caveat to this potential personalized approach is the need for mechanisms to eliminate specific members of the microbiota while leaving the rest of the microbiota intact. Future extensions of this study should seek to correlate specific host polymorphisms, such as in the *Muc19* or *NOD2* genes (Jostins et al., 2012) with specific IgA-targeted bacteria. Perhaps deficiencies in bacterial detection, as are predicted in individuals with NOD2 mutations, would lead to perturbations in IgA targeting and expansion of specific microbial groups, while mucous alterations may result in increased localization of microbes near intestinal epithelium and subsequently altered IgA targeting of different microbial populations. Other important questions include what processes dictate IgA specificity, and can these pathways be harnessed to develop therapies against colitogenic members of the microbiota. Much remains to be done; however, these findings suggest that following the immune system might help to identify the bacterial troublemakers.

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Tethering Viral Restriction to Signal Transduction

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Tetherin serves as an innate sensor of viral infection in addition to its role in inhibiting virus release from infected cells. In this issue, Galão et al. (2014) provide important insights into the mechanism of virus-induced signal transduction by tetherin.

Tetherin (BST-2 or CD317) is rapidly upregulated on the cell surface by type I interferons, where it prevents the detachment of virus particles from infected cells. This activity reflects the unique topology of tetherin, which includes a short cytoplasmic tail followed by an N-terminal transmembrane domain, an extracellular coiled-coil domain, and a C-terminal glycosyl-phosphatidylinositol anchor (reviewed in Neil [2013]). These structural features allow opposite ends of tetherin dimers to partition between viral and cellular membranes during viral budding, thereby linking nascent virions to the plasma membrane (Neil, 2013).

Although first identified as an HIV-1 restriction factor, tetherin is now understood to have broad activity against diverse families of enveloped viruses, many of which have in turn evolved countermeasures to tetherin (Neil, 2013). Among the primate lentiviruses, at least three different viral gene products have acquired the ability to counteract tetherin. Whereas Nef is used by the majority of simian immunodeficiency viruses (SIVs) to antagonize the tetherin proteins of their nonhuman primate hosts, HIV-1 Vpu and HIV-2 Env have acquired the ability to counteract human tetherin due to the absence of a five amino acid sequence in the cytoplasmic tail of human tetherin that confers susceptibility to Nef (Neil, 2013). While these observations imply that tetherin has potent antiviral activity, the immunological mechanisms contributing to the antiviral effects of tetherin are not fully understood.

Earlier reports by Galão et al. and others demonstrated a role for tetherin in signal transduction (Cocka and Bates, 2012; Galão et al., 2012; Tokarev et al., 2013). Under conditions of protein overexpression or virion-induced clustering, tetherin activates NFκB by a pathway involving the recruitment of the mitogen-activated protein kinase TAK1. NFκB activation was found to be dependent on sequences in the extracellular domain of tetherin that participate in virion retention, and on a dual-tyrosine motif in the cytoplasmic tail (Y_6DY_8CRV) previously implicated in the constitutive cycling of tetherin between the plasma membrane and the *trans*-Golgi network (Galão et al., 2012). However, signaling is separable from the role of this dual-tyrosine motif in tetherin internalization. Indeed,

inhibition of tetherin endocytosis enhances virion-induced signaling, pointing to the clustering of tetherin on the cell surface as the initial trigger for signal transduction (Galão et al., 2012).

In this issue of *Cell Host and Microbe*, Galão et al. (2014) build on their earlier work to define several of the key events leading to NFκB activation by tetherin. Their findings include Src-dependent phosphorylation of tyrosine residues 6 and 8 (Y6 and Y8) in the cytoplasmic tail of tetherin, the recruitment of Syk kinase, and a role for RICH2 in coupling tetherin to cortical actin. A mutational analysis revealed that NFκB activation is critically dependent on Y6 phosphorylation, whereas Y8 phosphorylation contributes to the overall efficiency of signaling. Notably, viral budding is essential for both tyrosine phosphorylation and NFκB signaling under conditions of constitutive tetherin expression, as illustrated by a comparison of these events in stable cell lines producing murine leukemia virus (MLV) with and without a late-domain mutation in Gag that prevents budding. Treatment with specific kinase inhibitors and RNAi depletion revealed that virus-induced phosphorylation of tetherin is dependent on Src-family kinases and on the recruitment of the tyrosine kinase Syk. Syk is in turn required for the assembly of a complex containing the signaling adaptors TRAF2 and TRAF6 as well as TAK1, as indicated by the loss of these factors from immunoprecipitates with tetherin after RNAi-depletion of Syk. Actin depolymerization or RNAi depletion of RICH2, a RacGAP reported to physically link tetherin to the actin cytoskeletal network (Rollason et al., 2009), also blocked tetherin phosphorylation and downstream NFκB activation without affecting virion retention, indicating that signaling is coupled to cortical actin by RICH2.

The authors propose a model in which the juxtaposition of a pair of YDYCRV sequences in adjacent cytoplasmic tails of a tetherin dimer serves as a noncanonical hemi-immunotyrosine activation motif (HemITAM). HemITAMs are found in many dimeric C-type lectin receptors and consist of a single YXXΦ motif per monomer that upon tyrosine phosphorylation creates a docking site for kinases that contain SH2 domains. Displacement of tetherin from cortical actin as a result of virion-induced clustering is sensed through a link to the underlying cytoskeletal network provided by RICH2 (Figure 1). This exposes Y6 and Y8 in the cytoplasmic tail of tetherin for phosphorylation by Src-family kinases, which leads to the recruitment of Syk, or possibly the related kinase Zap70, via their SH2 domains. Syk binding and activation in turn recruits TRAF2, TRAF6, and TAK1 into a signaling complex that leads to the downstream activation of NFκB and, ultimately, to the production of proinflammatory cytokines (Figure 1).

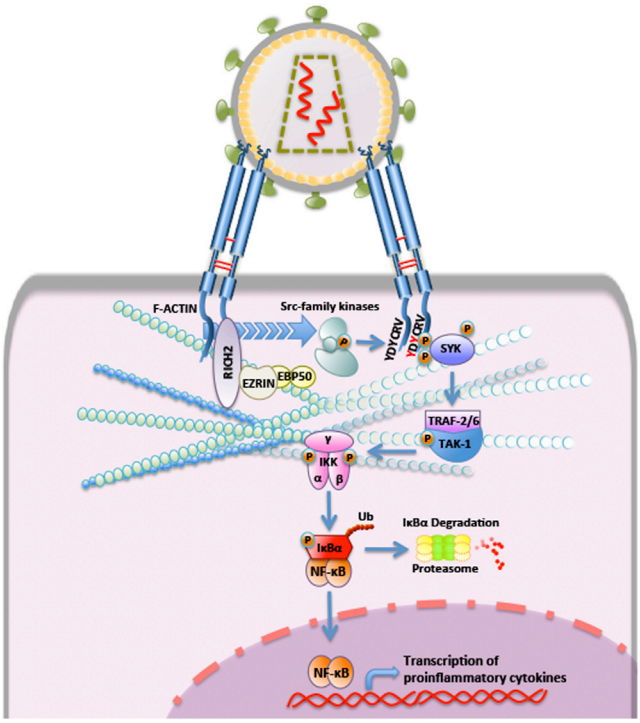

Figure 1. Virus-Induced Signal Transduction by Tetherin
Viral budding is detected by tetherin as a result of virion-induced clustering at the plasma membrane through a link to cortical actin provided by RICH2. This leads to the phosphorylation of tyrosine residues of the YDYCRV motif in the cytoplasmic tail of tetherin by Src-family kinases and the recruitment of spleen tyrosine kinase (Syk). Syk activation subsequently recruits TRAF2, TRAF6, and TAK1 into a complex that leads to the activation of NFκB and to the induction of proinflammatory cytokines.

Signaling appears to be a recently evolved functional activity of hominid tetherins. NFκB activation is approximately twice as efficient for human tetherin as it is for chimpanzee tetherin, but negligible for the tetherin proteins of gorillas and Old World monkeys. This study shows that these differences in signaling reflect species-specific differences in tetherin that determine the efficiency of tyrosine phosphorylation and Syk activation. Moreover, the lack of sequence variation in RICH2 or Syk argues against species-specific differences in these gene products as an explanation for the inability to detect signaling for tetherin proteins of other primates. Compared to the tetherin proteins of Old World monkeys, human and chimpanzee tetherin contain a two amino acid insertion in the transmembrane domain and four amino acid differences in the cytoplasmic tail that contribute to their greater susceptibility to phosphorylation by Src-family kinases. The additional increase in tyrosine phosphorylation of human tetherin can be explained by the absence of five amino acids corresponding to sequences in the cytoplasmic tail of chimpanzee tetherin that confer susceptibility to antagonism by SIV Nef. Thus, it is tempting to speculate that the evolution of hominid tetherin, including the recent loss of sequences from human tetherin that confer susceptibility to Nef, may have been shaped as much by the selective advantage gained by acquiring the ability to sense viral budding as by resistance to viral antagonism.

While this study reveals many of the essential features of signal transduction by tetherin, a few aspects of the mechanism remain to be fully defined. Compelling data showing an enrichment of phosphorylated tetherin and activated Syk in detergent-resistant microdomains of MLV-producing cells suggests that signaling is spatially distributed in the plasma membrane to cholesterol-rich "lipid rafts," thought to be preferential sites of virus assembly. In view of the proposed model, it will be interesting to know

whether this organization is a result of RICH2 coupling of tetherin to cortical actin. The molecular interactions between RICH2, tetherin, and cytoskeletal actin are also yet to be fully defined. However, intriguing data are provided to show that a natural polymorphism in human tetherin (R19H), which impairs signaling without affecting viral restriction (Sauter et al., 2013), disrupts the physical association of RICH2 with this tetherin variant.

The enhanced transcriptional activation of genes coding for the cytokines CXCL10, IL-6, and IFNβ in primary $CD4^+$ lymphocytes infected with *vpu*-deleted HIV-1 suggests that human tetherin serves as a pattern recognition receptor coupling viral restriction to the release of proinflammatory cytokines. This has fascinating implications with respect to the antiviral activity of tetherin, since it implies that the function of this molecule as a cell-autonomous restriction factor is integrally linked to other components of innate and adaptive immunity. Although the downstream effects of tetherin on immune activation are likely to be complex, the release of IFNβ, itself a type I interferon, may induce interferon-stimulated genes, including tetherin, that further impair virus replication in neighboring cells. Moreover, the chemotactic properties of cytokines such as CXCL10 may attract cellular mediators of antiviral immunity, perhaps magnifying the effects of tetherin on the susceptibility of HIV-infected cells to antibody-dependent cell-mediated cytotoxicity (Alvarez et al., 2014; Arias et al., 2014). Thus, insights into the mechanism of signal transduction by tetherin provided here suggest the antiviral activity of tetherin is not merely a function of its ability to impede virus release, but may be amplified by host immune responses as a function of its ability to signal in response to viral infection.

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Flavivirus NS5 Prevents the InSTATement of IFN

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Given the potency of interferon-α/β, viral evasion of this pathway is crucial for infection. In this issue of *Cell Host & Microbe*, Laurent-Rolle et al. (2014) report that during yellow fever virus infection, interferon-α/β stimulates the polyubiquitination of viral NS5, which binds to STAT2 and inhibits transcription of interferon-stimulated genes.

Pathogen-host interaction determines the outcome of an infection. Pathogens hijack host machinery to reproduce, while hosts activate the immune system to detect and eliminate pathogens. Innate immune response is the first line of host defense, among which type 1 interferon (IFN) production and signaling play a central role. Understanding the molecular interplay between pathogen and host is essential for development of novel vaccines and therapeutics. Flaviviruses, such as yellow fever virus (YFV), dengue virus (DENV), West Nile virus (WNV), Japanese encephalitis virus (JEV), and tick-borne encephalitis virus (TBEV), cause major epidemics and represent global public health threats. These viruses contain a single-strand, plus-sense RNA genome, which encodes three structure proteins (capsid, membrane, and envelope) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). Since many flaviviruses alternate their life cycle between mammalian and arthropod (mosquitoes and ticks), they have adapted to replicate and evade immune restriction in distinct host species.

Flaviviruses employ various strategies to evade innate immune detection. They encode their own capping machinery to form a cap structure at the 5′ end of genomic RNA, preventing the 5′ triphosphate from being detected by RNA sensor RIG-I. To suppress IFN production, the DENV NS2B/NS3 protease selectively cleaves and inactivates human STING (but not murine STING), which interacts with RIG-I to facilitate type-1 IFN production (Aguirre et al., 2012; Yu et al., 2012).

Flaviviruses have also developed distinct mechanisms to directly antagonize