triguingly, the Cde48 ATPase complex inhibits transcription in a proteolysis-independent manner by preventing ubiquitinated transcription factors from binding to their target sequences (38). Whether these functions of Cde48 and Asi complexes in gene regulation are linked is unclear. However, an appealing possibility is that the ERAD machinery at the INM has been co-opted to perform additional functions, such as controlling transcription factor activity.

REFERENCES AND NOTES


Supplementary materials:

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NOROVIRUS

Enteric bacteria promote human and mouse norovirus infection of B cells

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The cell tropism of human noroviruses and the development of an in vitro infection model remain elusive. Although susceptibility to individual human norovirus strains correlates with an individual’s histo-blood group antigen (HBGA) profile, the biological basis of this restriction is unknown. We demonstrate that human and mouse noroviruses infected B cells in vitro and likely in vivo. Human norovirus infection of B cells required the presence of HBGA-expressing enteric bacteria. Furthermore, mouse norovirus replication was reduced in vivo when the intestinal microbiota was depleted by means of oral antibiotic administration. Thus, we have identified B cells as a cellular target of noroviruses and enteric bacteria as a stimulatory factor for norovirus infection, leading to the development of an in vitro infection model for human noroviruses.

Noroviruses (NoVs) are nonenveloped plus-strand RNA viruses that are the leading cause of epidemic and sporadic gastroenteritis (1–5). The cellular tropism of human NoVs (HuNoVs), and thus the development of a cultivation system for their in vitro propagation, has long eluded the NoV research community (6–11). Several pieces of data led us to ask whether NoVs can infect B cells. First, interferon-deficient and interleukin 10–deficient mice infected with a mouse NoV (MuNoV) contained virus-positive cells in the B cell zones of Peyer’s patches (12, 13). Second, MuNoV-infected Rag2−/− mice (which lack B and T cells) and B cell–deficient mice had reduced virus titers compared with those of wild-type mice, suggesting the absence of a target cell (14). Last, chimpanzees infected

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Fig. 1. MuNoVs infect B cells in culture. (A) The indicated mouse cell lines were infected with MNV-1 (left) or MNV-3 (right) at multiplicity of infection (MOI) 5 and virus growth curves determined by using standard TCID\textsubscript{50} assay. The limit of detection is indicated by a dashed line. (B) The indicated cell lines were mock-inoculated (left) or infected with MNV-1 (middle) or MNV-3 (right) at MOI 5, and cell viability was determined at various times after infection by using propidium iodide staining. (C) M12 or WEHI-231 cells were infected with MNV-1 (black bars) or MNV-3 (gray bars) at MOI 20 for M12 cells or MOI 5 for WEHI-231 cells. At the indicated dpi on the x axis, cells were stained with antibody to ProPol and 4′,6-diamidino-2-phenylindole (DAPI) and imaged on a fluorescent microscope. The percentage of virally infected cells in each cell line was then quantified as the average ratio of ProPol+ cells per total cells. (D) Duplicate wells of M12 cells were infected with MNV-1 (black line) or MNV-3 (gray line) at MOI 5 and passaged every 2 days. At the first passage and every fifth passage, the virus titers in the supernatants were determined by using a standard TCID\textsubscript{50} assay (left). A portion of these cultures were analyzed by means of immunofluorescence assay for infectivity rates (middle). (Inset) Representative images merging the viral ProPol signal (red) and the DAPI staining of nuclei (blue) are shown from passage 10 (P10) cultures. A representative Western blot of cell lysates from persistently MNV-1– or MNV-3–infected M12 cultures (two independent cultures per virus strain) generated at passage 23 (P23) is shown. The MNV-1 virus stock used for initial infections was also tested (labeled as “+”). The blot was probed with antibody to VP1 and reprobed for actin as a loading control. For all, n = 3 to 5 experimental repeats. Error bars denote mean ± SD; Student’s t test in (C), *P < 0.05, **P < 0.01, ***P < 0.001.

Fig. 2. MuNoVs target Peyer’s patch B cells. (A and B) Groups (n = 5 mice) of B6 mice (black bars) and μMT mice (white bars) were infected with 10\textsuperscript{7} TCID\textsubscript{50} units (A) MNV-1 or (B) MNV-3 and harvested at 0.5 or 1 dpi. Virus titers were determined by performing plaque assay on homogenates of the indicated tissues. The data are presented as plaque-forming units (PFU) per gram of tissue on a logarithmic scale, and data for all mice in each group were averaged (n = 2 experiments). Limits of detection are indicated by dashed lines. Error bars denote mean ± SD; Student’s t test, *P < 0.05, **P < 0.01, ***P < 0.001. (C) Groups of B6 mice (n = 8 mice) were inoculated with either mock inoculum or 10\textsuperscript{7} TCID\textsubscript{50} units MNV1 or MNV-3. At 1 dpi, Peyer’s patches were harvested, and quantitative reverse transcription polymerase chain reaction (RT-PCR) was performed on bulk cells (black bars) and purified CD19\textsuperscript{+} cells (white bars) by using virus ORF1-specific primers (n = 5 experiments). Data are reported as viral genomes per cell on a logarithmic scale. The limit of detection is indicated by a dashed line. (D) Stat1\textsuperscript{−/−} mice (n = 2 mice) were inoculated with mock inoculum (gray bars) or 10\textsuperscript{7} TCID\textsubscript{50} units MNV1 (black bars). Intracellular staining for the MNV-1 nonstructural N-term protein was performed on B cells isolated from Peyer’s patches. CD19 or B220 are markers of B cells. Portions of cells were stained with preimmune sera in place of antibody to N-term as a background control. Data are presented as the percentage of B cells stained by N-term subtracted by the percentage of B cells stained by preimmune sera (n = 3 experiments).
with a HuNoV contained capsid protein–positive duodenal B cells (35). Thus, in this study we probed whether NoVs infect B cells.

To investigate whether MuNoVs infect B cells in culture, M12 and WEHI-231 mouse B cell lines were infected with either MNV-1 or MNV-3. These two MuNoV strains were selected because they display numerous pathogenic distinctions. Specifically, MNV-1 establishes an acute infection, whereas MNV-3 establishes persistence (16–18); MNV-3 is attenuated compared with MNV-1 (19); and MNV-3 elicits more robust protective immunity than does MNV-1 (14). Both MuNoV strains replicated efficiently in the B cell lines, although peak titers were reached ~1 day later than in mouse RAW264.7 macrophages, a cell line known to be permissive to MuNoVs (Fig. 1A) (30). The mouse intestinal epithelial CMT-93 cell line was nonpermissive. Synthesis of viral proteins—measured by means of Western blot analysis of the viral RNA-dependent RNA polymerase (RdRp), the VPI capsid protein, and the VP2 minor structural protein—also reflected the slower replication of MuNoVs in B cells as compared with macrophages (fig. S1). MuNoV infection of M12 cells did not result in visible cytopathic effect (CPE), a finding that was confirmed by using propidium iodide staining (Fig. 1B) and trypan blue exclusion. In contrast, MuNoV infection of WEHI-231 cells resulted in visible CPE and loss of cell viability. This difference in infection outcome may relate to the distinct nature of the B cell lines considering that WEHI-231 cells are immature B cells, whereas M12 cells are mature B cells. Whereas MNV-1 infection resulted in complete loss of viability in WEHI-231 cells, MNV-3 infection resulted in a transient loss of ~60% of cells followed by recovery of the culture. Consistent with this, at 2 to 4 days post infection (dpi) actin was undetectable in MNV-1-infected, but not MNV-3-infected, WEHI-231 cells (fig. S1).

To determine percent infectivity, cells were stained for the MuNoV protease-RdRp (ProPol) nonstructural proteins. Although 80 to 90% of RAW264.7 and WEHI-231 cells were productively infected, only 5 to 15% of M12 cells were productively infected (Fig. 1C and fig. S2). To determine whether M12 cultures cleared this low-level infection or instead became persistently infected, we measured virus titers in supernatant fluid after repeated passaging of infected cultures. We consistently detected 10^6 to 10^7 median tissue culture infectious dose (TCID50) per mL of each virus in the supernatant through 25 culture passages, which correlated with consistent low infection frequency and positive staining for the viral VPI protein (Fig. 1D). Similarly, MNV-3 established persistent infection in WEHI-231 cells after the initial drop in cell viability. Thus, MuNoVs can persistently infect B cells in culture.

We used several complementary approaches to confirm that B cells are bona fide NoV targets in vivo. First, MNV-1 and MNV-3 titers were significantly reduced in the distal ileum and mesenteric lymph node (ILN) or filtered (gray bars) stool inoculum was untreated (solid bars) or UV-treated (hatched bars) before inoculation onto B cells. Samples were analyzed as described above, and data were reported as the fold-increase in viral genome copy numbers from 0 to 3 or 5 dpi (n = 3 experiments) (D and E). Mock inoculum or 5 × 10^6 genome copy numbers of unfiltered (black bars) or filtered (gray bars) stool inoculum was untreated (solid bars) or UV-treated (hatched bars) before inoculation onto B cells. Samples were analyzed as described above, and data were reported as the fold-increase in viral genome copy numbers from 0 to 3 or 5 dpi (n = 3 experiments). (C and D) Mock inoculum or 5 × 10^6 genome copy numbers of unfiltered GII.4-Sydney HuNoV-positive stool was applied to BJAB cells, and the cells were washed after 2 hours. (C) Cell lysates were tested in Western blotting by using a polyclonal antibody to NS6. The asterisk indicates a band of the expected size for the HuNoV NS5-NS6 processing intermediate (35 kD) that was only observed in infected cells at 3 to 5 dpi. No mature NS6 protein was detected, which is consistent with a report demonstrating that the NS5-NS6 cleavage site of a HuNoV is processed very inefficiently by the viral protease (33). (D) Cells were stained with antibody to VPI (red) and DAPI (blue) and imaged on a fluorescent microscope. No VPI signal was detected in mock-inoculated cells at 5 dpi, nor infected cells stained with an isotype control antibody. (E) 5 × 10^5 genome copy numbers of a P0 inoculum was passaged onto naïve BJABs. At 0, 3, and 5 dpi, wells were collected and analyzed by means of genogroup II-specific quantitative RT-PCR (n = 4 experiments). The data are presented as the fold-increase in genomes from 0 to 3 or 5 dpi. The genome copy numbers detected at each time point were compared with 0 dpi for statistical purposes, indicated by black asterisks. (F) 1 × 10^6 genome equivalents of the unfiltered (black bars) or filtered (gray bars) GII.4-Sydney HuNoV-positive stool sample were applied to the apical side of a transwell with polarized HT-29 IECs grown on the membrane and BJAB B cells cultured in the basal compartment. At 0 and 3 dpi, the basal compartment was collected for viral genome analysis by means of quantitative RT-PCR (n = 5 experiments). The data are presented as the fold-increase in genomes from 0 to 3 dpi. In two experiments, unfiltered stool was applied to a coculture with no cells in the basal chamber as a control (white bars).
lymph nodes (MLNs) of B cell–deficient mice (μMT mice) as compared with B6 mice (Fig. 2, A and B). To test whether the reduced virus titers in μMT mice reflected decreased viral replication or increased clearance of input virus, we infected mice with light-sensitive MNV-1 to allow differentiation between input and newly replicated virus (this assay, along with complete methods, are described in (29)). No significant differences in the ratio of light-insensitive (newly replicated) to total virus titers were observed between B6 and μMT mice (fig. S5A), demonstrating that B cells are required for optimal viral replication in vivo. Further supporting in vivo B cell infection, B cells purified from Peyer’s patches of wild-type B6 mice contained viral genomes (Fig. 2C).

Although MuNoV nonstructural protein expression has not been demonstrable in any cell type in vivo during NoV infections, we asked whether HuNoVs infect B cells. The strain called GII.4-Sydney (24–26), which expresses H type HBGA (Fig. 4A) that the GII.4-Sydney HuNoV strain can bind (29), Filtered stool containing GII.4-Sydney virus displayed a dose-dependent restoration of infectivity when incubated with E. cloacae before inoculation of BJAB B cells (Fig. 4B). Neither Escherichia coli (which did not express H antigen) nor lipopolysaccharide (LPS, a component of the outer membrane of Gram-negative bacteria) rescued infectivity, whereas synthetic H antigen restored infectivity of filtered stool comparably with E. cloacae. Antibody to VP1 neutralized infectivity of the unfiltered stool, as expected. Providing insight into the mechanism of H antigen-mediated stimulation, filtration of GII.4-Sydney HuNoV-positive stool inoculum ablated virus attachment to B cells, and synthetic H antigen was sufficient to restore attachment (Fig. 4C). Overall, these results demonstrate that HuNoV interactions with enteric bacteria, likely
through binding to bacterially expressed HBGAs, facilitate productive attachment to, and infection of, B cells.

To examine whether intestinal bacteria contribute to NoV infection in vivo, we depleted the intestinal microbiota of wild-type B6 mice before MuNoV infection (fig. S7). Indeed, antibiotic depletion of normal intestinal flora resulted in a significant reduction in MuNoV titers before MuNoV infection (fig. S7). Indeed, anti-NoV neutralizing antibodies that permit enzymatically targeted incorporation of fluorophores at these sites provided a view of the dynamic features of the Env trimer in various conformations. Previous studies have shown that NoV infection is facilitated by bacterial LPS to the intestinal microbiota of wild-type B6 mice, in particular with the ability of bacterial LPS to contribute to NoV infection in vivo, we depleted the intestinal microbiota of wild-type B6 mice before MuNoV infection (fig. S7). Indeed, anti-NoV neutralizing antibodies that permit enzymatically targeted incorporation of fluorophores at these sites provided a view of the dynamic features of the Env trimer in various conformations.

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SUPPLEMENTARY MATERIALS
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Figs. S1 to S7
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HIV ENTRY

Conformational dynamics of single HIV-1 envelope trimers on the surface of native virions

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The HIV-1 envelope (Env) mediates viral entry into host cells. To enable the direct imaging of conformational dynamics within Env, we introduced fluorophores into variable regions of the glycoprotein gp120 subunit and measured single-molecule fluorescence resonance energy transfer within the context of native trimers on the surface of HIV-1 virions. Our observations revealed unliganded HIV-1 Env to be intrinsically dynamic, transitioning between three distinct prefusion conformations, whose relative occupancies were remodeled by receptor CD4 and antibody binding. The distinct properties of neutralization-sensitive and neutralization-resistant HIV-1 isolates support a dynamics-based mechanism of immune evasion and ligand recognition.

The HIV-1 envelope (Env) spike is a membrane-fusion machine that mediates viral entry into cells (7). HIV-1 Env, composed of three gp120 glycoproteins and three gp41 subunits, evades recognition by antibodies by favoring a neutralization-resistant ground-state conformation in which N-linked glycans cover most of the surface (2–5). Interaction with the CD4 receptor causes structural rearrangements in gp120, which lead to formation of a co-receptor–binding site (1, 2, 6, 7). These rearrangements include movement of the variable loops 1 and 2 (V1/V2) from their apical position in the unliganded trimmer, to the trimer periphery (8–10). Subsequent interactions with the co-receptor trigger additional Env remodeling, with gp41 rearranging into a stable six-helix bundle that facilitates fusion between viral and cellular membranes. Although static images of HIV-1 Env in various conformations have been obtained (6–9, 11–17), direct measurement of the dynamic features of the Env trimmer has been lacking.

To enable real-time observations of conformational transitions in the native HIV-1 Env spike on the surface of virions, we used single-molecule fluorescence resonance energy transfer (smFRET) (18). Short peptides were introduced that permit enzymatically targeted incorporation of fluorophores (19, 20) into the V1 loop and one of the following: the V4 loop, the E loop, or the V5 loop of gp120. The attachment of donor and acceptor fluorophores at these sites provided
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