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Helminth infection reactivates latent γ-herpesvirus via cytokine competition at a viral promoter — Source link

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gene. Also, mutations in *IZKF3*, which also had HIV integrations detected in two of our three participants, was recently associated with a form of acute lymphoblastic leukemia (*35*). Fourth, as somatic mutations that "drive" cancers are estimated to only convey a 0.4% growth advantage (*36*), HIV integration into genes with subtle enhancement of cell proliferation may be difficult to detect as clonal due to our limited sampling.

HIV-infected cells that express viral proteins are likely to be eliminated by immune surveillance, or virus replication may lead to cell lysis. Whether the proliferating and persisting HIV-infected cells that we describe harbor replication-competent virus is critical to defining their role in perpetuating the infectious virus reservoir. Undoubtedly, some clonal populations persist due to defects in expression of the proviral genome (*9*, *10*, *37*). Although we did not evaluate viral sequences for replication competency, lethally hypermutated viral genomes were linked to three integration sites (Fig. 2). However, cells producing viremias with identical *env* sequences have been shown to harbor replication-competent virus (*38*). Also, approximately 12% of proviruses refractory to in vitro induction were found to have intact genomes and may be infectious (*27*). Although transcriptional interference was not detected in the aforementioned noninduced viral transcripts (*26*, *27*), others have observed that the site of integration may cause transcriptional interference (*34*, *39*–*43*).

In conclusion, HIV integration into genes associated with cancer or cell cycle regulation appears to confer a survival advantage that allows these cells to persist during suppressive ART, with cell proliferation appearing to serve as an important mechanism of HIV persistence. To be defined are the mechanisms contributing to cell proliferation, the role of proliferating cells in perpetuating the infectious virus reservoir, and whether therapies that target HIV-infected proliferating cells, specific genes, or their products may contribute to a curative strategy for HIV infection.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6196/570/suppl/DC1
Materials and Methods
Figs. S1 to S3
Tables S1 to S3
References (*46*–*59*)

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COINFECTION

Helminth infection reactivates latent γ-herpesvirus via cytokine competition at a viral promoter

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Mammals are coinfected by multiple pathogens that interact through unknown mechanisms. We found that helminth infection, characterized by the induction of the cytokine interleukin-4 (IL-4) and the activation of the transcription factor Stat6, reactivated murine γ-herpesvirus infection in vivo. IL-4 promoted viral replication and blocked the antiviral effects of interferon-γ (IFNγ) by inducing Stat6 binding to the promoter for an important viral transcriptional transactivator. IL-4 also reactivated human Kaposi's sarcoma–associated herpesvirus from latency in cultured cells. Exogenous IL-4 plus blockade of IFNγ reactivated latent murine γ-herpesvirus infection in vivo, suggesting a "two-signal" model for viral reactivation. Thus, chronic herpesvirus infection, a component of the mammalian virome, is regulated by the counterpoised actions of multiple cytokines on viral promoters that have evolved to sense host immune status.

Mammals are populated by many chronic viruses, termed the virome, which can regulate host physiology and disease susceptibility (*1*). For example, more than 90% of humans are latently infected with herpesviruses that, after clearance of acute infection, produce little infectious virus and often cause no overt disease. Like the human γ-herpesviruses Epstein-Barr virus (EBV) and Kaposi's sarcoma–associated herpesvirus (KSHV), murine γ-erpesvirus-68 (MHV68) establishes lifelong latency. Studies in this model system showed that the cytokine

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interferon-γ (IFNγ) inhibits MHV68 replication and reactivation from macrophages, a major cellular site for latency (*2*–*5*); controls persistent replication in vivo (*6*); and is present at low amounts during latency (*7*).

Many people around the world are coinfected with herpesviruses and intestinal helminths. Although herpesviruses can modulate immunity to harm or benefit the host (*7*–*11*), the effects of helminth coinfection on chronic herpesvirus infection are unexplored. Intestinal helminths generate strong T helper 2 (T_H2)–driven cytokine responses, which counter the biological effects of IFNγ and drive the activation of macrophages with an M2 (immunoregulatory) rather than M1 (proinflammatory) phenotype (*12*). Parasitic worms may influence control of pathogens—including *Mycobacterium tuberculosis*, HIV, and *Plasmodium* species in humans—but there are few studies elucidating the mechanisms behind this immunomodulation (*13*). Thus, we considered the hypothesis that parasite infection would induce MHV68 reactivation in vivo.

We examined the effects of acute infection with *Heligmosomoides polygyrus* or *Schistosomiasis mansoni* (*Sm*) egg administration on MHV68

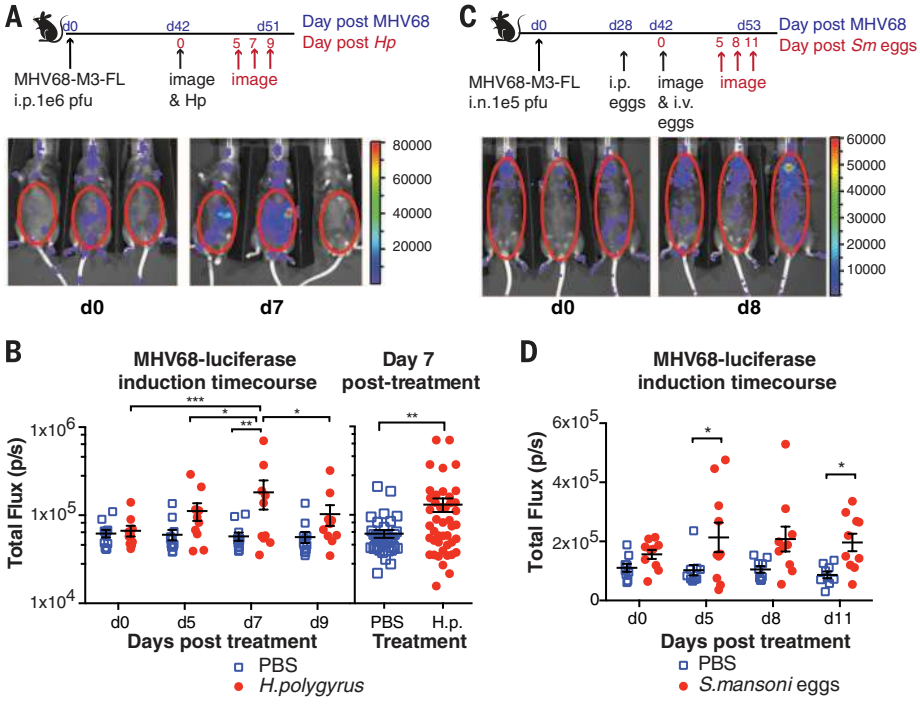

Fig. 1. Challenge with *H. polygyrus* and *S. mansoni* eggs reactivates MHV68. (**A**) C57BL6/J mice were infected intraperitoneally (i.p.) with MHV68-M3-FL and challenged with *H. polygyrus* 42 days later. Mice were imaged before *H. polygyrus* infection (day 0, d0) and 5, 7, and 9 days after. Three representative mice imaged on days 0 and 7 are shown. (**B**) Total flux (photons per second, p/s) was quantitated for mice in two independent experiments for the timecourse after infection with *H. polygyrus*. Data from four independent experiments at day 7 after *H. polygyrus* (H.p.) infection are also shown. (**C**) C57BL/6J mice were infected intranasally (i.n.) with MHV68-M3-FL. The diagram indicates the timecourse of the experiment and challenge with *Sm* eggs or phosphate-buffered saline (PBS) as a control. Mice were injected with D-Luciferin and imaged before intravenous (i.v.) challenge with *Sm* eggs (day 0). They were subsequently imaged 5, 8, and 11 days after challenge with *Sm* eggs. Three representative mice imaged on days 0 and 8 are shown. (**D**) Total flux was quantitated from mice in two independent experiments after *Sm* egg challenge. Symbols represent individual mice, and the mean and standard error are indicated. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ by two-way repeated measures analysis of variance (ANOVA) with Tukey's and Bonferroni's post-test.

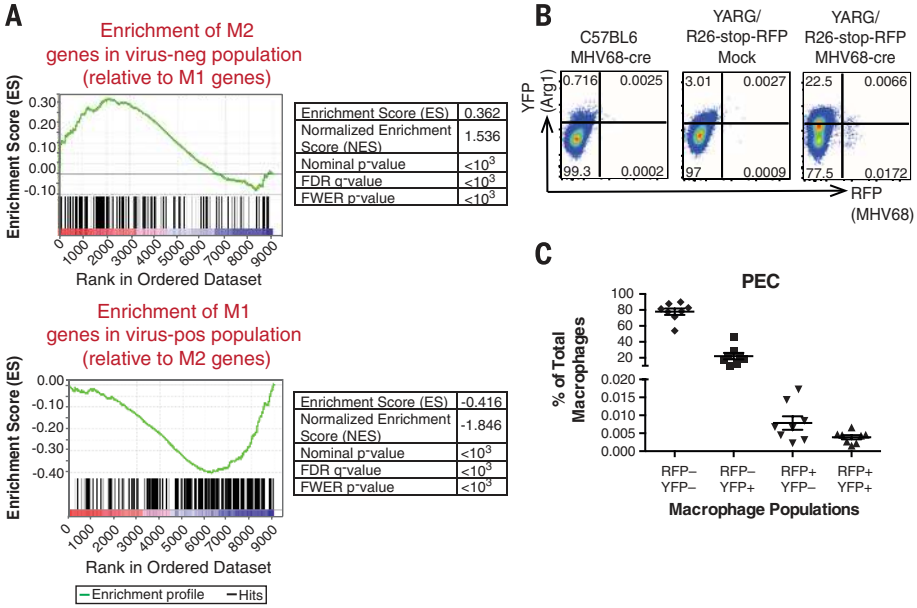

Fig. 2. IL-4 and IFNγ signatures identified in different macrophage populations during MHV68 infection. (**A**) GSEA of virus-positive and -negative cells sorted from the peritoneum of MHV68-infected mice compared to BMDMs stimulated with IL-4 or IFNγ/LPS. (**B**) C57BL/6J mice or YARG/R26-stop-RFP mice were either mock infected or infected with MHV68-cre. CD11b+F4/80+ cells were gated and examined for RFP and YFP expression. Representative plots from two independent experiments, with three to five mice per experiment, are shown. (**C**) Quantitation of fluorescence-activated cell sorting (FACS) analysis in (B), with each symbol representing a single mouse. PEC, peritoneal exudate cells.

reactivation from latency using a MHV68 virus expressing luciferase under the control of a lytic viral promoter upon reactivation from latency in vivo (MHV68-M3-FL) (*14*). Both acute *H. polygyrus* infection and *Sm* egg challenge reactivated MHV68 infection (Fig. 1, A to D). Mice latently infected for more than 100 days also showed increased luciferase expression after *Sm* egg challenge (fig. S1). By contrast, infection with the systemic bacteria *Listeria monocytogenes* did not stimulate viral reactivation (fig. S2). Thus, responses to either a nematode parasite or trematode eggs induced herpesvirus reactivation, suggesting a role for T_H2 cytokines in viral reactivation.

To determine whether T_H2 cytokines affect latently infected macrophages, we compared host gene expression patterns in virally infected and uninfected macrophages during chronic infection. We engineered MHV68 to express cre-recombinase (MHV68-cre) from a locus permitting heterologous gene expression without altering viral replication or reactivation (*15*) (fig. S3). Reporter mice in which fluorescent protein expression is induced by cre recombination [Rosa26-floxed stop-eYFP or tandem dimer (td)RFP (eYFP, enhanced yellow fluorescent protein; RFP, red fluorescent protein) (*16*)] were infected with MHV68-cre. Virus-positive and -negative cells sorted from latently infected mice (fig. S3G) were subjected to RNAseq analysis. Transcription in these cells was compared to that in bone marrow–derived macrophages (BMDMs) stimulated with interleukin-4 (IL-4) (M2) or IFNγ plus lipopolysaccharide (LPS) (M1) compared to untreated BMDMs (M0). Gene set enrichment analysis (GSEA) revealed that genes up-regulated in M1 BMDMs were enriched in

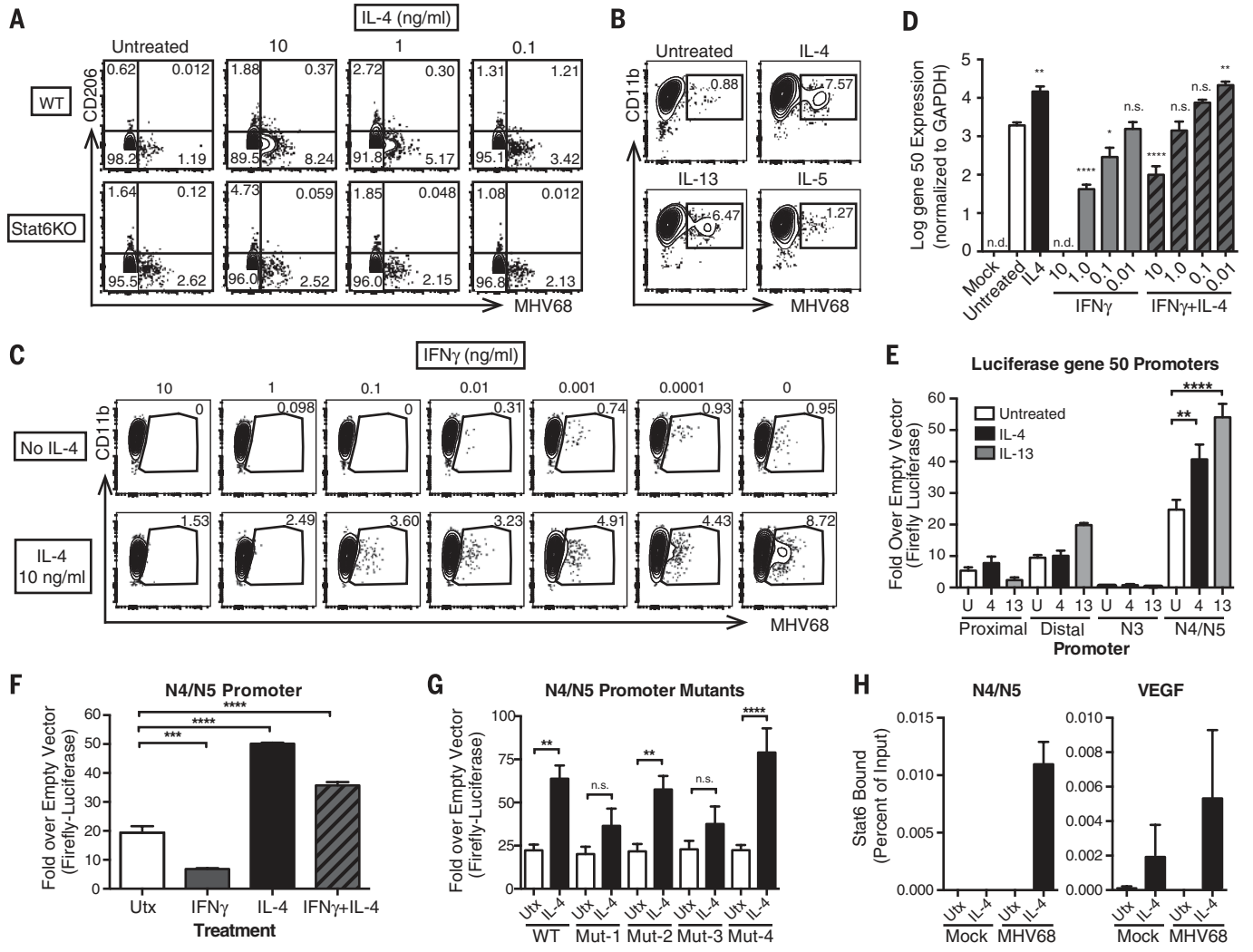

Fig. 3. IL-4 promotes viral replication and antagonizes IFNγ suppression of viral replication through direct binding to a viral promoter. (**A**) BMDMs were untreated or treated with the indicated doses of IL-4 and infected with MHV68. Twenty-four hours after infection, cells were analyzed for expression of MHV68 lytic viral proteins and CD206 expression. Data from three independent experiments are shown. WT, wild-type. (**B**) Pretreatment of BMDMs with IL-4, IL-13, or IL-5 before infection with MHV68 and FACS analysis. (**C**) BMDMs were pretreated with varying doses of IFNγ ± 10 ng/ml of IL-4. At 24 hours after infection, cells were analyzed for expression of lytic viral proteins. Data from three independent experiments are shown. (**D**) *Gene 50* expression was analyzed by reverse transcription polymerase chain reaction (RT-PCR) in BMDMs pretreated with IL-4 and/or IFNγ. Expression was normalized to *Gapdh* (glyceraldehyde phosphate dehydrogenase). Data from four independent experiments are shown. (**E**) RAW264.7 cells were transfected with vectors expressing luciferase under control of four different *gene 50* promoters (*23*, *24*). Cells were then treated with or without IL-4 or IL-13 for 24 hours, lysed, and assayed for luciferase activity. (**F**) Cells were transfected with the N4/N5 promoter luciferase construct and treated with IL-4, IFNγ, or both. (**G**) N4/N5 luciferase mutants were transfected into RAW264.7 cells and assayed for sensitivity to IL-4. (**H**) RAW264.7 cells were infected with MHV68 at multiplicity of infection = 5 and treated with IL-4 for 8 hours. After ChIP with Stat6 antibody, quantitative PCR (qPCR) was performed for the N4/N5 promoter region or vascular endothelial growth factor (VEGF). Percentage of input after normalizing to immunoglobulin G control was calculated for both N4/N5 and VEGF. One experiment representative of four independent experiments is shown. Error bars indicate SEM. n.d. not detected; n.s. not significant. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$ by *t* test or one-way ANOVA with Sidak's multiple comparisons test.

virus-positive macrophages, whereas genes upregulated in M2 BMDMs were enriched in virus-negative cells (Fig. 2A and table S1). This was consistent with the role of IFNγ, which drives M1 macrophage polarization, in inhibiting MHV68 replication and reactivation (*3*, *4*). We therefore tested whether latent MHV68 infection was restricted to M1-type macrophages by infecting tdRFP mice carrying the Arginase-1 (Arg1)–YFP reporter (YARG, a marker for macrophages stimulated with T_H2 cytokines) (*17*) with MHV68-cre. Surprisingly, virus-positive macrophages were either positive or negative for *Arg1* (Fig. 2, B and C), suggesting that despite the role for IFNγ in controlling chronic MHV68 infection, at least some virus-infected cells were exposed to cytokines that drive *Arg-1* expression in vivo.

Along with the observation that T_H2 cytokine-inducing parasites promote reactivation from latency, the presence of an IL-4 signature in some virus-infected macrophages suggests a role for IL-4 in viral infection. We tested this by determining the effect of IL-4 on MHV68 replication in BMDMs. Treatment with IL-4 increased *Arg1* expression (fig. S4A), consistent with M2 polarization (*18*). As expected, few infected BMDMs expressed lytic viral antigens upon MHV68 infection (*19*). However, IL-4 pretreatment increased the number of BMDMs expressing viral proteins and enhanced viral replication (Fig. 3A and fig. S4, B to D); it also increased infection of transformed RAW264.7 macrophages (fig. S4E). Treatment with IL-4 after MHV68 infection increased viral replication (fig. S4F), indicating that IL-4 acts on replication rather than by increasing the number of infected cells. Enhancement of replication was dependent on the T_H2-associated transcription factor Stat6 (Fig. 3A) and occurred with IL-13 stimulation, another T_H2-associated cytokine that uses the IL-4 receptor α chain and signals via Stat6 (Fig. 3B and fig. S4, D and G). The T_H2 cytokine IL-5, which does not signal through Stat6, did not promote MHV68 replication (Fig. 3B).

After treatment with IL-4, the majority of infected cells did not express the M2 markers CD206 or *Arg1* (Fig. 3A and fig. S4A), suggesting that not all IL-4-induced changes in macrophage differentiation are required for enhanced MHV68 replication (*20*). Etomoxir blocks IL-4–induced changes in fatty acid oxidation (*21*) and upregulation of CD206 (fig. S5A) but did not block enhancement of MHV68 replication by IL-4 (fig. S5B). Moreover, IL-4 enhanced replication in the absence of peroxisome proliferator–activated receptor γ (PPARγ) or ARG1, key proteins involved in M2 macrophage function, or inducible nitric oxide synthase, an essential protein in M1 macrophage function (fig. S5, C to G) (*20*). Importantly, IL-4 antagonized IFNγ-mediated suppression of viral replication (Fig. 3C) (*3*). Because Stat6 antagonizes Stat1 (*22*), we tested whether IL-4 promoted virus replication in the absence of Stat1.

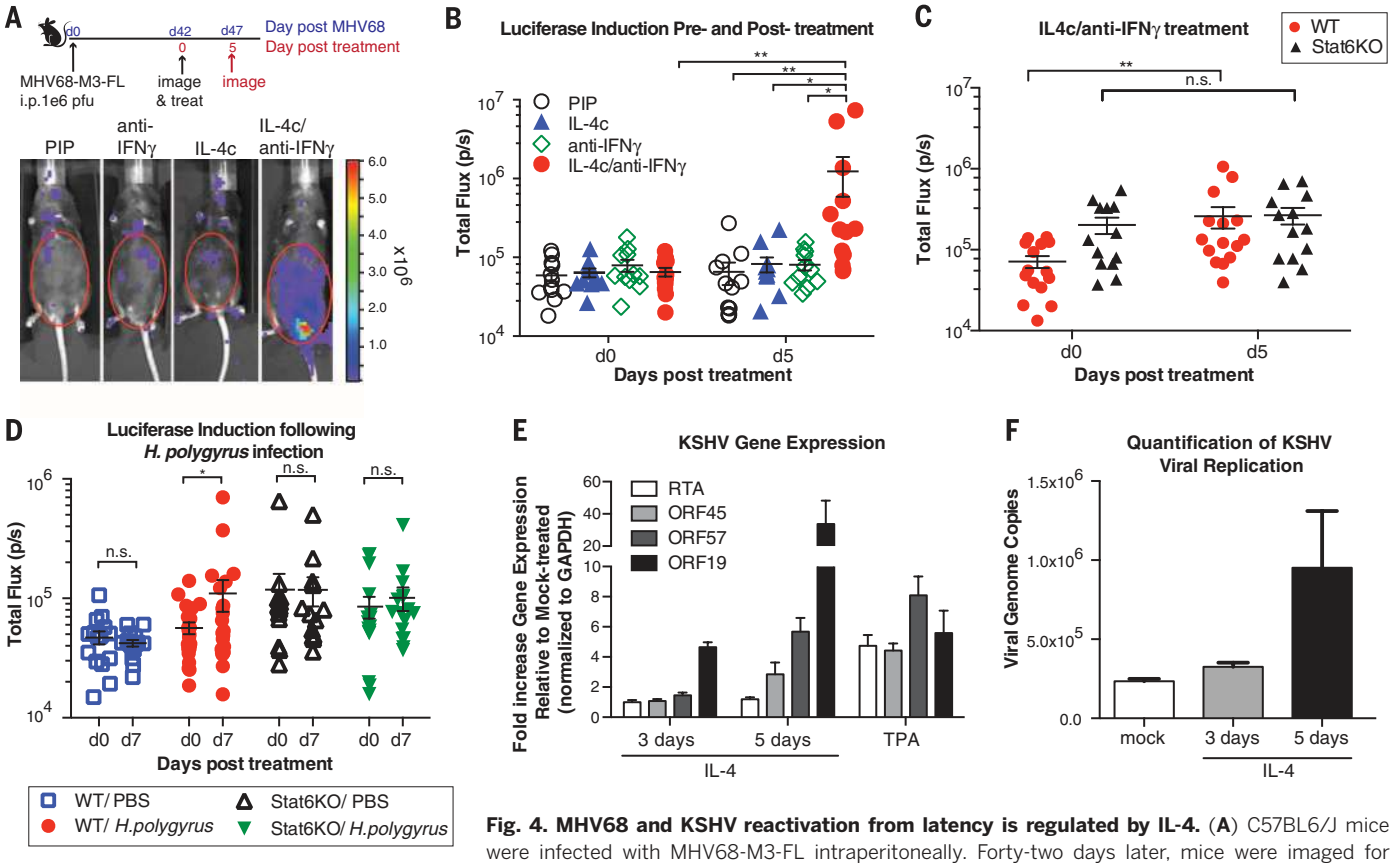

Fig. 4. MHV68 and KSHV reactivation from latency is regulated by IL-4. (**A**) C57BL6/J mice were infected with MHV68-M3-FL intraperitoneally. Forty-two days later, mice were imaged for luciferase expression (d0) and then received isotype control (PIP), anti-IFNγ (H22), IL-4c, or both anti-IFNγ and IL-4c. On day 44, mice received a second dose of IL-4c or PBS. Mice were imaged 5 days after the first treatment, and total flux from the abdominal region was quantitated. Data from four representative mice are shown. (**B**) Quantitation of total flux from three individual experiments described in (A) is shown. Each symbol represents an individual mouse. Bars denote means; and error bars represent standard errors. (**C**) WT or Stat6KO mice were treated as in (A) with anti-IFNγ/IL-4c and imaged 5 days later. Bars denote the means of individual mice (symbols); error bars represent standard errors. (**D**) The experimental set-up was the same as that of Fig. 1A. Latently infected WT and Stat6KO mice infected with MHV68-M3-FL were infected with *H. polygyrus* or treated with PBS on day 42, and reactivation was quantitated by luciferase induction 7 days later. (**E**) BCBL-1 cells were treated with IL-4 for 3 or 5 days or 12-*O*-tetradecanoylphorbol-13-acetate (TPA) for 48 hours, and viral gene expression was analyzed. The fold increase in gene expression over mock after normalization of GAPDH is shown. Data are from three independent experiments. (**F**) Supernatants from cells treated in (E) were collected, and virus was isolated by centrifugation. Viral genome copy number was assayed by qPCR using serial diluted LANA expression plasmid as a standard curve. One experiment representative of two independent experiments is shown. For luciferase experiments: $*P < 0.05$, $**P < 0.01$. n.s., not significant by two-way repeated measures ANOVA with Tukey's and Bonferroni's post-test. Error bars indicate SEM.

IL-4 increased virus replication in Stat1-deficient BMDMs (fig. S6).

Previously, we found that IFNγ-mediated suppression of viral replication was associated with inhibition of promoters driving expression of the essential viral latent-to-lytic switch gene (*gene 50*) (*3*, *23*). IL-4 antagonizes IFNγ-mediated suppression of *gene 50* transcription (Fig. 3D). This effect was specific to the viral promoter because IL-4 did not block IFNγ-mediated induction of *Nos2*, and IFNγ did not inhibit IL-4–mediated induction of *Arg1* and *Relma/Fizz1* (fig. S7). Furthermore, IL-4 and IL-13 transactivated the *gene 50* N4/N5 promoter (Fig. 3E) (*24*), and IL-4 antagonized IFNγ-mediated suppression of the N4/N5 promoter (Fig. 3F). The effect of IL-4 on the N4/N5 promoter was diminished by mutation of two of four putative Stat-binding sites in the promoter (Fig. 3G and fig. S8). Further, chromatin immunoprecipitation (ChIP) experiments revealed that Stat6 bound to the N4/N5 promoter after IL-4 treatment of MHV68-infected cells (Fig. 3H). Taken together, these data suggest that activated Stat6 induced by IL-4/IL-13 promotes viral replication by binding to and acting on a viral promoter to induce expression of *gene 50*.

These counterbalancing effects of IFNγ and IL-4 on virus replication and viral promoter activity suggest a potential mechanism by which IL-4–inducing pathogens such as helminths promote reactivation. Therefore, we treated mice infected with MHV68-M3-FL virus with a blocking antibody to IFNγ (anti-IFNγ) (clone H22) (*25*), an isotype control antibody (clone PIP), long-lasting IL-4 complexes (IL-4c) (*26*), or a combination of anti-IFNγ and IL-4c. No reactivation was observed after treatment with anti-IFNγ, IL-4c, or PIP alone, indicating that a single signal was insufficient to reactivate virus in vivo. However, robust reactivation was observed in mice that received a combination of IL-4c and anti-IFNγ (Fig. 4, A and B, and fig. S9A). We next assayed reactivation using an independent assay (*27*, *28*). Little or no preformed virus was detectable in tissues after treatment with PIP, IL-4c, or anti-IFNγ alone (*27*), whereas treatment with IL-4c plus anti-IFNγ increased infectious virus (fig. S9, B and C). Together, these data support a "two-signal" mechanism by which coinfections could induce reactivation via induction of IL-4 and inhibition of T_H1 responses (*12*).

Increased reactivation after treatment with both IL-4c/anti-IFNγ required Stat6 (Fig. 4C). We did not test the role of Stat1 or the IFNγ receptor because both are required to establish latency (*6*). To assess whether the effects of helminth infection on MHV68 reactivation also required Stat6, we challenged MHV68-infected Stat6KO mice with *H. polygyrus*. We found that helminth infection did not reactivate MHV68 from latency in Stat6KO mice, further supporting a two-signal model for control of γ-herpesvirus reactivation in vivo (Fig. 4D).

Our results suggested a possible role for IL-4 in human γ-herpesvirus reactivation. We therefore tested whether IL-4 could reactivate the human γ-herpesvirus KSHV in the BCBL-1 human B cell lymphoma cell line. We found that treatment with IL-4 increased immediate early (RTA, ORF45, and ORF57) and late (ORF19) viral transcripts (*29*) (Fig. 4E). RTA is the homolog in KSHV of MHV68 *gene 50*, and ORF45 and ORF57 are both transactivators, indicating a common role of IL-4 in regulating important viral transcriptional transactivators. Furthermore, IL-4 treatment of cells increased virus production (Fig. 4F), indicating that IL-4 is capable of inducing reactivation of KSHV.

A notable aspect of herpesvirus infection is its permanence despite ongoing immunity combined with the capacity to reactivate and spread to new hosts. This work illuminates one potential mechanism by which a γ-herpesvirus exhibits these two apparently disparate functions. Our data suggest that the virus evolved cytokine-responsive promoters to remain latent under some conditions (IFNγ-dominant) and reactivate under other conditions (IL-4–dominant). In this setting, coinfection may govern the outcome of reactivation by changing the balance in IL-4 and IFNγ, thus raising a potential issue with herpesvirus reactivation and proposed live helminth therapies (*12*). Additionally, our data illustrate one potential mechanism by which helminths and other type 2 immune response–inducing parasites influence host control of another pathogen through M2 macrophage polarization (*13*). The fact that viral promoters for an essential gene are responsive to host cytokines implies that the viral genome evolved to sense the infection status of the host. We speculate that a similar mechanism for IL-4–induced reactivation of KSHV could also be true. Although not extensively studied, seroprevalence to KSHV is associated with hookworm and other parasitic infections in Uganda (*30*). Intriguingly, certain Burkitt's lymphoma cell lines are reported to express EBV transcripts in response to IL-4 (*31*).

Although mouse studies are done in specific pathogen-free animals, our data suggest that there is added complexity when multiple pathogens infect the same host, particularly in situations where one pathogen has the capacity to respond to specific immune signals generated to another pathogen to regulate chronic infection. Previously, we showed that herpesvirus infection, a component of the mammalian virome (*1*), enhances resistance to some pathogens (*7*). Here, we demonstrate the opposite effect: that coinfection regulates herpesvirus reactivation. These studies emphasize that the spectrum of immunity to chronic infection is a dynamic equilibrium regulated by coinfections, in part through highly evolved pathogen genomes with the capacity to sense host cytokines.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6196/573/suppl/DC1
Materials and Methods
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Science

Helminth infection reactivates latent γ-herpesvirus via cytokine competition at a viral promoter

T. A. Reese, B. S. Wakeman, H. S. Choi, M. M. Hufford, S. C. Huang, X. Zhang, M. D. Buck, A. Jezewski, A. Kambal, C. Y. Liu, G. Goel, P. J. Murray, R. J. Xavier, M. H. Kaplan, R. Renne, S. H. Speck, M. N. Artyomov, E. J. Pearce and H. W. Virgin

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Parasites make it hard to fight viruses

Microbial co-infections challenge the immune system—different pathogens often require different flavors of immune responses for their elimination or containment (see the Perspective by Maizels and Gause). Two teams studied what happens when parasitic worms and viruses infect mice at the same time. Reese *et al.* found that parasite co-infection woke up a dormant virus. Osborne *et al.* found that mice already infected with parasitic worms were worse at fighting off viruses. In both cases, worms skewed the immune response so that the immune cells and the molecules they secreted created an environment favorable for the worm at the expense of antiviral immunity.

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