Early Control of H5N1 Influenza Virus Replication by the Type I Interferon Response in Mice

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Widespread distribution of highly pathogenic avian H5N1 influenza viruses in domesticated and wild birds continues to pose a threat to public health, as interspecies transmission of virus has resulted in increasing numbers of human disease cases. Although the pathogenic mechanism(s) of H5N1 influenza viruses has not been fully elucidated, it has been suggested that the ability to evade host innate responses, such as the type I interferon response, may contribute to the virulence of these viruses in mammals. We investigated the role that type I interferons (alpha/beta interferon [IFN-α/β]) might play in H5N1 pathogenicity in vivo, by comparing the kinetics and outcomes of H5N1 virus infection in IFN-α/β receptor (IFN-α/βR)-deficient and C57BL/6 mice using two avian influenza A viruses isolated from humans, A/Hong Kong/483/97 (HK/483) and A/Hong Kong/486/97 (HK/486), which exhibit high and low lethality in mice, respectively. IFN-α/βR-deficient mice experienced significantly more weight loss and more rapid time to death than did wild-type mice. HK/486 virus caused a systemic infection similar to that with HK/483 virus in IFN-α/βR-deficient mice, suggesting a role for IFN-α/β in controlling the systemic spread of this H5N1 virus. HK/483 virus replicated more efficiently than HK/486 virus both in vivo and in vitro. However, replication of both viruses was significantly reduced following pretreatment with IFN-α/β. These results suggest a role for the IFN-α/β response in the control of H5N1 virus replication both in vivo and in vitro, and as such it may provide some degree of protection to the host in the early stages of infection.

Highly pathogenic avian influenza H5N1 viruses continue to circulate and cause disease in domesticated and wild birds across Asia, Europe, and Africa. Increased exposure to infected avian species has resulted in over 400 laboratory-confirmed human cases and 250 deaths due to H5N1 virus infection since late 2003 (http://www.who.int/csr/disease/avian_influenza/country). Exacerbated cytokine and chemokine responses have been associated with high respiratory virus load and severe disease in H5N1 virus-infected patients (7, 8, 47, 48, 62, 71). Molecular determinants of H5N1 viruses have been proposed to contribute to the subversion of the host’s innate immune response, leading to the severe disease caused by these viruses (24, 34, 35, 52, 53). In particular, studies in a porcine model have suggested that H5N1 viruses are resistant to the effects of the type I interferon response (52, 53). We chose to use a mouse model to assess the role of the type I interferon response in a mammalian model in which H5N1 viruses exhibit a level of virulence similar to that seen in humans and other natural mammalian hosts.

Inbred mice, H5N1 viruses isolated from humans exhibit one of two general pathogenic phenotypes (11, 15, 28, 30, 36, 38, 60, 63). Highly pathogenic viruses replicate in both pulmonary and extrapulmonary organs and are highly fatal for mice, whereas viruses that exhibit a low pathogenicity phenotype replicate primarily in the respiratory tract and are generally not lethal. Recently, we demonstrated a role for several proinflammatory cytokines, specifically tumor necrosis factor alpha and interleukin 1β, in the severity or control of H5N1 virus disease in mice, respectively. However, the role of the type I interferons in the pathogenicity of these viruses has not been fully elucidated (60).

Influenza A viruses have been shown to induce alpha/beta interferon (IFN-α/β) in experimentally infected mice and humans (1, 18). Recently, ligation of cellular pattern recognition receptors that recognize double-stranded RNA (dsRNA), byproducts of influenza virus replication, have been shown to be a key initiator of the type I interferon response in infected epithelial cells. These pattern recognition receptors include helicases (such as retinoic acid inducible gene I [RIG-I] and melanoma differentiation-associated gene-5 [MDA-5]), important regulators of cytoplasmic dsRNA recognition and inducers of IFN response genes and toll-like receptor 3 (TLR3) and TLR7/8, which detect endosomal dsRNA and initiate alternate interferon induction pathways primarily in dendritic cells (18, 22, 29, 33, 40, 42, 50, 56, 70). The nonstructural protein 1 (NS1)
protein of influenza A viruses inhibits the antiviral effects of IFN-α/β by antagonizing this induction pathway by multiple mechanisms, including sequestering dsRNA, direct modulation of protein kinase R (PKR) activity, and inhibiting transcription and translation of host antiviral response genes (6, 20, 37, 45, 61, 67, 68). Although NS1 proteins from both human and avian influenza A viruses can inhibit type I interferon responses, there is considerable variation among virus strains or subtypes in their ability to induce or suppress type I interferon responses (19, 20, 32). Influenza A virus susceptibility to the host’s IFN antiviral response was demonstrated previously using mouse-adapted H1N1 viruses. A lack of functional NS1 rendered mutant influenza viruses incapable of replicating except in interferon-deficient cells, such as Vero cells (13). Following infection of mice deficient in IFN-α/β receptor or STAT1, influenza viruses could replicate to higher titers and spread outside of the respiratory tract (12).

Here we show that mice lacking IFN-α/β receptor (IFN-α/βR) signaling succumb to lethal disease more rapidly and have more extensive extrapulmonary spread of avian H5N1 viruses of both high and low pathogenicity. Furthermore, we demonstrate that H5N1 virus replication is initially suppressed in mouse lung epithelial cells pretreated with IFN-α or IFN-β but that viruses with high replication efficiency can ultimately overcome these effects. Thus, even highly pathogenic H5N1 viruses are susceptible to the murine host’s type I interferon response; however, the ability to overcome this antiviral effect likely contributes to the heightened virulence of these viruses in vivo.

MATERIALS AND METHODS

Viruses. The influenza viruses used in this study were the H1N1 virus A/WSN/33 (WSN); the H5N1 viruses A/Hong Kong/483/97 (HK/483), A/Hong Kong/486/97 (HK/486), A/Thailand/16/04 (Thai/16), and A/Thailand/SP/83/04 (SP83); and reassortant HK/483 (HK483PB2-486) and HK/486 (HK486 E627K) viruses containing substitutions in the PB2 protein. H5N1 virus stocks were propagated in the allantoic cavities of 10-day-old embryonated chicken eggs for 24 h at 37°C. The allantoic fluids were harvested, aliquoted, and stored at −70°C.

Virus titration by plaque assay. The numbers of PFU per ml in LA-4 culture supernatants were determined by standard plaque assay, as described previously (59). Virus titers were calculated and expressed as the mean log10 PFU/ml. Culture supernatants in which no virus was detected were given a value of 10^0 PFU/ml for calculation of the mean titer.

Virus titration by EID50 and histopathology. Tissues were homogenized in 1 ml of cold phosphate-buffered saline. Clarified tissue homogenates were infected for virus infectivity in eggs from an initial dilution of 1:10 for lungs and 1:2 for brain, spleen, and thymus. Virus titers were calculated by the method of Reed and Muench and are expressed as the average log10 EID50/ml ± the standard errors of the means (49). Tissues in which no virus was detected were given a value of 10^0.5 or 10^0.0 EID50/ml, the limit of virus detection for lung or other tissues, respectively, for calculation of the mean titer. In addition, lung, brain, and spleen from days 3 and 7 p.i. were fixed in 10% buffered formalin and routinely processed to hematoxylin and cosin-stained histological sections.

RESULTS

Increased virulence and dissemination of H5N1 viruses in IFN-α/βR-deficient mice. To determine whether the type I interferon response contributed to the control of avian H5N1 viruses in mice, we compared the relative susceptibilities of IFN-α/βR-deficient and SvEv129 control mice to two H5N1 influenza viruses previously characterized in the BALB/c, C57BL/6, and B6/12 mouse models as being of either high pathogenicity (HK/483 virus) or low pathogenicity (HK/486 virus) (36, 60, 63). Mice were infected i.n. with 1,000, 100, or 10 MLD50 of HK/483 or HK/486 virus and were monitored for weight loss and survival for 14 days. For all virus doses of HK/483 virus, IFN-α/βR-deficient mice had a significantly more rapid mean time to death (P < 0.001), succumbing to viral infection three days earlier than SvEv129 mice (Fig. 1). Unexpectedly, we observed that doses of 1,000 and 100 MLD50 of HK/486 virus were fatal for SvEv129 controls, indicating that this strain of mice was approximately 100 times more
susceptible to this H5N1 virus than other inbred strains of mice (Fig. 1A and B) (36, 60, 63). Despite the overall increased sensitivity of the SvEv129 wild-type mice, IFN-α/βR-deficient mice infected with HK/486 virus were significantly more susceptible to fatal disease, dying either more rapidly following infection with 1,000 MID₅₀ (P < 0.001) or more frequently following infection with 10 MID₅₀ (P < 0.01) than did wild-type mice (Fig. 1A and C). These data suggest that the type I interferon response contributes to the early control of H5N1 virus infection in these inbred mice, but it is not sufficient to protect against the fatal outcome of H5N1 virus infection.

To further examine the heightened lethality of H5N1 viruses in mice deficient in IFN-α/βR, we next evaluated viral replication on days 1, 3, 5, and 7 p.i. in mice inoculated i.n. with 10 MID₅₀ of HK/483 and HK/486 viruses (Fig. 2). On all days evaluated, there were no significant differences in lung viral titers between SvEv129 and IFN-α/βR-deficient mice infected with either H5N1 virus, and lungs from both mice had similar histological lesions of neutrophilic to histiocytic alveolitis and bronchiolitis, although the inflammatory lesions were slightly more severe with HK/483 than with HK/486. Interestingly, the IFN-α/βR-deficient mice had accompanying multiple foci of necrosis in their alveoli; this was lacking in SvEv129 mouse lungs (data not shown). There was a significant difference in the viral titers of HK/483 versus HK/486 in the lungs of mice on day 1 p.i. regardless of the background (Fig. 2A; P < 0.02). This enhanced pulmonary replication of HK/483 virus in either mouse strain early after infection distinguishes this more virulent strain from the less virulent HK/486 virus. As early as day 3 p.i. (Fig. 2B), HK/483 virus was detected to a greater extent in tissues outside of the lungs in IFN-α/βR-deficient mice, compared to the level in control mice; day 5 p.i. viral titers in all organs tested were significantly higher in IFN-α/βR-deficient mice than in SvEv129 mice (Fig. 2C; P < 0.05, P < 0.001). Such disseminated virus was commonly associated with moderate to severe necrosis and accompanying neutrophilic inflammation in the spleens of IFN-α/βR-deficient mice; such splenic lesions were a rare occurrence in SvEv129 mice. Le-
sions in the brain were lacking in all mice (data not shown). All IFN-α/βR-deficient mice succumbed to HK/483 virus infection by day 7 p.i. (Fig. 2D). Interestingly, the spread of HK/486 virus to multiple extrapulmonary organs was observed to be more extensive in IFN-α/βR-deficient mice on days 5 and 7 p.i. than with wild-type controls.

These results suggest that the type I interferon response controls the spread to and/or extent of viral replication in extrapulmonary organs in H5N1 virus-infected mice.

**H5N1 influenza viruses are sensitive to type I interferons in vitro.** We next determined whether H5N1 virus replication in vitro was affected by the exogenous addition of type I interferon. For these experiments, two pairs of H5N1 viruses from the 1997 (HK/483 and HK/486) and 2004 (Thai/16 and SP83) outbreaks that exhibit high or low pathogenicity phenotypes, respectively, in mice were used to account for the genetic divergence that has occurred in more-recent H5N1 viruses (38). Murine lung epithelial LA-4 cells were treated with 250 U/ml of recombinant mouse IFN-α, IFN-β, or media alone for 24 h, washed, and infected with the H5N1 viruses or a mouse-adapted H1N1 virus, WSN, at a MOI of 0.1. An earlier dose-response experiment had determined that a concentration of

### FIG. 2. Effect of IFN-α/βR deficiency on H5N1 virus replication and spread in mice.

SvEv129 and IFN-α/βR-deficient mice were infected with 10 MID₅₀ of HK/483 or HK/486 virus. Four mice from each virus-infected group were euthanized on either day 1 (A), day 3 (B), day 5 (C), or day 7 (D) p.i., and viral titers of individual tissues were determined. Data are expressed as log₁₀ mean viral titers ± standard errors. (*, *P < 0.001; **, P < 0.01; *** P < 0.05; †, no surviving animals).
250 U/ml of IFN-α or IFN-β resulted in maximal inhibition of virus replication at 24 h p.i. (data not shown). Culture supernatants were collected over the next 48 h, and titers were determined by plaque assay. Data are expressed as log_{10} PFU/ml and represent averages of three independent experiments ± standard errors. (**, P < 0.01; ***, P < 0.05).

FIG. 3. Effect of IFN-α/β treatment on virus replication in LA-4 cells. LA-4 cells were treated for 24 h with 250 U of IFN-β or media alone and then infected at an MOI of 0.1 with HK/483 (A), HK/486 (B), Thai/16 (C), SP83 (D), or WSN (E) virus. Culture supernatants were collected at 0, 8, 16, 24, 36, and 48 h p.i. and titers were determined by plaque assay. Data are expressed as log_{10} PFU/ml and represent averages of three independent experiments ± standard errors. (**, P < 0.01; ***, P < 0.05).
and Thai/16, and the H1N1 control virus, WSN. Culture supernatants were collected over a 72-h period, and titers were determined by plaque assay (Fig. 4). The amount of HK/483 virus produced in IFN-β/H9252-treated LA-4 cells at 72 h was similar regardless of the initial MOI (Fig. 4A, B, and C). In contrast, the difference in HK/483 virus titers between IFN-β- and mock-treated LA-4 cells was substantially greater as the MOI decreased, reaching an almost 1,000-fold reduction in virus at 24 h p.i. in interferon-treated cells infected with an MOI of 0.001 (Fig. 4C). Similarly, interferon pretreatment reduced levels of Thai/16 virus at 24 h p.i. by almost 10,000-fold compared with the levels in mock-treated cells at an MOI of 0.001 (Fig. 4F). Interestingly, the titers of both H5N1 viruses in interferon-treated cells increased substantially after 24 h p.i., regardless of the MOI, suggesting that these viruses were no longer affected by the IFN-β pretreatment of cells (Fig. 4A to F). In contrast, WSN virus consistently had only a 10-fold difference in viral titers between IFN-β- and mock-treated cells and exhibited only a modest, if any, increase in virus produced after 24 h p.i. (Fig. 4G to I). These results are similar to our observation with the H5N1 viruses of low pathogenicity, HK/486 and SP83 (Fig. 3B to D).

Effect of PB2 gene on sensitivity of H5N1 viruses in vitro and in vivo. The polymerase complex from highly pathogenic avian H5N1 viruses has been shown to be important for the virulence of these viruses in mammalian systems (17, 51, 55). In particular the E627K substitution in the PB2 protein has been shown to confer greater replicative efficiency and extended host range through the adaptation of the avian polymerase complex to one that is more permissive in mammalian hosts (39, 55, 58, 69). To examine whether this virulence factor contributed to IFN-α/β sensitivity of H5N1 viruses, we used two previously described reassortant viruses generated by reverse genetics: an HK/483 virus containing the PB2 gene segment from HK/486 (HK/483PB2-486) and a HK/486 E627K virus containing the E627K substitution in the PB2 protein (HK/486 E627K) (5). The introduction of the E627K substitution into the PB2 protein of HK/486 virus dramatically increased the
replication efficiency of the virus and also changed the phenotype of the virus in regard to IFN-α/β sensitivity (Fig. 5A versus Fig. 3B). Conversely, replacing the HK/483 for the HK/486 PB2 gene substantially reduced the replication efficiency of the HK/483PB2-486 reassortant virus and its ability to overcome the interferon pretreatment of LA-4 cells (Fig. 5B).

To assess the effect of the PB2 E627K mutation on virulence and replication in vivo, SvEv129 wild-type mice or IFN-α/βR-deficient mice were infected with the HK/486 E627K virus or the parental wild-type virus HK/486, also derived by reverse genetics (rgHK/486). The HK/486 E627K mutant virus exhibited enhanced virulence compared with that for the rgHK/486 virus, not only in the SvEv129 wild-type mice but also in the IFN-α/βR-deficient mice, which succumbed to infection 2 days earlier than wild-type mice (Fig. 6A; *P < 0.01). Furthermore, the HK/486 E627K virus was detected in tissues outside of the lungs in the IFN-α/βR-deficient mice on day 5 p.i. to a greater extent than with control mice (*P < 0.05, **P < 0.01) or with the parental rgHK486 virus in IFN-α/βR-deficient mice (P < 0.05) (Fig. 6B). These results are similar to those achieved with the more virulent wild-type HK/483 virus, which possesses 627K in PB2 (Fig. 1C and Fig. 2C), and suggest that in vivo, viruses

Figure 5. Effect of IFN-α/β treatment on reverse genetics-generated H5N1 virus replication in LA-4 cells. LA-4 cells were treated for 24 h with 250 U of IFN-β or media alone and then infected at an MOI of 0.1 with HK483PB2-486 (A) or HK486 E627K (B). Culture supernatants were collected at 0, 8, 16, 24, 36, and 48 h p.i., and titers were determined by plaque assay. Data are expressed as log_{10} PFU/ml and represent averages of three independent experiments ± standard errors. (*, P < 0.001; **, P < 0.01; ***, P < 0.05.)

Figure 6. Effect of IFN-α/βR deficiency on H5N1 virus disease outcome, replication, and spread in mice. SvEv129 and IFN-α/βR-deficient mice were infected with 10^3 EID_{50} of either HK/486 E627K or parental HK/486 virus, both derived by reverse genetics. Groups of three to six mice were observed for survival for 14 days p.i. (A). An additional three mice per group were euthanized on day 5, and viral titers of individual tissues were determined (B). Data are expressed as log_{10} mean viral titers ± standard errors. (**, P < 0.01; ***, P < 0.05.)
possessing the PB2 E627K mutation exhibit enhanced lethality and extrapulmonary spread, even though they are still sensitive to early, limited control through the type I interferon response. Taken together, these data support the hypothesis that H5N1 viruses are sensitive to the antiviral effect of type I interferons in vitro, although H5N1 strains that exhibit enhanced replication in vitro and virulence in vivo eventually overcome the antiviral state.

**DISCUSSION**

Fatal human H5N1 virus disease has been associated with elevated respiratory viral loads and exacerbated cytokine responses (4, 7, 62, 71). The type I interferons are key components of the host antiviral immune response and modulators of adaptive immune responses. Produced within hours of viral infection, IFN-α/β induces an antiviral state in uninfected cells that helps to contain the spread of progeny virions to neighboring cells (14, 25, 41, 43, 46, 57, 72). In this study, we observed that H5N1 virus-infected IFN-α/βR-deficient mice had a more rapid time to death, increased viral replication in extrapulmonary organs, and severe splenic lesions, in contrast to immunocompetent SvEv129 mice. Furthermore, we directly tested the sensitivity of H5N1/1997 and H5N1/2004 viruses in vitro and found that their replication was significantly reduced in LA-4 cells pretreated with either IFN-α or IFN-β. Therefore, in a mouse model in which avian H5N1 viruses exhibit either high or low virulence, we have demonstrated that the type I interferon response contributes to control of H5N1 virus replication both in vivo and in vitro. However, our in vitro studies suggest that the high efficiency of replication of specific H5N1 viruses overcomes the initial protective effect of type I interferon, leading to increased virulence in vivo.

Both H5N1 viruses used in this study, like other influenza A viruses, induce elevated amounts of IFN-α/β following productive replication of the virus in the lungs of mice (our unpublished data). Our data indicate that avian H5N1 viruses are similar in their interferon sensitivity to mouse-adapted H1N1 influenza A viruses, as IFN-α/βR- or STAT1-deficient mice succumbed to infection more rapidly with high titers of disseminated virus (13). Direct intracranial inoculation of BALB/c mice with the less-pathogenic HK/486 virus results in replication at this site, neuronal tropism, and lethal disease that is indistinguishable from infection with the more virulent HK/483 virus administered either i.n., intracranially, or intravenously (3). From these data, we conclude that the type I interferon response can play a role in limiting replication and systemic spread of H5N1 viruses. As suggested by Hayman et al., this may have consequences for limiting transmission of avian H5N1 viruses in hosts for which these viruses are not currently well adapted (19).

Using a porcine lung epithelial cell line, Seo et al. demonstrated that H5N1 viruses from 1997 were shown to resist the effect of porcine interferon at doses similar to those used in this study (52). The NS1 of the H5N1/1997 virus and, in particular, a novel substitution at residue D92E were implicated in this resistance and were associated with enhanced virulence in pigs when reassorted into a H1N1 background (52, 53). However, our results reveal that in the absence of IFN-α/βR signaling in vivo, H5N1 viruses are more virulent. Similarly, H5N1 virus replication was suppressed in interferon-pretreated murine LA-4 cells, confirming the sensitivity of H5N1 viruses to murine interferon. Consistent with our findings, Hayman et al. demonstrated that avian influenza viruses exhibited enhanced replication in human cells deficient in the type I interferon system, suggesting that human interferon can also limit avian virus replication in vitro (20). This same group demonstrated that the NS1 gene from A/Teal/Hong Kong/1997, which is identical to the H5N1/1997 virus NS1 gene including the D92E substitution, did not confer resistance to the induction and expression of interferon-induced genes in a human epithelial cell line (19). Using an in vitro minireplicon system, others have demonstrated that avian H5N1 virus polymerase activity is controlled by mammalian Mx proteins (9). The mechanism of inhibition of influenza virus replication by the antiviral interferon-induced gene products may also include suppression of viral assembly, a decrease in viral budding, or release of less-infectious progeny virions, which have been observed in other viral systems (2, 10, 16, 26, 44, 66). Preliminary work from our laboratory demonstrated decreased levels of viral RNA copies and viral gene products in interferon-pretreated H5N1 virus-infected cells, suggesting that interferon inhibition occurs early in the virus replication cycle (data not shown). The specific interferon response element(s) that contributes to this inhibition remains to be identified.

While the NS1 gene product has been implicated in contributing to the virulence of some H5N1 viruses in mammalian systems, the NS gene does not contribute substantially to the differential virulence of HK/483 and HK/486 viruses in mice (17, 27, 65). Because of the differential sensitivity to interferon observed between H5N1 viruses of high and low pathogenicity in vitro, we sought to determine the contribution of the PB2 gene, known to confer enhanced virulence for H5N1 viruses in the murine model, to the overall sensitivity to IFN-α/β in vitro. We demonstrated that the E627K substitution conferred increased replication efficiency and a more interferon-resistant phenotype in the HK/486 virus background. In contrast, replacing the HK/483 PB2 gene with that from the HK/486 virus substantially reduced the replication efficiency of the HK/483PB2-486 reassortant virus and its ability to overcome the interferon pretreatment of LA-4 cells. These results suggest that the replication efficiency of the H5N1 viruses contributes to their relative sensitivity to interferon in vitro. However, in vivo, the more virulent HK/486 E627K virus was still sensitive to the early antiviral effect of the type I interferon response. It should be noted that not all H5N1 viruses characterized to be highly pathogenic for mice possess the E627K substitution, and as such other substitutions in PB2 or other polymerase genes that confer enhanced replicative ability may also contribute to the high pathogenicity phenotype (21, 51). The optimal constellation of viral genes that confer virulence for a given strain may also be dependent on various interactions of viral gene products with host proteins (31, 54, 55).

Type I interferons activate numerous interferon-stimulated genes which encode proteins with antiviral activities such as PKR, 2′-5′ OAS, RNase L, and Mx proteins. Because the inbred mice, and the respiratory epithelial cells derived from them, that were used in this study lack a functional Mx1 protein (which is specifically associated with conferring resistance to orthomyxoviruses [57]) but do express other antiviral pro-
teins, they are likely to be more sensitive to the effects of type I interferon than strains that express a functional Mx gene. Previous work from this laboratory has demonstrated enhanced resistance to H5N1 viruses in \textit{Mx1}^{+/+} BALB.A2G mice compared with that in BALB/c \textit{Mx1}^{−/−} mice (64). Furthermore, prophylactic treatment with type I interferon was shown to protect H5N1 virus-infected \textit{Mx1}^{+/+} but not \textit{Mx1}^{−/−} mice from fatal disease (64).

Results from the present study reveal that IFN-α/βR signaling can control H5N1 virus replication and spread in extrapulmonary organs in vivo, supporting the hypothesis that even highly pathogenic avian H5N1 influenza viruses are sensitive to the antiviral effects of the type I interferon response in a susceptible mammalian host system. Control of virus replication was recapitulated in vitro with LA-4 cells treated with exogenously added interferon. In this system, we observed that highly virulent H5N1 viruses could ultimately overcome this early control through more-efficient replication in host cells, in contrast to viruses of low virulence. Therefore, prophylactic therapies that include interferon, such as pegylated interferon, may be more sensitive to the effects of type I interferon than strains that express a functional Mx gene.

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in H5N1 patients, and thus they warrant further investigation.

Therapies that include interferon, such as pegylated interferon, alone or in combination with antiviral drugs could limit influenza infection. Relation to symptom formation and host defense. J. Clin. Investig. 101:643–649.


