell line was stained with the likely concentration of Ag-transfected positive control cells. The specificity of staining was assessed using the appropriate control cells. The intensity (scored on a 4-point scale from minimal to marked intensity), distribution, and/or characteristics from the isotype control were assessed. Any lesions noted during gross examination.

### Processing for histopathology and immunohistochemistry

Tissue sections or cell cultures were fixed in 10% buffered formalin, embedded in paraffin, cut into 4- or 5-μm sections, and mounted onto Superfrost Plus slides (Fisher Scientific, Pittsburgh, PA). Immunohistochemistry was performed by loading onto the Ventana XT Autostainer (Roche Diagnostics, Indianapolis, IN). The system dewaxes the slides; pretreats them with a Tris/EDTA, pH 8.0, Ag retrieval system (Ventana mCC1) for 16 min; blocks endogenous peroxidase; and then stains with a primary Ab for 1 h at room temperature, and subsequently a biotinylated secondary Ab (Merck KGaA, Darmstadt, Germany) was applied according to the manufacturer’s instructions. After removal of the slides from the Ventana system, they were dehydrated, treated with xylene to clear, and mounted using DPX. Primary Abs were validated on fixed eGFp- or Ag-transfected HEK 293 cells. Each cell line was stained with the likely concentration of Ag-transfected positive control cells. The specificity of staining was assessed using the appropriate control cells. The intensity (scored on a 4-point scale from minimal to marked intensity), distribution, and/or characteristics from the isotype control were assessed. Any lesions noted during gross examination.

### Clinical chemistry, hematology, and urinalysis

The complete blood count, differential, and reticulocyte parameters were measured using whole blood (K2 EDTA) on the Siemens Advia 120 Hematology Analyzer. Standard clinical chemistry parameters were measured in serum on the Siemens Advia 2400 Chemistry Analyzer. Serum insulin was determined using the Siemens Advia Centaur automated immunoassay platform. Glucagon was measured in plasma (K2 EDTA and aprotonin) by the BioPlex Lumex Suspension Array System. Urinalysis was performed using the Clinitek Atlas Chemistry Analyzer (Merck KGaA, Darmstadt, Germany). Urinary creatinine and N-acetylgalactosamine studies were also performed on the Advia 2400. Light microscopy was used for microscopic analysis of urine sediment in all urinalysis samples.

### Peptides

Peptides

For mL1O2, peptides were synthesized by New England Peptide (Gardner, MA). Two sets were obtained: 15-mer peptides overlapping by 10 residues; and a predicted MHC class I binding epitope peptide. Twelve peptides were generated using the MHC I processing method at Immune Epitope Database (artificial neutral network). Seventeen predicted peptides were identified from Fr1, 18 from Fr2, and 27 from Fr3. For the macaque studies, the following were obtained: 15-mers overlapping by 11 aa spanning the entire protein sequence of mL1O2 (JPT, Berlin, Germany) and SERV-K Gag and Mamu-A*B*11, Mamu-B*07, Mamu-B*04, Mamu-B*05, Mamu-B*07, Mamu-B*08, Mamu-B*17, and Mamu-B*29, using sequence-specific primers (51, 52). We excluded Mamu-B*17* and Mamu-B*08* animals from this study because these alleles are associated with spontaneous control of SIV replication.
Peptides were divided into pools of ≤10 peptides.

**T cell cytotoxicity assay**

Ex vivo CTL assay was conducted using the Cr-release assay, as described previously (56), with modifications. Briefly, splenocytes (3 × 10^6 cells) from individual mice were cultured in complete RPMI 1640 medium supplemented with 10% (v/v) FCS, 2 mM l-glutamine, 10 U/ml penicillin G, 10 mg/ml streptomycin, and 0.025 mg/ml amphotericin B (HyClone, Logan, UT) to prevent protein transport into the Golgi apparatus, with or without peptide stimulation with 10 μM peptide. For experiments with CD107a, we added anti-CD107a at the beginning of the assay and added monensin according to the manufacturer’s directions with the Brefeldin A. After the incubation period, we washed and stained the cells for selected surface markers (CD8 and CD4) and fixed them overnight in 2% paraformaldehyde at 4˚C. On the following day, we permeabilized the cells with 0.1% saponin in PBS containing 10% FCS–secreting cells in whole unfractionated serum (compare with Supplemental Fig. 1, E&H). For rhesus macaque ELISPOTs, fresh PBMCs isolated from EDTA-anticoagulated blood were used for the detection of IFN-γ secretion (data not shown). Thus, we used the RT peptide affinity-purified anti-hL1O2 RT IgG polyclonal Ab (pAb), which showed an enhanced specificity of staining of L1O2 Fr2-transfected HEK 293 cells when compared with the whole unfractionated serum (compare with Supplemental Fig. 1, E&H).

**Statistical analysis**

All statistical analyses were performed using GraphPad Prism software (v5.01, GraphPad Software, San Diego, CA) and were vetted by a statistician. Groups were compared using the Kruskal–Wallis test, followed by two-tailed, unpaired Mann–Whitney tests, except in the case of ELISA titer data, which were log transformed and analyzed by ANOVA and the unpaired Student t test.

**Results**

**Target colocalization studies in human, rhesus and cynomolgus macaque, and mouse tissues**

To predict the potential risks of ERE vaccination in humans and how well these might be modeled in nonhuman primates (SERV-K and L1O2) and mice (L1O2 only), we first performed immunohistochemical studies of mouse, human, and rhesus and cynomolgus macaque healthy tissue arrays. Our reagent Abs were the commercial HERV-K Env mAb HERM-1811-5 and our own Abs raised against one hL1O2 peptide and two HERV-K Gag peptides. We validated the Abs extensively for our purposes, demonstrating specificity and cross-species reactivity (Supplemental Figs. 1–3).

Using unfractionated L1O2 RT antisera, we identified staining in normal healthy testes similar to that reported previously (39). However, the presence of both the immunogen RT peptide and an irrelevant peptide abrogated staining, calling the specificity of this staining into question (data not shown). Thus, we used the RT peptide affinity-purified anti-hL1O2 RT IgG polyclonal Ab (pAb), which showed an enhanced specificity of staining of L1O2 Fr2-transfected HEK 293 cells when compared with the whole unfractionated serum (compare with Supplemental Fig. 1, E&H).

Indeed, we observed no staining of healthy testes with this validated Ab. Upon probing of the tissue arrays from the four species with this purified pAb only, the medullary region of the adrenal gland demonstrated specific staining (Fig. 1A, 1B, Table I). In mice, specific L1O2 staining was also seen in the adrenal medulla, as well as in the β islets of the pancreas and in the brain (Table I). Within the brain, the immunostaining was seen in the pituitary gland (occasional pituitocytes in the pars distalis), cerebellum (Purkinje cells), and cerebrum (occasional neurons). These studies suggest macaques would more closely model safety and immunogenicity aspects of L1O2 vaccination in humans, whereas mice might overestimate the potential autoimmunity risks and/or show greater immune tolerance of L1O2 owing to wider tissue expression.

We next probed tissue arrays from human and the nonhuman primate species with four pAbs against two HERV-K Gag peptides (p15 and capsid [CA] regions). The tissues demonstrating the most consistently specific staining for all four HERV-K Gag Abs in one or more species were as follows: CNS (neurons, Purkinje cells, and occasionally ependymal cells) (Fig. 1C, 1D); endocrine pancreas (few cells in the β islets of Langerhans) (Fig. 1E); and exocrine pancreas (ductal epithelial cells). The other tissues with specific staining (kidney, adrenal gland, male and female genital tract, vasculature, stomach, small intestine, and bone marrow) reacted with one or both Abs, recognizing either just the p15 or the CA
regions (Table I). The high degree of interspecies concordance between humans and macaques suggests that vaccination with SERV-K Gag in macaques would be a good model for HERV-K in humans.

For HERV-K Env we used a commercially available mAb, HERM-1811-5, to probe the tissue arrays. HERV-K Env expression was not detected in the human tissue array. However, in nonhuman primates, SERV-K Env expression was observed in several tissues (Table I), most notably in the kidneys (Fig. 1F). These findings suggest that macaques might overestimate the risks of HERV-K Env vaccination in humans.

Safety and immunogenicity of vaccination with mL1O2 in mice

Because L1O2 expression is tightly regulated in vivo, we tested a variety of mL1O2 constructs (Fig. 2A, upper left) in initial experiments to determine whether self L1O2 immunization could elicit immune responses. Female BALB/C mice were immunized at weeks 0 and 4 with plasmid DNA encoding FL or Fr1, Fr2, Fr3, or Fr1+2+3 mL1O2 constructs using PMED (Fig. 2A). At 2 wk after the boost, Fr2-elicited Ag-specific killing was superior to the other fractions of mL1O2 (Fig. 2A, upper right). Vaccination with the mixture of Fr1+2+3 elicited a relatively lower magnitude CTL response. In contrast, mice immunized with FL, Fr1, or Fr3 mL1O2 showed background levels of CTL response close to that of naive mice. After an additional boost, the IFN-γ ELISPOT response was assessed in whole splenocytes and enriched CD8 T cells. In accordance with CTL activity, only Fr2 induced an Ag-specific CD8 T cell response, and this response was increased 10-fold by CD8 T cell enrichment (Fig. 2A, bottom).

To further our initial observations, we next conducted a series of experiments testing the mL1O2 fragments separately in the context of the following homologous and heterologous prime-boost regimens: DNA or rAd5 alone; DNA, then rAd5; DNA twice; DNA twice, then rAd5; or three DNA immunizations (Fig. 2B). To enhance the sensitivity of our readouts, we used mL1O2 fragment-specific peptide pools, rather than pools comprising all 57 predicted peptides. Fr1 was poorly immunogenic irrespective of the vector or regimen. Fr2 induced modest levels of CTL activity but consistently strong IFN-γ ELISPOT responses against a pool of predicted 9-mer peptides and, to a lesser degree, to pools of overlapping 15-mers. Regimens including rAd5 alone or preceded by PMED DNA were the most immunogenic in terms of IFN-γ responses. We fractionated the pooled splenocytes from this group and found CD8 T cell IFN-γ responses to the 9-mers, but little or no response to the 15-mers from either T cell subset (data not shown). We conclude that no CD4 T cell IFN-γ responses were elicited; that the 15-mers were inefficient at stimulating purified CD8 T cells; and that the response measured in whole splenocytes with 15-mers might be largely of NK cell origin, as has been observed elsewhere (59). Therefore, we tested only fractionated CD4 and CD8 T cells in subsequent mouse experiments. With use of our fragment-specific pools, Fr3 induced strong CTL activity but minimal IFN-γ production. No clear pattern of immunogenicity emerged from the different prime-boost regimens with Fr3 (p = 0.1607 by Kruskal–Wallis analysis). Overall, these studies demonstrated that mL1O2 Fr2 and Fr3 could elicit strong CD8+ T cell-dominant immune responses, with CTL activity more potent against Fr3 and IFN-γ production much stronger against Fr2.

Having established that Fr2 and Fr3 were immunogenic in BALB/C mice, we next conducted an additional immunization study using a PMED-prime rAd5-boost regimen for Fr2 and a homologous rAd5 prime-boost regimen for Fr3. As before, Fr2 elicited much more potent IFN-γ ELISPOT responses than did Fr3 (Fig. 2C). Fractionation of the T cells demonstrated that for both Fr2 and Fr3 the response was entirely CD8+ T cell mediated. We conducted full histopathological evaluations, using mice from the experiments represented in Fig. 2B and 2C. These studies revealed minimal to mild, multifocal aggregates of lymphocytes and monocytes at the injection site (skeletal muscle) in all groups receiving i.m. injection of rAd5, but not in mice vaccinated exclusively with the PMED device or naive mice (Fig. 3A–F). These aggregates were often found around small arteries and adjacent connective tissue (perimysium) (Fig. 3C). The nature of this histopathological change (almost a pure population of lymphocytes and monocytes), its predilection for the rAd5-vaccinated groups, and its tendency to be less evident the more time had elapsed between rAd5 vaccination and necropsy indicated that it was not consistent with an autoimmune response to mL1O2; rather, the finding was most likely the result of an immune response against the rAd5 vector itself. Inflammation of the superficial dermis was noted in the skin of mice vaccinated with the PMED device (Fig.
animals. The control group received an empty plasmid PMED DNA. Rhesus macaques were randomly assigned into three groups of eight human primate species of rhesus macaque. Twenty-four Indian rhesus macaques were subjected to the study to compare the number of mL1O2 expression in healthy mice tissues. Likely to result in autoimmune disease owing to low or absent immunogenicity of hL1O2 and of SERV-K Gag and Env in non-human primate species of rhesus macaque. Twenty-four Indian rhesus macaques were randomly assigned into three groups of eight animals. The control group received an empty plasmid PMED DNA prime followed by rAd5 encoding eGFP. Vaccine group 1 consisted of a PMED DNA prime, rAd5 boost regimen, and vaccine group 2 received a reversed modality consisting of a rAd5 prime, PMED DNA boost regimen (Fig. 4A). Both vaccine groups received hL1O2, SERV-K Gag, and SERV-K Env as Ags encoded by separate constructs. PMED vaccinations were given at six weekly intervals, with at least 8 wk between PMED and rAd5. Vaccine group 2 was modified to include an additional PMED DNA booster (four in total versus three PMED immunizations in vaccine group 1). DNA priming substantially improves the immune response to foreign Ags encoded by rAd5 vaccines. Whether DNA priming prior to rAd5 vaccination would improve the immune response to self-antigens is unclear. To determine whether PMED DNA immunizations affected the breadth of responses made following rAd5 vaccination with self-antigens, we compared the number of mL1O2 expression in healthy mice tissues.

**Immunogenicity of hL1O2 and of SERV-K Gag and Env in Indian rhesus macaques**

We next expanded our study to the more clinically relevant non-human primate species of rhesus macaque. Twenty-four Indian rhesus macaques were randomly assigned into three groups of eight animals. The control group received an empty plasmid PMED DNA prime followed by rAd5 encoding eGFP. Vaccine group 1 consisted of a PMED DNA prime, rAd5 boost regimen, and vaccine group 2 received a reversed modality consisting of a rAd5 prime, PMED DNA boost regimen (Fig. 4A). Both vaccine groups received hL1O2, SERV-K Gag, and SERV-K Env as Ags encoded by separate constructs. PMED vaccinations were given at six weekly intervals, with at least 8 wk between PMED and rAd5. Vaccine group 2 was modified to include an additional PMED DNA booster (four in total versus three PMED immunizations in vaccine group 1). DNA priming substantially improves the immune response to foreign Ags encoded by rAd5 vaccines. Whether DNA priming prior to rAd5 vaccination would improve the immune response to self-antigens is unclear. To determine whether PMED DNA immunizations affected the breadth of responses made following rAd5 vaccination with self-antigens, we compared the number of mL1O2 expression in healthy mice tissues.

**Table I. Summary of L1O2 and ERV-K Env and Gag immunohistochemistry findings**

<table>
<thead>
<tr>
<th>Ag mAb or pAb</th>
<th>L1O2</th>
<th>HERV-K Env</th>
<th>HERV-K Gag</th>
<th>P15-4141</th>
<th>p15-4142</th>
<th>CA-4143</th>
<th>CA-4144</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS</td>
<td>Pituitary, Purkinje cells, cerebromedulla</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pancreas</td>
<td>–</td>
<td>Tubular epithelial cells</td>
<td>Medulla (collecting ducts)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Kidneys</td>
<td>–</td>
<td>Tubular epithelial cells (macula densa and proximal convoluted tubules)</td>
<td>Medulla (collecting ducts)</td>
<td>–</td>
<td>–</td>
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<td>–</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>Medulla (neuroendocrine cells)</td>
<td>Capsule (endocrine and stromal cells)</td>
<td>Medulla (neuroendocrine cells)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Q tissues</td>
<td>Cervical epithelium</td>
<td>Endometrial stroma</td>
<td>Cervical epithelium</td>
<td>–</td>
<td>–</td>
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<td>–</td>
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<td>Vascularure</td>
<td>Ductal epithelium (epididymis)</td>
<td>Smooth muscle</td>
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<tr>
<td>Stomach</td>
<td>–</td>
<td>Stomatal epithelium (media)</td>
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<tr>
<td>Small intestine</td>
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<td>Enterocytes</td>
<td>Epithelium (crypts)</td>
<td>Brunner glands</td>
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In mice only, L1O2 immunohistochemistry was conducted, as they lack ERV-K. Human and nonhuman primate tissue arrays were probed with all Abs. Bold indicate tissues stained by more than one Ab.

1Staining observed in nonhuman primate but not humans.
2Not tested on mouse tissues.
3Staining observed only in mice.
4Human adrenal gland samples on tissue arrays had insufficient medulla for assessment.
5Staining observed in nonhuman primate but not humans.
6A lack of specific staining, as defined by being greater in intensity than the isotype control and reduced upon specific but not control peptide competition.
cell responses following either a single DNA or rAd5 prime and throughout the vaccine phase (Fig. 4C). Of interest, a distinct hierarchy emerged in the magnitude with which each Ag was targeted throughout the vaccine phase. hL1O2-specific responses dominated the cellular immune response, with SERV-K Env recognized less frequently and SERV-K Gag being the least targeted of the three Ags (Fig. 4C). To further investigate the kinetics of the T cell response to vaccination with these self-antigens, we performed a longitudinal weekly analysis of vaccine group 2 following the third DNA boost. We observed anamnestic T cell responses at 1 wk following the third DNA boost, and these responses mostly persisted over the 4-wk monitoring period, although the number of peptide pools recognized varied from week to week, peaking with seven of eight animals responding to a total of 18 pools at week 3 (Fig. 4D).

Following the final DNA or rAd5 boost, we observed expansion of anamnestic T cells in seven of eight animals that received a DNA prime (vaccine group 1) and all of the animals that received an rAd5 prime (vaccine group 2) (Fig. 4E). Of note, the single nonresponder in vaccine group 1 consistently had extremely high background levels of IFN-γ, making it difficult to identify positive responses. Although this animal did not make a clear response following the rAd5 boost, we observed a strong IFN-γ T cell response following the third DNA prime (Fig. 4C, post boost 2). Therefore, every vaccinated animal in our study mounted at least one T cell response to the vaccine encoding self-antigen, whereas no responses were observed in animals that received only empty vectors (Fig. 4E). The total magnitude and breadth of T cell responses did not vary significantly between the two regimens at the end of the vaccination phase (p = 0.1853 and 0.2477, respectively). However, vaccine group 2 included an additional higher dose DNA boost using a next-generation PMED platform. This boost increased the magnitude 10-fold and breadth 4.4-fold, although these increases were not quite significant (p = 0.0547 and 0.0625, respectively). If vaccine group 1 is compared with vaccine group 2 following the same number of total vaccinations (i.e., week 35 versus week 22), it had superior magnitude and breadth (p = 0.0111 and 0.0093, respectively). Heterologous prime-boost regimens are clearly affected by dose, timing, and platform, with each component requiring optimization.

To further investigate the T cell response to vaccination with self-antigens, we next selected the strongest T cell response against L1O2, SERV-K Gag, and SERV-K Env and performed intracellular cytokine staining assays using 15-mer peptides. The results mirrored the ELISPOT assay, as L1O2 was again targeted with the highest magnitude (Fig. 5A). The self-antigen–specific CD8+ T cells displayed no responses following the third DNA boost. We observed anamnestic T cell responses at 1 wk following the third DNA boost, and these responses mostly persisted over the 4-wk monitoring period, although the number of peptide pools recognized varied from week to week, peaking with seven of eight animals responding to a total of 18 pools at week 3 (Fig. 4D).

FIGURE 2. The immunogenicity of mL1O2 in BALB/c mice. (A) BALB/C mice (n = 10 per group) were administered 2 μg plasmid DNA encoding various mL1O2 constructs (upper left panel: CMV, promoter; EN, endonuclease; DUF, domain of unknown function 1725 [commonly found in eukaryotic proteins]) by PMED at weeks 0, 4, and 8. At week 6, IFN-γ production in whole splenocytes (bottom left) and enriched CD8+ T cells (bottom right) was determined for the remaining five mice. Targets and APCs pulsed with a pool of 57 peptides (9-mers, H2Kb) derived from mL1O2 were used to determine CTL activity and IFN-γ spot-forming cells, respectively. (B) BALB/C mice (n = 5 per group) were immunized with a truncated form of mL1O2 Fr1 (top), Fr2 (middle), or Fr3 (bottom) according to the indicated schedule, using heterologous prime-boost regimens. At week 6, CTL activity (left panel, at an E:T ratio of 50:1) and IFN-γ production (right panel) were determined. Splenocytes were pooled per group for Fr2 and kept separate for Fr1 and Fr3. (C) BALB/C mice (n = 5 per group) were immunized with mL1O2 Fr2 by DNA prime–rAd5 boost and for Fr3 by rAd5 prime-boost regimen. At week 6, IFN-γ spot-forming cells were quantified in enriched CD4+ or CD8+ T cells. Cells in (B) and (C) were stimulated with a pool of 9-mers and two pools of 15-mers (56–57 peptides per pool) at 10 μg/ml−1 per peptide, derived from appropriate fragments. Bars indicate the mean ± 95% confidence interval; N.D., Not done.
functional deficiencies, as they were able to produce multiple cytokines (IFN-γ and TNF-α) and degranulate (CD107a) in response to cognate Ag (Fig. 5B). Next, we further explored the characteristics of our self-antigen vaccine-induced T cell responses by mapping their minimal T cell epitope and restricting MHC molecules (Fig. 5C). Knowing the restricting allele allowed us to determine if vaccinated animals expressing these alleles would commonly target the same epitope. Indeed, vaccinees sharing an MHC molecule often mounted the same T cell response, as five of five and three of five Mamu-A*02* animals targeted the mapped Mamu-A*02 restricted L1O2 and SERV-K Env CD8+ T cell epitopes, respectively. Furthermore, six of eight vaccinated animals expressing the MHC II molecule Mamu-DPβ1*06 mounted the same CD4+ T cell response against SERV-K Env (Fig. 5D). Finally, knowledge of the minimal CD8+ T cell epitope and MHC I-restricting allele allowed us to fold MHC I tetramers and stain these cells directly (Fig. 5D). Cumulatively, these data indicate that both CD8+ and CD4+ T cells against self-antigen can be generated via vaccination in primates and that they are able to perform multiple effector functions.

We next investigated IgG responses to ERE vaccination. Our studies were constrained by the significant technical challenge of deriving protein of sufficient quality (>90% purity, soluble, low proportion of aggregates and degraded product). This was achieved for the CA subprotein of SERV-K Gag and for the SU and TM subproteins of HERV-K Env. We determined that HERV-K Env is 84% identical to SERV-K Env, favoring sero-cross-reactivity, although potentially underestimating the true response. Sera taken at baseline and 2 wk following the final vaccination were used in our ELISA studies. In control animals, spontaneous weak IgG responses to both SERV-K Gag CA and HERV-K Env were detected in up to half of the animals, but their levels remained constant throughout the study. Conversely, among the vaccinated animals, 14 of 16 macaques made IgG responses cross-reacting to HERV-K Env ranging in titer from 1:28 to 1:15,700 (Fig. 6A). The IgG responses detected by both HERV-K SU and TM ELISAs were significantly greater in vaccine group 2 than in the controls (Fig. 6A [ANOVA, p < 0.0001]). Vaccine group 2 also attained greater titers of IgG against HERV-K TM protein than did vaccine group 1 (p = 0.0160). When the fold ELISA titer change from baseline was examined, vaccine group 2 achieved statistically significant 145-fold and 4.2-fold increases in HERV-K Env TM and SU-specific IgG titers, whereas vaccine group 1 demonstrated a median 3-fold increase in HERV-K Env TM titer only (Fig. 6B [Kruskal–Wallis, p = 0.0041]). Only two macaques made modest vaccine-induced IgG responses to SERV-K Gag CA (data not shown). Although we were not successful at purifying recombinant protein for hL1O2, we were able to screen the baseline and final sera by Western blot on lysates of hL1O2 Fr2-transfected HEK293 cells. We detected a clear vaccine-induced response in a single animal (r99080) from vaccine group 1 that both CD8+ and CD4+ T cells against self-antigen can be generated via vaccination in primates and that they are able to perform multiple effector functions.

ERE vaccination is safe in Indian rhesus macaques

Blood samples were taken for clinical chemistry and hematological analysis twice before vaccination commenced (baseline values) and...
after each vaccination to provide correlation between any findings and the increasing immune response magnitude or duration. No vaccine-related changes from baseline or standard reference ranges for rhesus macaques were evident (data not shown). Emerging immunohistochemistry data showing HERV-K Env expression in the kidneys prompted us to include urinalysis during the experiment, with urine samples taken at weeks 20 and 35. Although we lacked a true urinalysis baseline, no significant changes were detected over the 15-wk period, which included the rAd5 boost in vaccine group 1 and two PMED DNA boosts in vaccine group 2. These in-life safety findings encouraged us to transfer the animals to an SIV-challenge protocol to determine the safety and efficacy of immune response to these Ags at preventing, controlling, and eradicating SIV infection. Animals were repeatedly challenged until they became infected. After 10-12 wk of SIV infection, the animals were culled for detailed safety readouts before chronic SIV disease could become a confounding factor. Extensive analysis of the challenge outcome is under way and will be reported shortly (N.C. Sheppard et al., manuscript in preparation).

Following necropsy, the animals underwent extensive histopathological analysis focusing on tissues that had been flagged by our immunoprobe (Fig. 1, Table I) and by prior reports as potential target tissues (37–39, 42). Early-stage SIV infection could be eliminated as a confounding factor because we had unvaccinated controls for comparison and SIV-related disease has been extensively characterized (61). Even in animals with the strongest immune responses, the tissues were morphologically normal (Fig. 7). Lesions in all animals other than r99002 (see below) were mild and consistent, with the common background pathological features of rhesus macaques maintained in colony at the Wisconsin National Primate Center, combined with their early-stage SIV infection status. Such background lesions include mild focal capsular fibrosis of the liver, minimal focal lymphocytic myocarditis, mild prostatitis, mild cholecystitis of the gallbladder, minimal to mild esophagitis, mild inguinal hernia, and very mild gastritis. We observed no histological evidence of disease induced by an immune response (vasculitis, glomerulonephritis, or retinitis) or by immune complex formation and/or deposition (vascular, glomerular, and ocular inflammation). In one case (macaque r99002), an adenocarcinoma involving the ileum and ileocecal junction was diagnosed. However, intestinal neoplasia accounts for ~48% of all neoplasms diagnosed in this colony; thus, this is not thought to be vaccine related (62). In summary, we observed no adverse safety effects from vaccination with ERE.
Discussion

Mounting evidence suggests that HERV-K and L1 may encode tumor- or viral infection-associated Ags (1–7, 10–12, 14, 16, 17, 20–25). As such, the ORFs of these EREs are novel candidates for targeting with vaccines or immunotherapeutics. Their utility will depend on the consistency of their association with the targeted pathological state and a lack of expression in healthy somatic cells accessible to the immune system. Although evidence for the former has emerged from various laboratories in recent years, evidence of the latter is particularly sparse when one considers only proof of expression at the protein level or direct evidence of increases in genomic copy number within a given tissue. To our knowledge, ours is the first report of somatic tissue array analysis by immunohistochemistry in mice, macaques, and humans for our three candidate Ags: L1O2 and HERV/SERV-K Gag and Env, using Ab reagents carefully selected and validated to enable cross-species investigation.

Ergün and colleagues (39) had earlier reported on L1O2 expression in human Leydig, Sertoli, and vascular endothelial cells of the testes, using chicken IgY pAbs to the L1O2 endonuclease domain. We did not observe the same pattern of staining with our affinity-purified anti-L1O2 RT pAb. The discrepancy with the results of Ergün and colleagues may be due to differential Ab specificity (endonuclease peptide versus RT peptide), avidity, host species (chicken IgY versus rabbit IgG), or procedural differences. The principal procedural difference was that we conducted peptide competition by premixing Ab with peptide and adding the mixture to the slide, whereas Ergün and colleagues first depleted their anti-L1O2 endonuclease IgY preparation with an ∼1:270 molar excess of the endonuclease peptide coupled to Sepharose beads before using cleared supernatant to repeat the stain. Such a procedure may deplete specific Ab entirely from the staining reaction, and in our opinion this does not prove the specificity of their staining.

The use of HERV-K Env mAb HERM-1811 in Western blots, immunofluorescence, and immunohistochemistry has been reported previously (44, 63) (and in R.B. Jones et al., submitted for publication). We conducted our own validation for this mAb and proved that it could recognize SERV-K Env. We did not observe any specific staining of human tissues; however, we did observe staining in macaques.

We developed and validated four of our own pAbs against HERV-K Gag that cross-reacted with SERV-K Gag. The most convincing staining was that shown by Abs to both the p15 and CA components of HERV-K Gag, which occurred in the Purkinje cell body and axons of the brain, and in the β islets of the pancreas of both human and nonhuman primates. We also observed differential staining whereby only the p15 or the CA Abs stained a par-
reference ranges. Moreover, at postmortem there was no evidence of significant changes from baseline values or standard clinical chemistry and hematology studies and urinalysis did not reveal any vaccine-Ag–related findings. Infiltrates at the injection site of the rhesus macaque study, the potential target tissues of mice. Infiltrates at the injection site of the rhesus macaque study, the potential target tissues of macaques with the strongest immune responses to the vaccine Ags were examined at postmortem (10–12 wk following SIV infection). No autoimmune or SIV-related pathological changes are evident. (A) Adrenal gland of r01061. (B) Hippocampus of r01061. (C) Cerbellum of r99080. (D) Pancreas of r99080. (E) Kidney of r01359. (A–D) Original magnification ×10. Scale bar, 100 μm. (E) Original magnification ×20. Scale bar, 50 μm.

There are several potential explanations for the apparent safety observed in these studies. First, it is possible that all the observed staining is caused by cross-reactive proteins. However, we were unable to find any close matches for our immunogen peptides by Blast searches, making this possibility less likely. Second, the immune responses induced might not have been potent enough or of the correct CD4/CD8 balance to drive autoimmunity, especially if Ag-specific CD4 T cell responses are required to provide the cue for migration of CD8 CTLs into tissues expressing the self-antigens, as shown for virus-specific CTLs (64). This is potentially the case for SERV-K Gag, to which only modest CD8 T cell responses were shown for virus-specific CTLs (64). This is potentially the case for SERV-K Gag, to which only modest CD8 T cell responses were observed in 6 of 16 macaques in the absence of detectable CD4 response, and for L1O2, which elicited strong CD8 responses without detectable CD4 responses in both mice and macaques. However, the lack of CD4 responses cannot explain the safety of L1O2, which elicited strong CD8 responses without detectable CD4 responses in both mice and macaques. However, the lack of CD4 responses cannot explain the safety of L1O2, which elicited strong CD8 responses without detectable CD4 responses in both mice and macaques.

The potential targeting of HERV-K Env SU, whereas ○ indicates the response to HERV-K Env TM. Medians are indicated. Statistical significance was determined for: titer data by log-transformation, followed by ANOVA, then Student t tests; fold-change data by Kruskal–Wallis analysis and then Mann–Whitney tests.

There are several potential explanations for the apparent safety observed in these studies. First, it is possible that all the observed staining is caused by cross-reactive proteins. However, we were unable to find any close matches for our immunogen peptides by Blast searches, making this possibility less likely. Second, the immune responses induced might not have been potent enough or of the correct CD4/CD8 balance to drive autoimmunity, especially if Ag-specific CD4 T cell responses are required to provide the cue for migration of CD8 CTLs into tissues expressing the self-antigens, as shown for virus-specific CTLs (64). This is potentially the case for SERV-K Gag, to which only modest CD8 T cell responses were observed in 6 of 16 macaques in the absence of detectable CD4 response, and for L1O2, which elicited strong CD8 responses without detectable CD4 responses in both mice and macaques. However, the lack of CD4 responses cannot explain the safety of SERV-K Gag, to which only modest CD8 T cell responses were observed in 6 of 16 macaques in the absence of detectable CD4 response, and for L1O2, which elicited strong CD8 responses without detectable CD4 responses in both mice and macaques. However, the lack of CD4 responses cannot explain the safety of SERV-K Gag, to which only modest CD8 T cell responses were observed in 6 of 16 macaques in the absence of detectable CD4 response, and for L1O2, which elicited strong CD8 responses without detectable CD4 responses in both mice and macaques.
The immunogenicity and safety of mL1O2 in mice. Finally, for the staining related to the brain, the existence of the Ag within an immune-privileged site might be sufficient to prevent autoimmune disease, as the responding T cells cannot access the tissue. Our use of different vaccination schedules in rhesus macaques revealed that the DNA prime, rAd5 boost regimen showed greater immunodominance of hL1O2 than did the reverse regimen. Because all Ag-encoding plasmids were coformulated onto gold particles and delivered to the same anatomical site, whereas the rAd5 vectors each encoded a single Ag and were delivered at separate anatomical sites, this pattern might reflect competition at the level of the APCS in the local draining lymph nodes. Alternatively, the SERV-K Ags might require more potent danger signals to prime responses, and these would be better provided by our rAd5 vectors than our DNA vaccines. We conclude that future multi-antigen studies should consider regimens that overcome immunodominance, such as rAd5 prime, DNA boost.

In summary, we have expanded the knowledge regarding protein level expression of HERV-K Gag and Env and L1O2 in healthy tissues. Further, we have shown that it is possible to induce both T and B cell responses to self and near-self ERE Ags in preclinical species, and the responses we induced did not cause autoimmune disease. These findings enable the investigation of ERE Ags in relevant efficacy models as tumor- or HIV infection-association targets and form a foundation for preclinical safety studies of lead candidates emerging from such efficacy studies.

Acknowledgments
We thank Prof. Deborah Fuller of Albany Medical College, Fred Immerman of Pfizer for assistance with statistics, Prof. David Watkins and Dr. Nancy Wilson-Schleier of the University of Wisconsin for helpful discussions and guidance, the Wisconsin Primate Research Center Immunology Services and Virology Services for experimental assistance, and members of the Wisconsin National Primate Research Center Animal Care, Scientific Protocol Implementation, and Pathology units for nonhuman pri-

Disclosures
This study was sponsored by Pfizer and comprises both internal research and funding. R.B.J., D.F.N., and M.A.O. are coauthors of two patent applications related to this work. This study was conducted in collaboration with Pfizer funding. R.B.J., D.F.N., and M.A.O. are coauthors of two patent applications related to this work. This study was conducted in collaboration with Pfizer funding.
Figure S1. **Validation of the anti-hL1O2 RT antibody.** A) A multisequence alignment of the RT peptide from rhesus macaques, mice and humans reveals a single conservative substitution at position 14 in the mouse RT peptide, which was shared with rhesus macaques that also had one additional semi-conservative substitution at position 10. B) Polyclonal anti-hL1O2 RT antibody immunoprecipitation from hL1O2 fr2 (46kDa) or full-length (153 kDa) transfected HEK 293 cell lysates yields bands of the correct molecular mass in western blots, lanes 1 & 3 are the mass ladders, lane 2 hL1O2 fr2, lane 4 eGFP-transfected lysates, and lane 5 the full-length protein. C-H) Immunohistochemistry of HEK 293 cells: C) transfected with hL1O2 fr2 and incubated with 1:100 rabbit baseline serum; D) transfected with hL1O2 fr1 and incubated with 1:100 rabbit anti-hL1O2 RT immune serum; E) transfected with hL1O2 fr2 and incubated with 1:100 rabbit anti-hL1O2 RT immune serum alone, F) in the presence of 10μg/ml EN peptide, G) or RT peptide, H) or with affinity-purified anti-hL1O2 RT IgG at 1 μg/ml. I) The band detected in part B) was cut from a replica gel and subjected to tryptic digest and MALDI TOF peptide mass fingerprinting analysis, identifying 16 peptides from the hL1O2 fr2 sequence (labeled 1-16), two trypsin autolysin peaks used to internally calibrate the spectrum (labeled T) and peaks from contaminating proteins (unlabelled). m/z denotes mass-to-charge ratio. J) The table, lists experimentally determined mass, assigned mass according to the predicted sequence and the difference (delta in parts per million). M* residues are oxidized methionines. K) The 16 peptides identified are highlighted in red and the RT peptide is underlined at the C-terminus of fr2.

Figure S2: **Validation of anti-HERV-K Gag p15 and CA antibodies.** (A) A multisequence alignments of the p15 and CA peptides from SERV-K and HERV-K reveals two substitutions in p15, one conservative at position two and one non-conservative at position 14 in the p15 peptide; whereas the CA peptide has three conservative substitutions at positions 7, 10 and 11. (B) Western blots of SERV-K Gag protein constructs MA-Sp1-p15 (40kDa) or CA
(36kDa) proteins using HERV-K Gag p15 and CA whole antisera (left panel), or of full-length HERV-K Gag using affinity purified anti-p15 4142 (right panel) demonstrates specificity and species cross-reactivity: lane 1 – 4 SERV-K Gag proteins probed with 4141 (p15), 4142 (p15), 4143 (CA) and 4144 (CA) respectively; lane 5 – ladder, lane 6 full-length HERV-K Gag probed with affinity purified 4142 (p15). (C) Staining of HEK 293 cells transfected with full-length HERV-K Gag with 10 μg/ml anti-HERV-K Gag p15 4142 antibody alone, (D) with 25 μg/ml CA competing peptide, or with p15 competing peptide at (E) 2.5, (F) 12.5 or (G) 25 μg/ml. (H) Following immunoprecipitation of full-length HERV-K Gag from the lysate of transiently-transfected HEK293 cells using affinity-purified anti-p15 antibody 4142, a band equating that seen in part B was cut from a replica gel and subjected to tryptic digest and MALDI TOF peptide mass fingerprinting analysis, identifying 12 peptides from the HERV-K Gag sequence (labeled 1-12), two trypsin autolysin peaks used to internally calibrate the spectrum (labeled T) and peaks from contaminating proteins (unlabelled). m/z denotes mass-to-charge ratio . (I) The table, lists experimentally determined mass, assigned mass according to the predicted sequence and the difference (delta in parts per million). The peptide sequence marked with an asterisk contains an oxidized methionine residue. Q* indicates a pyroglutamine residue. (J) The 12 peptides identified are highlighted in red and the p15 and CA peptides are underlined.

**Fig S3. Validation of Anti-HERV-K Env HERM-1811-5 mAb for immunohistochemistry.** **A)** To demonstrate the suitability for staining macaque tissues, lysates of FLAG-tagged SERV-K Env transfected HEK 293 cells were probed by western blot using either HERM-1811-5 (lane 2) or anti-FLAG M2 tag antibody (lane 3). **B)** Anti-HERV-K Env mAb HERM-1811-5 was tested at 2.5 μg/ml on mock transfected, and **C)** HERV-K Env transfected HEK 293 cells.
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Figure S1

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### Figure B

![Graph](image)

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### Figure D

![Image](image)

### Figure E

![Image](image)

### Figure F

![Image](image)

### Figure G

![Image](image)

### Figure H

![Graph](image)

### Figure I

![Image](image)

### Figure J

```
1  MASGQTKSKI KSKYASYSF IKILLKRGGV KVSTKNLIK FQIIEQFCPW FPEQGTDLKL
61  DWKIRGEKL KQAGRGINKIP LTVWMDWAI KAALEFPQTE EDSISVSDAP GSCIIIDCEN
121  TRKKSQKETE SLHCXEYVAEP VMAQSTQNVD YNQLQEVITP ETLKLEGKGP ELVGSQESI
181  RGTSPLPAGQ VYVTLPQPOK VKNKTQPVPV AYOYMPAPEL QYRPPPEQSY GPYPMMPAPQ
241  GRAPYPQPT RRLNPTAPPS RQGSELHEII DKSREKGDTE AQWFPVTLEP MPPGEGAQEG
301  EPPTVEARYK GSF0KLLKDL KEVKQYGPAP SPYPRTLDDS IA0GHRILIPY DWEILAKSSL
361  SPQQLFTKFT WWDGQTVQOV RRRAANPPV NIDADQLLGI GQNWSTISQQ ALM0NEAIEQ
421  VRACLRWAKE KIQDQGSCP SFNTRQGSK EPYDFVARL QDVQAKSIAD EKARKVIVEL
481  MAYENANPEC QSAIKLPLKGK VPAGSDLVSE YVKAISIGNQ AMHKAMLMAQ AITGVVGLGQ
541  VRTFQGKCIY CPQXQLKKN CPVQNKQNTT IAQASTTREP PDLCPRCKKG KHNASQCRSK
601  FDKNCQPLSEG NNQRPQGQAP QQTGAFPIQXF VFPQQGFQGQQ PPLS7QFQGI SQQLPQSYMCP
661  PPQA0VQQ
```
Figure S3

A

[Image of gel electrophoresis with molecular weight markers (kDa) and lanes 1, 2, 3, with an arrow pointing to a band near 300 kDa]

B

[Images of cellular staining with blue and brown coloration]