2012

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Adaptive Immunity Restricts Replication of Novel Murine Astroviruses

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The mechanisms of astrovirus pathogenesis are largely unknown, in part due to a lack of a small-animal model of disease. Using shotgun sequencing and a custom analysis pipeline, we identified two novel astroviruses capable of infecting research mice, murine astrovirus (MuAstV) STL1 and STL2. Subsequent analysis revealed the presence of at least two additional viruses (MuAstV STL3 and STL4), suggestive of a diverse population of murine astroviruses in research mice. Complete genomic characterization and subsequent phylogenetic analysis showed that MuAstV STL1 to STL4 are members of the mamastrovirus genus and are likely members of a new mamastrovirus genogroup. Using Rag1−/− mice deficient in B and T cells, we demonstrate that adaptive immunity is required to control MuAstV infection. Furthermore, using Stat1−/− mice deficient in innate signaling, we demonstrate a role for the innate immune response in the control of MuAstV replication. Our results demonstrate that MuAstV STL permits the study of the mechanisms of astrovirus infection and host–pathogen interactions in a genetically manipulable small-animal model. Finally, we detected MuAstV in commercially available mice, suggesting that these viruses may be present in academic and commercial research mouse facilities, with possible implications for interpretation of data generated in current mouse models of disease.

Recent studies in mouse models have highlighted complex interactions between viruses, genetic susceptibility, and disease (reviewed in reference 59). To characterize the enteric virome of specific-pathogen-free (SPF)-housed research mice in an unbiased manner, we used shotgun sequencing, which revealed the presence of multiple novel astroviruses. Astroviruses are nonenveloped, positive-sense, polyadenylated RNA viruses often associated with gastrointestinal (GI) disease (11, 44). Human astrovirus 1 (HAstV1) to HAstV8 are an important cause of gastroenteritis in pediatric inpatients and outpatients, HIV-infected and immunocompromised populations, and the elderly (9, 10, 12, 16, 21, 22, 24, 28, 39, 44, 48, 53, 63). In addition, human astroviruses have been associated with sporadic outbreaks of gastroenteritis in immunocompetent adults (4, 44, 47). As a viral agent of pediatric gastroenteritis, HAstV is reportedly second only to rotavirus (12, 24, 28, 39, 44, 48, 53, 63). In addition, human astroviruses have been isolated from a number of hosts, including wild and domestic animals, marine mammals, and birds, and new astrovirus-susceptible hosts continue to be identified (11, 34, 42, 49, 52).

Astrovirus-related disease is not limited only to the GI tract. Astroviruses are implicated as the cause of hepatitis in ducks and encephalomyelitis in minks (5, 18, 20). In humans, an astrovirus was identified in the central nervous system of an immunocompromised child with X-linked agammaglobulinemia who died of encephalitis (51). Furthermore, we previously reported the presence of human astrovirus MLB2 in the plasma of a febrile child (27). Despite the prevalence of human astrovirus infection and the potential for extraintestinal disease, there are no specific treatment protocols for astrovirus infection, and no vaccine exists. In addition, little is known about the molecular mechanisms of astrovirus infection, replication, disease pathogenesis, or immunity.

The family Astroviridae is divided into the mamastrovirus and avastrovirus genera, which are characterized by the ability to infect mammals and avian species, respectively (44). The mamastrovirus genus is further subdivided into two genogroups based on the complete amino acid sequence of open reading frame 2 (ORF2) and distinguishes genogroups I and II based on a mean amino acid genetic distance (p-dist) of 0.671 ± 0.016 between the groups, with intragenogroup distances ranging from 0.338 to 0.783 (31).

Across both mamastrovirus and avastrovirus genera, the astrovirus genome ranges from 6.1 to 7.7 kb in length, not including the 3′ polyadenylated tail, and contains three ORFs as well as 5′ and 3′ untranslated regions (UTRs) (44). ORF1α encodes a polypeptide of 920 to 935 amino acids (aa) containing several conserved motifs, including a serine protease (11, 44). A highly conserved heptanucleotide motif and downstream hairpin structure at the ORF1α-ORF1β junction generates a −1 frameshift, leading to the translation of an ORF1α-ORF1β polypeptide which is later cleaved into polypeptides corresponding to ORF1α and ORF1β (11, 29, 41, 44). ORF1β encodes a polypeptide of approximately 515 to 528 aa, containing the RNA-dependent RNA polymerase (RdRP) (40, 44). ORF2 encodes a polypeptide consisting of 672 to 816 aa which contains the viral capsid (11, 40, 44).

Human case studies have highlighted the importance of the adaptive immune system in the control of mamastrovirus disease (51, 63). The turkey model of avastrovirus pathogenesis has been used to characterize astrovirus disease and immune response (32, 33) but lacks the ability to easily dissect host determinants of astrovirus infection and immunology. Furthermore, while mamastrovirus tissue culture systems do exist (23, 38), the ability to elu-
cidate molecular mechanisms that control mamastrovirus infection and pathogenesis has been hampered by a lack of a small-animal model.

In this paper, we report the first complete genome sequences of astroviruses in research mice, murine astrovirus (MuAstV) STL1 and STL2. Subsequent analysis revealed the presence of at least two additional MuAstV STL viruses, suggestive of a diverse pop-ulation of related astroviruses. Phylogenetic analysis of ORF2 indicated that the MuAstV STL viruses group with several other newly described astroviruses in a genetic cluster distinct from ma-mastrovirus genogroups I and II. MuAstV STL is capable of in-fecting laboratory mice, which represent a genetically manipula-ble small-animal model in which to study astrovirus infection. Initial experiments using this model of mamastrovirus infection revealed a role for both innate and adaptive immunity in the con-trol of astrovirus replication. Finally, we show that MuAstV is present in commercially available mice, with potential implica-tions for existing disease models.

MATERIALS AND METHODS

Nucleic acid preparation and 454 sequencing. Shotgun sequencing was performed as follows: stool samples were collected from C57BL/6j mice and resuspended in 6 volumes of phosphate-buffered saline (PBS) (14). The sample was centrifuged to pellet particulate matter, and the superna-tant fluids were then passed through a 0.45-μm-pore-size filter. Total nucleic acid was isolated from 200 μl primary stool filtrate using an Ampliprep DNA extraction machine (Roche) according to the manu-facturer’s instructions. To enable subsequent detection of both RNA and DNA viruses, total nucleic acid from each sample was reverse transcribed and amplified as previously described (61). Briefly, RNA templates were reverse transcribed using primerA containing a 16-nucleotide (nt) specific sequence followed by 9 random nucleotides for random priming. The 16-nt specific sequence is unique for each sample and served as a barcode in assigning sequencing reads to a sample. Sequenase (United States Bio-chemical) was used for second-strand cDNA synthesis and for random-primed amplification of DNA templates using PrimerA. Each sample was then subjected to 40 cycles of PCR amplification using PrimerB containing the same 16-nt specific sequence as in the corresponding PrimerA. Amplification products were pooled, adaptor ligated, and sequenced on the 454 GS-FLX Titanium platform (454 Life Sciences). Detection and analysis of viral sequences using VirusHunter soft-ware. Sequences were analyzed using VirusHunter, a customized pipe-line, as described previously (64). Briefly, sequence reads were assigned to samples based on the unique barcode sequences (i.e., PrimerB sequences). For further analysis, primer sequences were trimmed off and the sequence reads were clustered using CD-HIT (43) to identify redundant reads. Sequences were clustered on the basis of 95% identity over 95% sequence length, and the longest sequence from each cluster was picked as the representative sequence. Then, sequences were masked by RepeatMasker (http://www.repeatmasker.org). If a sequence did not contain a stretch of at least 50 consecutive non-“N” nucleotides or if greater than 40% of the total length of the sequence was masked, it was removed from further analysis (i.e., “filtered”). Good-quality sequences after filtering were sequentially compared against (i) the human genome using BLASTn; (ii) the GenBank nt database using BLASTn; or (iii) the GenBank nr database using BLASTx (2). Minimal e-value cutoffs of 1e−10 and 1e−5 were ap-plied for BLASTn and BLASTx, respectively. Sequences were phylopeted as human, mouse, fungal, bacterial, phage, viral, or other based on the identity of the top BLAST hit. Sequences without any significant hit to any of the databases were placed in the “unassigned” category. If a sequence aligned to both a virus and a member of another kingdom (e.g., a bacte-rium or fungus) with the same e-value, it was classified as “ambiguous.” All eukaryotic viral sequences were further classified into viral families based on the taxonomy identification of the best hit.

All viral sequences and unassigned sequences from each sample were assembled into contigs using Newbler (454 Life Sciences) with default parameters. If a sample was sequenced multiple times, all available se-quencing data were used to try to obtain the best assembly.

Genome amplification and confirmatory sequencing. Total RNA was extracted from the murine stool samples previously used for shotgun sequencing with an RNeasy minikit (Qiagen, Valencia, CA) to generate complete genomic sequences. To generate partial genome sequences corresponding to ORF2, total RNA was extracted from murine stool samples harvested from immunocompetent mice housed in different SPF facilities at Washington University. One microgram of RNA was used as the template for rapid amplification of cDNA ends (RACE) reactions to generate the 5′ and 3′ genome ends with 5′ RACE and 3′ RACE kits (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. To generate full genomic sequences, one microgram of RNA was used as the template for cDNA synthesis using a SuperScript III first-strand synthesis kit and an oligo(dT)12-20 primer (Invitrogen) according to the manufacturer’s in-structions. Sequences were then amplified using Elongase enzyme mix (Invitrogen) and primers 5′-CCAGAAAGAGGAGCATGTGCGACT C-3′ and 5′-GGTITTTTTTTTTTTTTTTTTTTGCGAATTTATTACCCA-3′. Partial genome sequences corresponding to ORF2 were amplified using a 3′ RACE kit and gene-specific primer 5′-CTTTGG AGGGGHHGGACAA-3′. The resulting PCR products were gel purified and ligated into a pcR4-TOPO TA sequencing vector (Invitrogen). Universal M13 forward and reverse primers were used for sequencing, and primer walking was applied as needed. Four clones were used to construct consensus sequence MuAstV STL1 with 2-to-4-fold redundancy using Geneious Pro v5.0.4. Three clones were used to construct the MuAstV STL2 consensus sequence with 2-to-4-fold redundancy. MuAstV STL3 and STL4 consensus sequences were each generated from three clones with 2-to-4-fold redundancy. Predicted ORFs were identified using Geneious. All protein motifs were predicted using Pfam (17).

Phylogenetic analysis. Sequences from astrovirus genomic segments encoding ORF1a, -1b, and -2, available in GenBank as of 26 July 2012, were translated. These sequences were aligned using ClustalX 2.0.12 (37). Phylogenetic inference was performed with maximum parsimony using PAUP 4b10 (57) and maximum likelihood using RAxML (55) and a BLOSUM62 transition matrix with 1,000 bootstrap replicates. The resulting phylogenetic trees were visualized using FigTree 1.3.1 (http://tree.bio .ed.ac.uk/software/figtree). MEGA 5.05 was used for distance estimation (uncorrected p-dist) (58).

Mice. C57BL/6j, B6.Rag1−/− (45), and Stat1−/− (13) mice were bred and housed in an enhanced-barrier SPF facility at Washington University in St. Louis in compliance with federal and institutional guidelines (8). B6.Rag1−/− mice were also purchased from The Jackson Laboratory (Bar Harbor, ME). All studies were performed using age-matched female mice between 8 and 12 weeks of age.

For MuAstV STL detection in commercially available mice, B6.Rag1−/− mice (B6.129S7-Rag1tm1Mom/J; catalog no. 002216) (45) were ordered from The Jackson Laboratory facility AX30, Rag2−/− mice (129S6/SvEvTac-Rag2m1Pfox, catalog no. RAG2-F) (54) were ordered from Taconic facility IBU25, and Fox Chase SCID mice (CB17/ lcr-Pkd−/−; lcrCtrl; catalog no. 236) (6) were ordered from Charles Riverfacility W09. Upon arrival, mice were maintained in their commercial transport container until they were sacrificed for sample collection on the same day.

Measurement of viral RNA by TaqMan qPCR. Total RNA was ex-tracted from individual stool pellets using an RNeasy minikit (Qiagen) or from tissue samples using TRIzol reagent (Invitrogen). One-tenth of the total stool RNA or 1 μg of tissue RNA was reverse transcribed using ImpprolI reverse transcriptase (RT) (Promega, Madison, WI) and random primers (Invitrogen) to yield cDNA. Triplet quantitative PCRs (qPCR) were performed using 1/10 of the cDNA, primers specific to an 80-nt region of ORF1b (sense, 5′-TACATCGAGGGGTGTGCTGC-3′; antisense, 5′-GTCATTACCAACGGCTACACCTTTCCA-3′), and a TaqMan
probe (Applied Biosystems, Foster City, CA) with the sequence 5′-TGTG
GCAATGGGGTTA-3′ containing a 5′-carboxyfluorescein (FAM) dye
label, 3′ nonfluorescent quencher (NFQ), and minor groove binder
(MGB). The number of genome copies per sample was determined by
comparison to a standard curve (generated by a 10-fold dilution of target-
containing plasmid in yeast tRNA [Invitrogen]). For stool samples, the
number of genome copies per sample was multiplied by 100 to account for
dilution from total RNA originally extracted from the stool pellet and is
reported as the number of genome copies per stool pellet.

Nucleotide sequence accession numbers. The sequences for MuAstV
STL1, STL2, STL3, and STL4 have been deposited in GenBank and have
been assigned accession numbers JX544743 to JX544746.

RESULTS
Detection of novel astroviruses using shotgun sequencing. To
examine the mouse virome in an unbiased manner, we generated
cecal RNA and DNA libraries from three immunocompetent wild-
type mice, shotgun sequenced them, and used VirusHunter (14–
16, 25, 26, 50, 64) to analyze the resulting reads. We identified 132
sequences (76, 21, and 35 from mice A, B, and C, respectively)
to analyze the resulting reads. We identified 132
sequences (76, 21, and 35 from mice A, B, and C, respectively)
with significant similarity to known astrovirus sequences. No
other viral reads were identified. The viral reads, as well as reads
with no detectable homology to sequences in public databases,
delected in the feces of mouse A were used to assemble a 6,748-
nucleotide (nt) contig with 9-fold coverage. A BLASTx (2) search
detected in the feces of mouse A were used to assemble a 6,748-
nucleotide (nt) contig with 9-fold coverage. A BLASTx (2) search
from these samples, here called MuAstV STL3 and STL4. MuAstV
STL3 and STL4 showed 95.3% nt identity to each other and 89.6%
to 91.4% nt identity to MuAstV STL1 and STL2. These data demon-
strate the presence of at least four novel astroviruses in research
mouse facilities at Washington University.

The genome organization of MuAstV STL is consistent with
those of other mamastroviruses. MuAstV STL1 and STL2 were
predicted to contain a 5′ UTR, three ORFs (ORF1a, -1b, and -2), a
3′ UTR, and a poly(A) tail. The 5′ UTR was determined to be 46 nt
in length. The 3′ UTRs of MuAstV STL1 and STL2 were deter-
ded to be 90 and 91 nt in length, respectively. ORF1a was pre-
dicted to encode a 298 (MuAstV STL1)- or 924 (MuAstV
STL2)-aa protein, with each containing a trypsin-like peptidase
domain as revealed by Pfam analysis (17) and showing significant
similarities to those of known
in astroviruses by BLASTp (2). The differences in four amino acids
occurred downstream of the putative protease motif. The 58-nt
ORF1a-ORF1b junction of MuAstV STL1 and STL2 contained the
heptanucleotide frameshift signal (AAAAAC) conserved in all
astroviruses (11, 29, 41, 44). Furthermore, FSFinder analysis (46)
confirmed that the downstream sequence may form a stem-loop
structure required for a −1 ribosomal frameshift to lead to
ORF1a-ORF1b translation (7, 19, 44). The first amino acid in
frame with the frameshift signal was predicted to be the start po-
nition for ORF1b of MuAstV STL1 and STL2. ORF1b of MuAstV
STL1 and STL2 is predicted to encode a 502-aa protein containing
an RdRP domain, consistent with other members of the Astroviri-
dae family (11, 44).

MuAstV STL contained a sequence that is highly conserved
among mammalian astroviruses upstream of ORF2, C UUU GGA

![FIG 1 Identification of novel astrovirus sequences. (A) Comparison between the longest contig assembled from mouse A and HAstV6 isolate Katano
(HM237363), the most closely related virus. *, percent amino acid identity and length of the best aligned homologous region compared to the reference
sequence. (B) Comparison between the longest contig assembled from mouse A and contigs assembled from mouse B. **, percent nucleotide identity across
the indicated contig.](http://jvi.asm.org/Downloaded from December 15, 2014 by Washington University in St. Louis)
Characterization of Novel Murine Astroviruses

To evaluate the relationship between astrovirus genogroups I and II, respectively. Intragroup p-dist values of 0.762, 0.010, 0.629, 0.011, and 0.641 ± 0.009 for mamastrovirus genogroups I and II and the MuAstV STL-containing clade, respectively. These p-dist values suggested that the MuAstV STL viruses may be members of a new genogroup within the mamastrovirus genus.

A sensitive and robust qRT-PCR assay for MuAstV detection. As MuAstV STL1 and STL2 were originally identified in the feces of asymptomatic wild-type mice, we examined whether MuAstV STL was present in the feces of other mice from the same SPF research mouse colony. To quantify the number of MuAstV STL1 and STL2 genome copies in tissues and feces, we designed a TaqMan-based quantitative reverse transcriptase PCR (qRT-PCR) assay (Fig. 4A), which targeted a 72-bp region of the RdRP conserved between MuAstV STL1 and STL2. Across multiple experiments, the assay consistently detected from 10^6 to 10^7 genome copies, and 1 genome copy was detected in 2 of 3 technical replicates consistent with Poisson distribution statistics, demonstrating that this assay was both sensitive and robust.

Innate and adaptive immunity contribute to the control of MuAstV replication. Given that previous human studies have implicated the adaptive immune system as essential in the control of astrovirus pathogenesis (63), we measured the number of astrovirus genome copies present in the feces of mice deficient in B and T cells due to a mutation in Recombination Activating Gene 1 (Rag1) (45). While these mice exhibited no overt signs of illness, we detected up to 10^9 astrovirus genome copies per fecal pellet. Notably, 21/21 Rag1^−/− mice that we screened were positive for astrovirus (data not shown). These data suggest that adaptive immunity is important for restricting MuAstV replication.

To assess the role of innate and adaptive immunity in restricting MuAstV replication, we examined the time course of natural astrovirus infection in wild-type mice and mice deficient in signal transducer and activator of transcription 1 (Stat1) (13). As previous studies have shown that astrovirus infections may spread beyond the GI tract (5, 51), we chose to examine infection of extraintestinal tissues as well as intestinal infection and fecal shedding. In order to ensure infection by the same viruses, we cohoused wild-type and Stat1^−/− mice with infected Rag1^−/− mice for 14 days.

Consistent with our screening data from Rag1^−/− mice, we observed high levels of MuAstV shedding in fecal samples from...
FIG 3 MuAstV STL is a member of a putative third genogroup of mamastroviruses. Genomic sequences corresponding to ORF2 were translated and aligned. Phylogenetic analyses were performed using maximum-parsimony (MP) and maximum-likelihood (ML) methods with 1,000 bootstrap replicates. The bootstrap support (ML/MP) is indicated adjacent to each branch of the best-scoring ML tree where significant. Corresponding sequences from representative avastroviruses were included for comparison. GenBank accession numbers immediately precede each sequence name.
Rag1\(^{-/-}\) mice at day 0 and at all time points tested (Fig. 4B). In contrast, low levels of MuAstV were observed in the feces of both wild-type and Stat1\(^{-/-}\) mice at day 0, prior to cohousing. After 2 days of cohousing, elevated levels of MuAstV genome copies were detected in feces from wild-type and Stat1\(^{-/-}\) mice, with Stat1\(^{-/-}\) mice shedding significantly more MuAstV than wild-type mice at day 2 (\(P < 0.05\)). Overall, levels of MuAstV shedding over the course of the experiment differed significantly by genotype (\(P < 0.0001\) for two-way comparisons). These data suggest that both the innate and adaptive immune systems contribute to the control of MuAstV replication.

We next analyzed the tissue distribution of MuAstV STL after 14 days of cohousing. We detected high levels of genome copies in the GI tract of Rag1\(^{-/-}\) mice (Fig. 4C), consistent with our observation that Rag1\(^{-/-}\) mice shed up to \(10^9\) genome copies per stool pellet. The quantity of viral genome copies detected in the GI tract of wild-type and Stat1\(^{-/-}\) mice was significantly lower than in the Rag1\(^{-/-}\) mice (\(P < 0.001\) for all GI tract tissues tested). MuAstV RNA was detected in the liver and kidney of Rag1\(^{-/-}\) mice but not in the liver or kidney of wild-type or Stat1\(^{-/-}\) mice. While we occasionally detected low levels of MuAstV RNA in the spleen of Stat1\(^{-/-}\) mice, we did not detect any genome copies in the spleen of wild-type mice. We were unable to detect MuAstV STL in the brain of any mouse tested. In aggregate, these data suggest a role for both the innate and adaptive immune systems in the control of astrovirus infection and replication.

MuAstV is present in mice purchased from commercial mouse vendors. Since we had detected MuAstV STL in our research mouse facility, we assessed whether MuAstV was present in mice purchased from commercial vendors. As we had observed extremely high levels of MuAstV STL in the feces of Rag1\(^{-/-}\) mice, we decided to assess the presence of MuAstV in commercially available mice with adaptive immune deficiencies. Of the available options from three commercial vendors, we purchased Rag1\(^{-/-}\) mice, Rag2\(^{-/-}\) mice (54), and SCID mice (6).

Consistent with our previous findings in Rag1\(^{-/-}\) mice from our academic research mouse facility, we observed extremely high levels of MuAstV STL in the feces of Rag1\(^{-/-}\) mice, we decided to assess the presence of MuAstV in commercially available mice with adaptive immune deficiencies. Of the available options from three commercial vendors, we purchased Rag1\(^{-/-}\) mice, Rag2\(^{-/-}\) mice (54), and SCID mice (6).

Consistent with our previous findings in Rag1\(^{-/-}\) mice from our academic research mouse facility, we observed extremely high levels of MuAstV in fecal and tissue samples from Rag1\(^{-/-}\) and Rag2\(^{-/-}\) mice from commercial mouse facilities (Fig. 5). While we did detect a limited number of MuAstV genome copies in the feces of two out of three SCID mice, none were detected in tissue samples. These mice may have been truly negative for MuAstV or may have been infected with a strain that we were unable to detect using our MuAstV STL-specific primers and probe. Overall, the presence of MuAstV in both academic and commercial mouse facilities strongly suggests that MuAstV is a
DISCUSSION

We present here the complete genome sequence of two novel astroviruses, MuAstV STL1 and STL2, initially discovered in immunocompetent mice housed in a specific-pathogen-free academic mouse facility. MuAstV STL is a member of the mamastrovirus genus and highly divergent from the previously described astrovirus found in a wild house mouse (49). Analysis of MuAstV natural infection revealed a requirement for adaptive immunity in the control of MuAstV replication and spread. The discovery and complete genomic characterization of MuAstV STL, an astrovirus capable of infecting research mice, provide an opportunity to investigate biology and immunology of a mammalian astrovirus, with implications for our understanding of human astrovirus disease mechanisms.

A new model of astrovirus infection. Turkey astrovirus 2 (TAstV2) has been used to study astrovirus pathogenesis in young turkeys and has been the only small-animal model of astrovirus infection and pathogenesis to date (33, 44). TAstV2, as a member of the avastrovirus genus, is genetically distinct from the mamastroviruses, including human astrovirus. Here, we have shown that the mamastrovirus MuAstV STL is capable of infecting research mice, an ideal system in which to examine astrovirus infection and host-virus interactions. Indeed, our initial studies during the course of natural MuAstV infection in mice demonstrated that the adaptive immune system is essential in restricting murine astrovirus replication, as has been reported for human astrovirus replication and persistence (51, 63). In addition, we also used this new model to identify a role for Stat1 in controlling astrovirus replication. Thus, this animal model is the first genetically manipulable small-animal model with which the molecular mechanisms of astrovirus infection may be elucidated.

MuAstV STL belongs to a potentially novel mamastrovirus genogroup. Our phylogenetic analysis of the mamastroviruses, including several recently described astroviruses, provides support for the addition of a third potential genogroup which comprises MuAstV STL as well as astroviruses that infect rats, pigs, deer, and cattle but not (to date) humans. The significance of the viruses within this putative new genogroup remains to be seen. At least one strain within this new putative genogroup has been associated with diarrheal disease in its host (36), suggesting that members of this proposed genogroup may have the potential to exhibit signs of classical human astrovirus disease.

In addition to the putative genogroup III to which MuAstV STL belongs, our analysis provides support for the creation of up to three additional mamastrovirus genogroups. AstV MLB1 and STL2, bottlenose dolphin astrovirus, and ovine astrovirus 2 and porcine astrovirus 5 all have deep branches and p-dist values greater than 0.671 with respect to the other genogroups. The creation of three new genogroups would serve to reduce the intragenogroup p-dist values, which previously overlapped with the intergenogroup values, likely due to the more limited number of astrovirus sequences available for analysis at the time the current taxonomy was established. The recent characterization of a number of novel astroviruses added resolution to our analysis of phylogenetic relationships and allowed us to detect a total of six potential genogroups within the mamastrovirus genus.

Immune modulation of astrovirus infection. The mechanisms by which the immune system responds to astrovirus infection are not well defined. A role for T- and B-cell-mediated immunity in controlling AstV infection is supported by case studies describing chronic astrovirus infection in two T-cell-deficient children and astrovirus-associated encephalitis in a child with X-linked agammaglobulinemia (51, 63). Experiments using TAstV2 have demonstrated that antibodies are not essential to controlling viral infection (32), but the importance of the humoral response itself has not been examined for mamastroviruses. Using MuAstV, we demonstrated that B and T cells are required to control astrovirus replication in multiple tissues as well as fecal shedding. Consistent with observations in T-cell-deficient children, we detected persistent astrovirus infection and shedding in the absence of B and T cells. Further studies are needed to assess the importance of each cell type in the modulation of astrovirus infection, but it is clear that the adaptive immune system is required for control of astrovirus replication.

While adaptive immunity plays a dominant role in the control of MuAstV infection, our results also provide evidence for a role for innate immunity. We observed increased MuAstV replication and shedding in Stat1−/− mice compared to wild-type mice. Stat1 is required for type I and II interferon signaling, and Stat1−/− mice are more susceptible to viral infection (13). Stat1 has also been shown to be essential for the expression of inducible nitric oxide synthase (iNOS) in intestinal epithelial cells (56), and nitric oxide activity is induced in the intestines of TAstV2-infected turkey embryos, thus suppressing astrovirus replication (32). It will be interesting to assess the role of iNOS during the course of mamastrovirus replication as well.

Research implications of prevalent astrovirus infection. The ability to interpret data generated in small-animal models of disease pathogenesis requires an accurate representation of the immune response to perturbation. Subclinical infection may alter immune phenotypes and baseline homeostasis (1, 3, 8). For example, in the case of mouse hepatitis virus (MHV) infection, in which clinical signs of disease are not apparent in immunocompetent adults, the inadvertent exposure of nonobese diabetic mice to MHV led to a decrease in diabetes incidence (62). Due to the potential for confounding experimental outcomes, homeostasis-
altering agents such as MHV are generally excluded from SPF facilities.

MuAstV was identified in mice purchased from several commercial vendors in the United States as well as in mice in several mouse research facilities at Washington University, suggesting that it may be present in many academic and commercial research facilities. Indeed, the sequence of murine astrovirus strain TF18LM isolated from a research facility in Cincinnati, OH, was similar to that of MuAstV STL by phylogenetic analysis.

While MuAstV was found in asymptomatic mice, its effects on host immunity and physiology are not known. Importantly, differences in astrovirus presence between facilities, or even between groups of experimental and control animals, may contribute to altered outcomes. Our previous studies have demonstrated the importance of the combination of genetic susceptibility and environmental factors in disease phenotypes. In particular, we showed that murine norovirus, which was originally discovered as a common contaminant in research mouse facilities, triggers Crohn’s disease-like pathology in mice hypomorphic for the autophagy protein Atg16L1 (8, 30). Standard methods for animal health monitoring currently account only for known pathogens; however, our identification of multiple viruses in research mice suggests that this approach does not detect all possible contributors to disease phenotypes. An unbiased approach to animal health monitoring might reveal additional members of the mouse virome and contribute to the reliability and interpretation of data from the animal models on which our understanding of disease mechanisms so heavily relies.

ACKNOWLEDGMENTS

This research was supported by National Institutes of Health grants RO1 AI054483 and AI084887 (H.W.V.), Crohns and Colitis Foundation grant 3132 (H.W.V.), and training grant T32AI007163 (C.C.Y.).

We thank members of the Virgin laboratory for their comments during the preparation of the manuscript and Darren Kreamalmeyer for his assistance with mouse husbandry.

A patent disclosure for MuAstV STL1, STL2, STL3, and STL4 has been filed by H.W.V., D.W., J.L., G.Z., T.S.S., L.B.T., and C.C.Y.

REFERENCES