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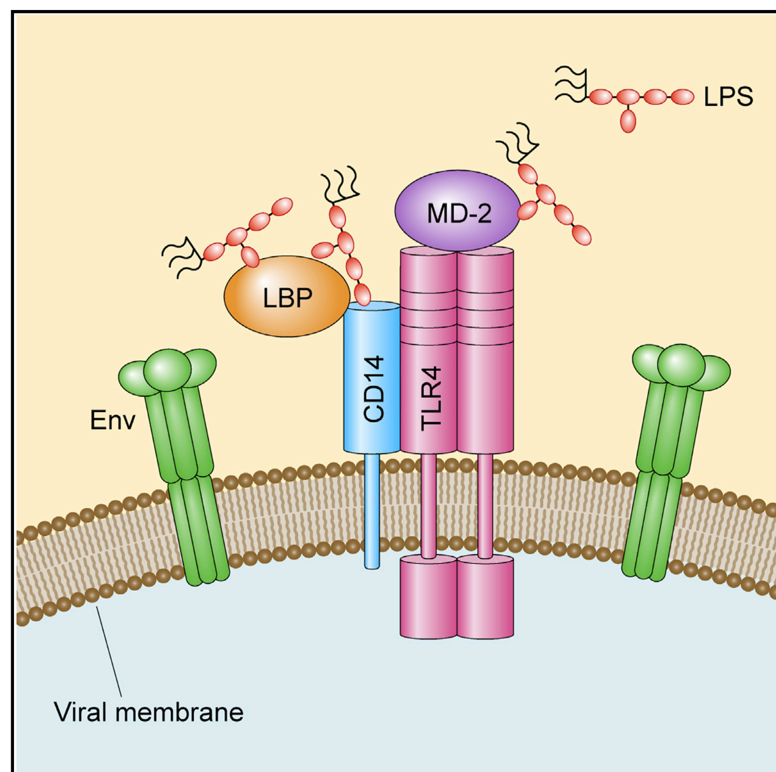
Mammalian Lipopolysaccharide Receptors Incorporated into the Retroviral Envelope Augment Virus Transmission

Graphical Abstract



Highlights

- The enveloped retrovirus MMTV acquires host LPS-binding factors during egress
- LPS-binding factors incorporated into the viral membrane enable MMTV to bind LPS
- Virally bound LPS augments virus transmission
- MMTV binding augments the immunopotency of commensal LPS

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In Brief

The products of commensal bacteria promote transmission of several unrelated enteric viruses. Wilks et al. find that an orally transmitted retrovirus incorporates mammalian lipopolysaccharide (LPS)-binding factors into its membrane, which bind LPS and counteract the anti-viral immune response. Thus, viruses exploit mammalian receptors for bacterial products to achieve efficient transmission.

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Mammalian Lipopolysaccharide Receptors Incorporated into the Retroviral Envelope Augment Virus Transmission

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SUMMARY

The orally transmitted retrovirus mouse mammary tumor virus (MMTV) requires the intestinal microbiota for persistence. Virion-associated lipopolysaccharide (LPS) activates Toll-like receptor 4 (TLR4), stimulating production of the immunosuppressive cytokine IL-10 and MMTV evasion of host immunity. However, the mechanisms by which MMTV associates with LPS remain unknown. We find that the viral envelope contains the mammalian LPS-binding factors CD14, TLR4, and MD-2, which, in conjunction with LPS-binding protein (LBP), bind LPS to the virus and augment transmission. MMTV isolated from infected mice lacking these LBPs cannot engage LPS or stimulate TLR4 and have a transmission defect. Furthermore, MMTV incorporation of a weak agonist LPS from Bacteroides, a prevalent LPS source in the gut, significantly enhances the ability of this LPS to stimulate TLR4, suggesting that MMTV intensifies these immunostimulatory properties. Thus, an orally transmitted retrovirus can capture, modify, and exploit mammalian receptors for bacterial ligands to ensure successful transmission.

INTRODUCTION

Mouse mammary tumor virus (MMTV) is a retrovirus that is transmitted as an endogenous, stably integrated provirus (*Mtv*) or as an exogenous virus (Goff, 2007). Exogenous MMTV is spread via the milk of infected females and is acquired by suckling pups. In the gut, the virus transcytoses through M cells in Peyer's patches (Golovkina et al., 1999) and infects the underlying lymphoid cells, including T and B lymphocytes. Infected T and B cells traffic to the mammary gland and pass the virus to the mammary gland epithelia (Finke and Acha-Orbea, 2001). During lactation, the virus is secreted into the milk and is transmitted to the progeny.

Persistence of the virus requires commensal bacteria and functional TLR4, as the virus is eliminated in both TLR4-deficient specific-pathogen-free (SPF) (Jude et al., 2003) and wild-type (WT) germ-free (GF) mice (Kane et al., 2011). A series of genetic and biochemical experiments established that, after infiltrating the host, the virus cloaks itself in lipopolysaccharide (LPS). The LPS-associated virus activates TLR4, which leads to IL-6-mediated production of the immunosuppressive cytokine IL-10 and blockage of the antiviral response (Jude et al., 2003; Kane et al., 2011). However, the nature of the virus-LPS interaction has remained unknown. Here we report that mammalian LPS receptors are integrated in the viral envelope and bind LPS, thus enabling the virus to activate the immune evasion pathway.

RESULTS

To address whether MMTV directly binds LPS, a modified capture ELISA was used. Both anti-envelope (Env) glycoprotein antibodies (Abs) and biotinylated LPS detected plate-bound MMTV virions equally well (Figure S1), suggesting the virions directly interact with LPS.

Mammals have multiple LPS-binding proteins (LBPs); some transfer LPS to other proteins, while others are directly involved in transmitting LPS-induced signals (Park and Lee, 2013). Specifically, the membrane-anchored protein CD14 binds LPS and transfers it to the MD-2-TLR4 complex (Viriyakosol et al., 2001). MD-2 directly binds LPS, and LPS-bound MD-2 triggers TLR4 dimerization and activation of downstream signaling (Park et al., 2009). Notably, in the absence of TLR4, MD-2 is not present on the cell surface. MMTV, an enveloped retrovirus, targets cells that express and display these LBPs (Lee et al., 2009; Miyake, 2006; Figure S2). Thus, we proposed that the virus acquires these proteins from the host and utilizes them to bind

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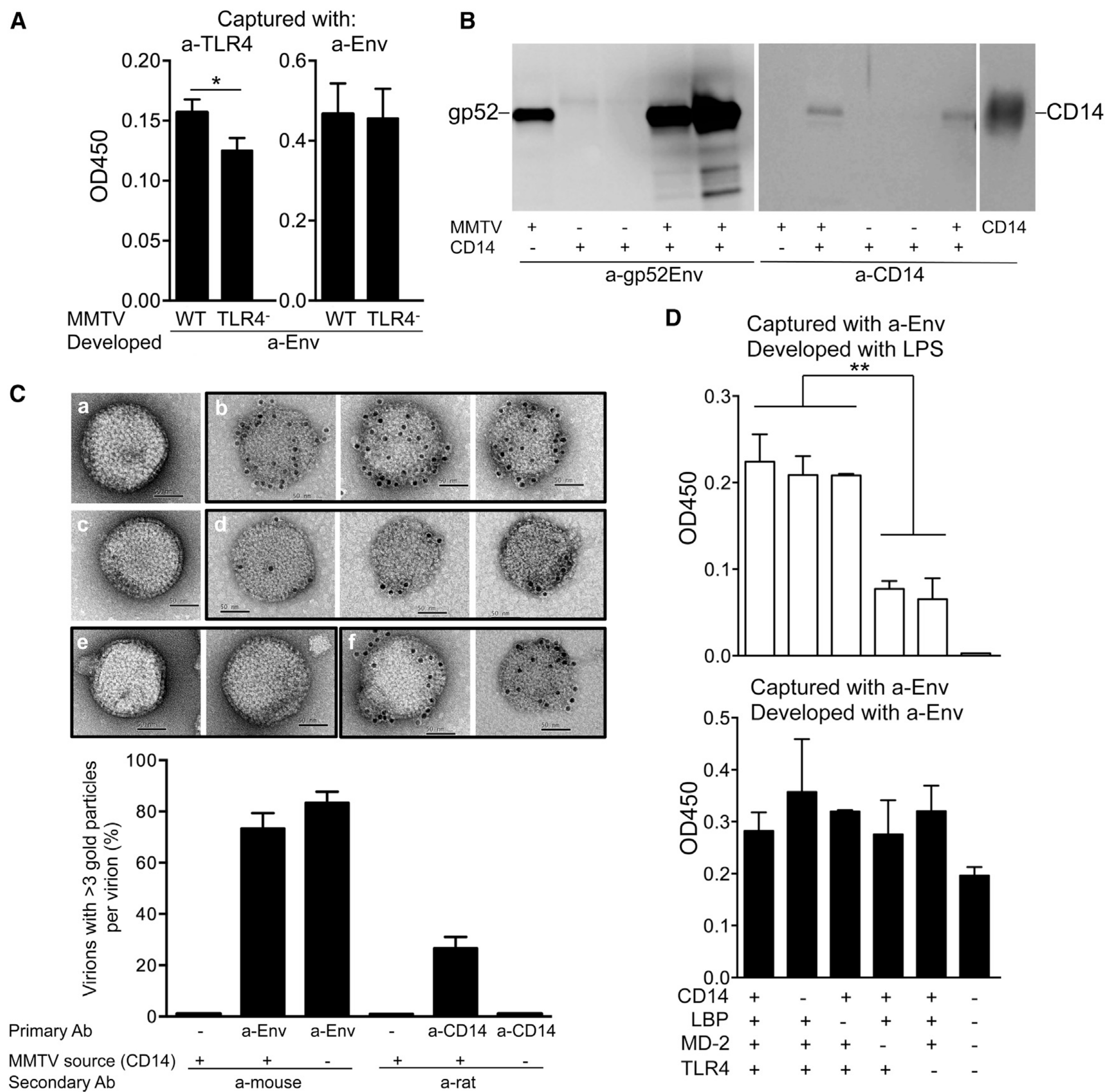

Figure 1. Mammalian LPS-Binding Receptors Are Present in MMTV Virions

(A) MMTV virions isolated from the milk of B6 wild-type (WT) and TLR4$^{-/-}$ mice were captured either by an anti-mouse TLR4 or anti-gp36Env Abs and detected with biotinylated anti-gp36Env Abs. Error bars represent the SE of three independent experiments. Significance was calculated using a two-sample t test (*$p < 0.03$).

(B) Western blot of proteins from virions purified from the milk of B6 (WT) and CD14$^{-/-}$ females. The same density fraction isolated from uninfected milk (MMTV^{-}) was used as a negative control. Anti-mouse CD14 Abs and anti-gp52Env Abs were used to detect CD14 and viral Env, respectively. Mouse CD14 recombinant protein was used as a positive control for anti-CD14 Abs.

(C) Immunogold labeling of MMTV virions. (Top) MMTV virions purified from B6 (WT) (a, b, c, and d) or CD14$^{-/-}$ (e and f) mouse milk were stained with anti-gp52Env Abs followed by anti-mouse Ab coupled to gold particles (b and f) or anti-mouse Ab alone (a) or anti-CD14 Ab followed by anti-rat Ab coupled to gold particles (d and e) or anti-rat Abs alone (c). (Bottom) Quantification of three independent experiments with over 50 virions examined per condition in each experiment. Error bars represent the SE.

(D) MMTV virions isolated from the milk of mice lacking CD14, LBP, MD-2, or TLR4 and from animals deficient in all four factors were captured with anti-gp36Env Abs bound to plastic. The ELISA was developed either with biotinylated LPS (*E. Coli*, serotype EH100) (top) or anti-gp36Env Abs (bottom). Error bars represent SE of three independent experiments. Significance was calculated using a two-sample t test (**$p < 0.001$).

LPS. In fact, both TLR4 and CD14 were detected on purified MMTV virions (Figures 1A–1C), supporting this idea.

To investigate the role of the mammalian LPS-binding machinery in viral acquisition of LPS, we used a genetic approach. WT females and females devoid of MD-2, TLR4, CD14, and another well-characterized LPS-binding factor, LBP (Park et al., 2009), were infected with MMTV via intraperitoneal injection as adults. The virions isolated from their milk were subjected to the LPS-binding assay. Whereas virus titers were comparable among all isolates (Figure 1D, bottom), virions produced by both TLR4$^{-/-}$ and MD-2$^{-/-}$ females exhibited a significant reduction in LPS binding (Figure 1D, top). This reduction was not observed

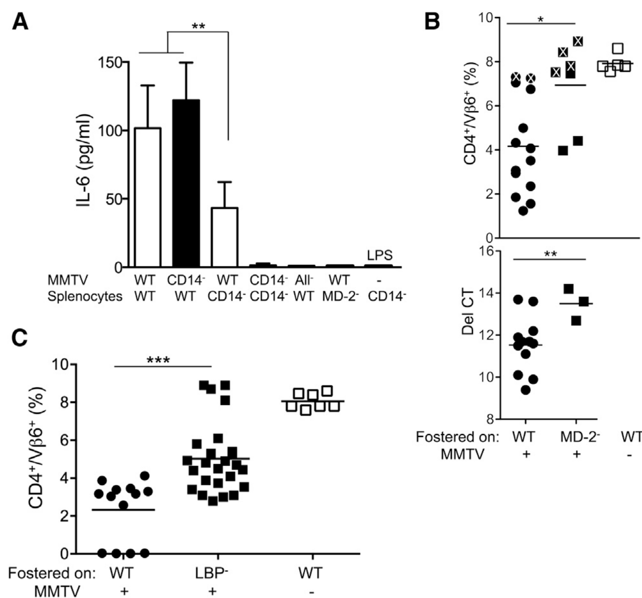

Figure 2. LPS-Binding Proteins Carried by the Virus Are Essential for Immune Activation and Efficient Transmission

(A) MMTV was isolated from the milk of the B6 (WT), CD14$^{-/-}$ (CD14^{-}), and all-LPS-binding-factor (All^{-})-deficient females. Viral isolates with LPS content normalized to 1 ng/ml via the LAL assay were added to either WT, CD14$^{-/-}$, or MD-2$^{-/-}$ (MD-2^{-}) splenocytes. IL-6 in tissue culture supernatants was quantified by ELISA 16 hr later. Error bars represent SE of three independent experiments. Significance was calculated using a two-sample t test (**$p < 0.001$). LPS, 1 ng/ml of LPS from *E. Coli*, serotype 055:B5.

(B) (Top) B6 (WT) mice were fostered on either WT or MD-2$^{-/-}$ (MD-2^{-}) viremic females for 48 hr. WT pups fostered on uninfected milk were used as a negative control. The deletion of SAg-cognate Vβ6^{+}CD4^{+} T cells among CD4^{+} T cells was used as a readout for viral infection. X, animals confirmed as uninfected by qPCR. (Bottom) Splenic DNA only from infected mice (not marked with X) was analyzed for integrated proviruses by qPCR. Data are presented as delta between the cycle threshhold (CT) obtained with MMTV-specific primers and IFN-gamma-specific primers. Significance was calculated using a two-sample t test (*$p < 0.03$, **$p < 0.001$).

(C) B6 mice were fostered on either BALB/cJ (WT) or BALB/cJ.LBP$^{-/-}$ (LBP^{-}) viremic females for 48 hr. B6 pups fostered on uninfected BALB/cJ milk were used as a negative control. The deletion of SAg-cognate Vβ6^{+}CD4^{+} T cells among CD4^{+} T cells was used to assess whether mice were infected. Error bars represent SE. Significance was calculated using a two-sample t test (***$p < 0.0001$).

with either the LBP- or CD14-deficient virions. However, virions obtained from all LPS-binding-factor-deficient mice completely lost their LPS-binding properties (Figure 1D, top). Thus, even though the MD-2-TLR4 complex is the primary LPS-binding factor, LBP and/or CD14 also contribute to LPS binding. In fact, the virus isolated from TLR4$^{-/-}$ mice was able to bind LPS (Figure 4C).

Activation of the TLR4 pathway, specifically production of IL-6 and IL-10, is required for MMTV's replication and successful transmission, and mice lacking TLR4, CD14, IL-6, or IL-10 eliminate the virus through successive generations (Jude et al., 2003; Kane et al., 2011). Therefore, the ability of MMTV virions lacking LPS-binding factors to activate TLR4 was tested. Virions lacking all LPS-binding factors on their membrane failed to activate the TLR4 pathway (Figure 2A). In contrast, CD14-deficient virions induced signaling in WT cells (Figure 2A). Interestingly, the presence of CD14 on either the viral particle or the host cell enabled LPS-bound virions to elicit TLR4 signaling. However, this activation was ablated when CD14 was absent altogether (Figure 2A), indicating that CD14 must be present in the system to facilitate the transfer of the virus's LPS cargo to the target cell.

To determine whether the reduced ability to bind LPS would affect virus transmission in vivo, we followed the virus's fate in mice infected with WT and MD-2^{-} virions. MMTV encodes for a superantigen (v-SAg), which is essential for the virus life cycle (Ross, 2008). Presentation of v-SAg results in the activation of SAg-cognate T cells and their subsequent deletion (Marrack et al., 1991). Deletion of SAg-cognate T cells is used as a readout of successful infection, and the degree of T cell deletion roughly correlates with the virus load (MacDearmid et al., 2006). Accordingly, WT neonates were fostered by either WT or MD-2$^{-/-}$ viremic females, and the presence of infectious virus was defined by the percentage of SAg-cognate CD4^{+} T cells among all CD4^{+} T cells (Figure 2B, top) and quantified by qPCR (Figure 2B, bottom). Enveloped viruses, such as retroviruses, acquire their membrane as they egress from infected cells. Therefore, virions secreted by MD-2$^{-/-}$ females acquire MD-2 upon one round of infection in WT mice, diminishing the phenotypic difference between WT and MD-2^{-} viruses. To solve this problem, we reduced the amount of incoming virus by exposing WT mice to infected foster mothers for only 2 days. Even though the viral titers secreted by WT and MD-2$^{-/-}$ females were comparable (Figure 1D), the virus titer was reduced in mice infected with the MD-2^{-} virus (Figure 2B). In addition, more mice fostered by MD-2$^{-/-}$ mothers remained uninfected (4/7) compared to mice fostered by WT mothers (2/14). Thus, MD-2^{-} virions were significantly compromised in their ability to establish infection, definitively linking the virus's LPS-binding capacity with successful transmission.

It is widely accepted that LBP solubilizes LPS aggregates in vivo, making this bacterial ligand accessible to CD14 and the MD-2-TLR4 receptor complex (Gutsmann et al., 2001; Hailman et al., 1994; Tobias et al., 1995). Even though virions secreted by LBP$^{-/-}$ females still bound LPS (Figure 1D), this in vitro assay was performed with solubilized LPS. Thus, the potential physiological significance of LBP in the virus-LPS interaction may have been masked. To test the significance of LBP in virus transmission, we followed the fate of the virus secreted by LBP$^{-/-}$ females. WT mice fostered on LBP$^{-/-}$ mothers had significantly reduced virus titers compared to mice fostered on WT mothers, and 4/24 were uninfected (Figure 2C). This strongly suggests that, like mammalian cells, virion-associated CD14 and MD-2-TLR4 require LBP to bind LPS monomers in vivo.

LPS consists of three chemically distinct components as follows: O-antigen, core oligosaccharide, and lipid A (Raetz et al., 2007). There is significant structural heterogeneity in lipid A molecules, with acyl chain length number, acyl chain length, and phosphorylation state being the primary determinants of their immunostimulatory properties (Trent et al., 2006). Notably, the structure of LPS and its stimulatory properties vary drastically among Gram-negative bacteria, making the biological properties of virus-bound LPS a necessary avenue of investigation. To address whether MMTV binds purified lipid A from diverse bacterial species, LPS-free MMTV isolated from GF

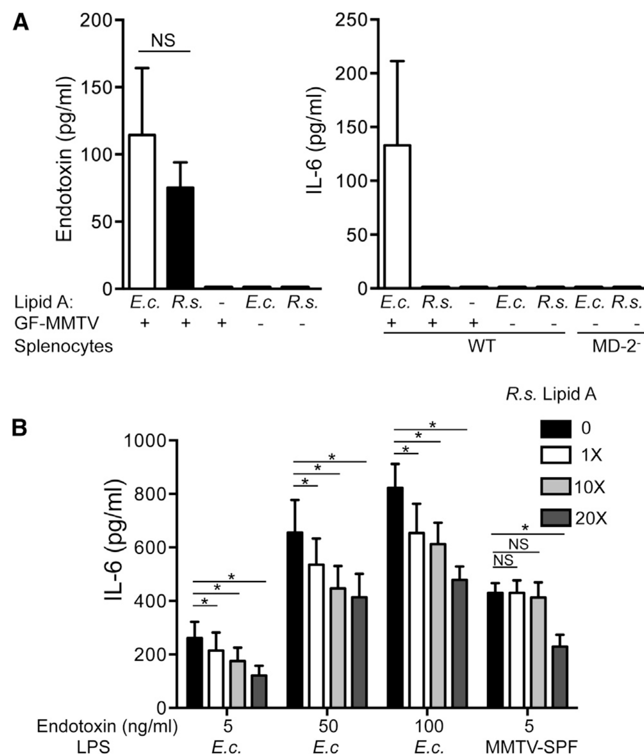

Figure 3. MMTV Binds the Lipid A Component of LPS from Diverse Species and Enables Agonist LPS to Resist Inhibition by an Antagonist LPS

(A) LPS-free MMTV from GF MyD88/TLR4 double-deficient mice was incubated with 40 ng/ml lipid A (*E. coli*, serotype R515) (*E.c.*) or *R. sphaeroides* (*R.s.*) and pelleted through a 30% sucrose cushion. The amount of bound endotoxin was quantified by the LAL assay (left). The ability of virus-bound or free lipid A (normalized to 1 ng/ml via the LAL assay) to stimulate IL-6 production by B6 (WT) splenocytes was measured by ELISA (right). Neither *E.c.* or *R.s.* lipid A elicit IL-6 in MD-2$^{-/-}$ splenocytes. Error bars represent SE of three independent experiments. Significance was calculated using a two-sample t test. NS, non-significant.

(B) LPS from *E. coli* serotype 055:B5 (at 5, 50, and 100 ng/ml) or intact SPF-MMTV virions (at 5 ng/ml endotoxin) was added to B6 splenocytes alone or together with increasing concentration of *R.s.* lipid A. IL-6 in tissue culture supernatants was quantified by ELISA 16 hr later. X is the factor by which *R.s.* lipid A concentration was increased relative to *E.c.* LPS. Error bars represent SE of three independent experiments. Significance was calculated using a paired t test (*p < 0.05).

mice was incubated with lipid A from either *Escherichia coli* (strong TLR4 agonist [Raetz and Whitfield, 2002]) or *Rhodobacter (R) sphaeroides* (TLR4 antagonist [Kutuzova et al., 2001]). MMTV bound both *E. coli* and *R. sphaeroides* lipid A to an equal degree (Figure 3A). However, only virus-bound *E. coli* lipid A activated TLR4 signaling (Figure 3A). Thus, MMTV does not discriminate between agonist and antagonist LPS/lipid A types for binding, but requires a TLR4 agonist to trigger TLR4 signaling.

MMTV-bound LPS is more immunopotent than virus-free LPS (Kane et al., 2011), indicating that binding of LPS to the viral membrane amplifies LPS-induced TLR4 activation. With this in mind and recognizing that *R. sphaeroides* lipid A antagonizes activation of TLR4 by agonist LPS (Kutuzova et al., 2001), we sought to determine whether virally bound LPS was protected from this inhibition. Indeed, *R. sphaeroides* lipid A blocked TLR4 activation by free *E. coli* LPS, but virus-bound LPS resisted this inhibition (Figure 3B). As competitive inhibition of free LPS by *R. sphaeroides* lipid A is concentration dependent (Manthey et al., 1993), the most parsimonious explanation for this result is that binding of LPS to the virion increases the effective local concentration, making MMTV-bound LPS more resistant to inhibition by *R. sphaeroides* lipid A.

The majority of Gram-negative commensals that populate the mouse and healthy human gut are of the *Bacteroides* genus (Human Microbiome Project Consortium, 2012) and synthesize relatively weak TLR4 agonists (Figure 4A). Since MMTV must activate TLR4 to propagate within the host, we hypothesized that the virus must potentiate weak agonist LPS. First, it was determined that MMTV binds physiologically relevant LPS from the human commensal *Bacteroides thetaiotaomicron (B. theta)* to the same extent as *E. coli* LPS, one of the strongest TLR4 agonists (Figure 4B). Second, GF-MMTV virions bound to *B. theta* LPS significantly strengthened the immunopotency of this commensal ligand similar to *E. coli* LPS (Figure 4C). Moreover, this phenomenon was observed with LPS species purified directly from MMTV virions (Figure 4D), suggesting that MMTV has the ability to intensify the immunostimulatory properties of agonistic commensal LPS found in the mouse gut.

Internalization of Gram-negative bacteria or LPS by innate immune cells activates a non-canonical inflammasome pathway (Kayagaki et al., 2013). This activation leads to the production and secretion of the inflammatory cytokine IL-1β, which is known to induce IL-6 (Cahill and Rogers, 2008). To determine whether the non-canonical inflammasome pathway was responsible for the increased immunopotency of virus-bound LPS, we examined virus-elicited IL-6 induction in WT and caspase 1/4-deficient mice, which lack both canonical and non-canonical pathways (Figure 4E). Virus-bound LPS maintained its immunopotency in caspase1/4-deficient splenocytes, suggesting that inflammasome-dependent activation does not explain the enhanced immunopotency of virus-bound LPS.

DISCUSSION

Although it has been noted previously that enveloped viruses acquire host proteins as they bud from the cell (Bubbers and Lilly, 1977; Cantin et al., 2005), few studies have linked the significance of this incorporation to virus replication, transmission, and pathogenesis. HIV-1 selectively incorporates the MHC class II glycoprotein within its membrane and uses it to accelerate entry into human T lymphocytes (Bastiani et al., 1997; Cantin et al., 1997). HTLV-1 displays the complement regulatory proteins CD59 and CD55 on its surface to evade complement-mediated lysis (Spear et al., 1995). In addition, virion-associated MHC class I and class II molecules impact the ability of HIV virions to trigger cell death (Esser et al., 2001). In the case of both HIV-1 and HTLV-1, however, the effects of the host-derived membrane proteins on virus infectivity and pathogenesis were demonstrated only in vitro. In this study, we show that incorporation of mammalian LBPs by an enveloped retrovirus enables the virus to bind LPS and evade the anti-retroviral immune response.

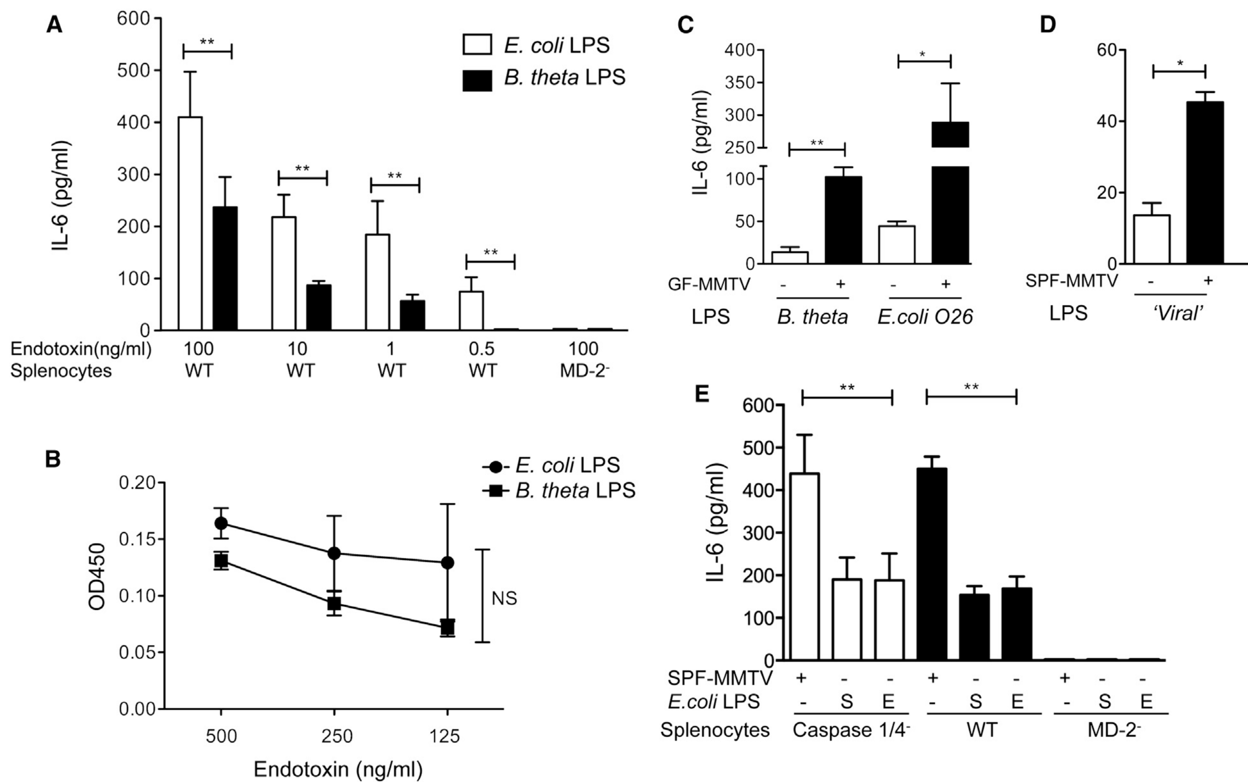

Figure 4. MMTV Augments the Immunopotency of Commensal LPS

(A) The immunostimulatory properties of LPS from *B. theta* and *E. coli* (serotype 055:B5) were compared by IL-6 ELISA after the addition to B6 (WT) and MD-2^{-} splenocytes. Error bars represent SE of three independent experiments. Significance was calculated using a two-sample t test (**$p < 0.001$).

(B) Binding of biotinylated *B. theta* and *E. coli* (serotype O26) LPS by SPF-MMTV virions captured to plastic with anti-gp36Env Abs was measured in serial dilutions. Error bars represent SE of three independent experiments. Significance was calculated using a two-way ANOVA. NS, non-significant.

(C) *B. theta* or *E. coli* (serotype O26) LPS (at 40 ng/ml endotoxin) was incubated with or without GF-MMTV and spun down via 30% sucrose cushion. Endotoxin levels were measured in pelleted fractions using LAL assay. Fractions with no virus added had no detectable LPS (not shown). Virus-LPS pelleted fractions were added to B6 splenocytes at an endotoxin concentration of 71 pg/ml. The amount of secreted IL-6 was measured in tissue culture supernatants 16 hr later. In separate cultures, virus-free *B. theta* and *E. coli* LPS were added to B6 splenocytes at the same endotoxin concentration. Error bars represent SE of three independent experiments. Significance was calculated using a paired t test (*$p < 0.03$ and **$p < 0.001$).

(D) LPS species isolated from SPF-MMTV virions were added to B6 splenocytes as free form (viral LPS) or bound to SPF-MMTV virions at 35 pg/ml. The amount of secreted IL-6 was used as a readout for TLR4 activation. Error bars represent SE of three independent experiments. Significance was calculated using a paired t test (*$p < 0.03$).

(E) IL-6 secretion elicited by SPF-MMTV virions in B6 (WT), Caspase 1/4$^{-/-}$ (Caspase 1/4^{-}), or MD-2$^{-/-}$ (MD-2^{-}) splenocytes or by *E. coli* (serotype 055:B5) LPS from Sigma (S) or Enzo Life Sciences (E) was measured by ELISA. Endotoxin concentration of 1 ng/ml in all samples was verified by the LAL assay. Error bars represent SE of three independent experiments. Significance was calculated using a paired t test (**$p < 0.001$).

The most abundant commensal Gram-negative bacterial species within the human and mouse gastrointestinal tract produce LPS that are weak TLR4 agonists (Human Microbiome Project Consortium, 2012). However, when MMTV binds commensal LPS derived from *B. theta*, it strengthens this LPS immunopotency to such an extent that it exceeds the immunopotency of *E. coli* LPS, a potent TLR4 agonist (Figure 4C). How the virus modulates LPS potency remains to be elucidated and will require further investigation. Nevertheless, a few possibilities can be considered. First, LPS aggregates on the viral surface might strengthen TLR4 activation, as aggregated but not monomeric endotoxin activates TLR4 signaling at similar concentrations (Müller et al., 2003). Second, virion bound LPS may have a greater effective concentration than free LPS, resulting in enhanced immunopotency. Third, delivery of LPS bound to the surface of the viral particle stabilizes or prolongs the ligand's interaction with the cell membrane, facilitating dimerization of MD-2-TLR4 complexes. The latter events potentially can occur on the cell surface or inside the endocytic compartment where functional TLR4 also is located (Kagan et al., 2008; Tanimura et al., 2008).

The phenomenon of LPS exploitation is not restricted to MMTV, as other enteric viruses from completely different genera, such as poliovirus, also utilize LPS to enhance their environmental fitness and infectivity (Kuss et al., 2011; Robinson et al., 2014). Many non-enveloped enteric viruses target cells that express LBPs (Iwasaki et al., 2002; Neal et al., 2006), and some were reported to encase themselves within the host cell membrane (Chen et al., 2015; Feng et al., 2013). Thus, it is possible that even naked viruses could potentially exploit host LPS receptors for their benefit. Further studies of this phenomenon may lead to approaches for the prevention and treatment of some viral infections.

EXPERIMENTAL PROCEDURES

Mice

All animals were bred and maintained at the animal facility of The University of Chicago. SPF MMTV-infected and uninfected C3H/HeN were maintained in our colony. TLR4$^{-/-}$ C57BL10/ScNJ (Poltorak et al., 1998), B6.CD14$^{-/-}$ (Moore et al., 2000), B6.IL-1R1$^{-/-}$ (Glaccum et al., 1997), BALB/cJ.LBP$^{-/-}$ (Jack et al., 1997), B6.Caspase 1/4$^{-/-}$ (Kuida et al., 1995), BALB/cJ, and B6 mice were obtained from The Jackson Laboratory. B6.MD-2$^{-/-}$ mice (Nagai et al., 2002) were crossed to B6 mice for four generations before the MD-2$^{-/-}$ was fixed at homozygosity. BALB/cJ.LBP$^{-/-}$ were crossed to B6 mice for six generations and then crossed to B6.CD14/MD-2/TLR4 triple-deficient mice and intercrossed to generate B6.LBP/CD14/MD-2/TLR4 quadruple-deficient mice. GF C3H/HeN.MyD88/TLR4 double-deficient mice have been reported previously (Kane et al., 2011). The studies described here were reviewed and approved by the Animal Care and Use Committee at The University of Chicago.

Detection of TLR4 on MMTV Virions

MMTV(LA) viral variant (Golovkina et al., 1997) was used in these studies. A capture ELISA was developed to detect TLR4 in virions. Briefly, anti-mouse TLR4 Abs (BioLegend) and anti-gp36Env Ab (Purdy et al., 2003) were bound to a 96-well plate overnight. After blocking, purified virions were added to the capture Abs and the reactions were developed with biotinylated anti-gp36Env Abs.

Detection of CD14 in Purified Virions

MMTV virions isolated from the milk of infected B6 and B6.CD14$^{-/-}$ mice, as well as virus density fractions isolated from the milk of uninfected B6 females, were subjected to western blot analysis using anti-mouse CD14 (Santa Cruz Biotechnology) and anti-gp52Env Abs (Purdy et al., 2003).

Transmission Electron Microscopy

Purified milk-borne virions were placed on glow discharge carbon-coated gold grids and incubated with either mouse anti-gp52Env or rat anti-CD14 Abs followed by anti-mouse or anti-rat Abs coupled to gold-conjugated Abs, respectively. Samples were fixed with glutaraldehyde and stained with uranyl acetate and examined under 300KV at FEI Tecnai F30.

MMTV-LPS Capture ELISA

Anti-gp36Env Abs were bound to 96-well plates and incubated with purified SPF-MMTV virions. ELISA was developed with either biotinylated LPS or biotinylated anti-gp36Env Abs.

MMTV Infection

To compare transmission of MD-2^{+} and MD-2^{-} virions, as well as LBP^{-} and LPB^{+} virions, newborn B6 mice were allowed to suckle on infected WT, MD-2$^{-/-}$, or LBP$^{-/-}$ females. Deletion of SAg-cognate T cells was used as a readout for MMTV infection, and qPCR was used to quantify viral titers.

LPS Binding to GF-MMTV Virions

Binding of TLR4/MyD88 double-deficient GF-MMTV virions to *E. coli* lipid A, *R. sphaeroides* lipid A, *B. theta* LPS, or *E. coli* LPS was performed as described previously (Kane et al., 2011). Endotoxin concentration was quantified via Limulus amebocyte lysate (LAL) assay.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and three figures and can be found with this article online at http://dx.doi.org/10.1016/j.chom.2015.09.005.

AUTHOR CONTRIBUTIONS

J.W. produced all LPS-binding-factor-deficient mice and performed most of the experiments. T.V.G. performed electron microscopy and western blot analysis. A.N.J and M.A.F. purified *B. theta* LPS. N.Q. and E.L. purified *R. sphaeroides* lipid A. E.L. and A.V.C. contributed to experimental design. J.W. and T.V.G. wrote the manuscript. T.V.G. conceived the project and analyzed the results. All authors discussed the results and commented on the manuscript.

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