A novel broad-spectrum treatment for respiratory virus infections: influenza-based defective interfering virus provides protection against pneumovirus infection in vivo

Andrew J. Easton*, Paul D. Scott, Nicole L. Edworthy, Bo Meng, Anthony C. Marriott, and Nigel J. Dimmock

School of Life Sciences, University of Warwick, Coventry CV4 7AL, UK

* Corresponding author: Professor Andrew J. Easton, School of Life Sciences, University of Warwick, Coventry CV4 7AL, UK. Tel: +44 (0)2476 524727; Fax: +44 (0)2476 523568

Email address: a.j.easton@warwick.ac.uk

AJE and PDS contributed equally to the work

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ABSTRACT

Respiratory viruses represent a major clinical burden. Few vaccines and antivirals are available, and the rapid appearance of resistant viruses is a cause for concern. We have developed a novel approach which exploits defective viruses (defective interfering (DI) or protecting viruses). These are naturally occurring deletion mutants which are replication-deficient and multiply only when coinfection with a genetically compatible infectious virus provides missing function(s) in trans. Interference/protection is believed to result primarily from genome competition and is therefore usually confined to the virus from which the DI genome originated. Using intranasally administered protecting influenza A virus we have successfully protected mice from lethal in vivo infection with influenza A viruses from several different subtypes [1]. Here we report, contrary to expectation, that protecting influenza A virus also protects in vivo against a genetically unrelated respiratory virus, pneumonia virus of mice, a pneumovirus from the family *Paramyxoviridae*. A single dose that contains 1 μg of protecting virus protected against lethal infection. This protection is achieved by stimulating type I interferon and possibly other elements of innate immunity. Protecting virus thus has the potential to protect against all interferon-sensitive respiratory viruses and all influenza A viruses.

1. Introduction

A wide range of viruses cause respiratory illness that is a major burden on the human population. The severity of respiratory disease ranges from the approximately 40 million fatalities of the 1918 influenza pandemic to the mild rhinitis of the common cold. Two groups of viruses responsible for large numbers of respiratory infections around the world are the influenza viruses (family *Orthomyxoviridae*) and the pneumoviruses (sub-family *Pneumovirinae* of the family *Paramyxoviridae*). Both the *Orthomyxoviridae* and *Paramyxoviridae* have single-strand, negative sense RNA genomes, but are genetically distinct with the *Orthomyxoviridae* having segmented genomes while those of the *Paramyxoviridae* comprise a single molecule [2, 3]. Prophylaxis against influenza viruses is achieved through vaccines which have to be developed annually because of antigenic variation. Antivirals (oseltamivir, zanamivir) are now seen as a significant weapon in the armoury in protecting against new outbreaks, but appearance of antiviral-resistant influenza viruses is a major concern [4, 5]. The only antiviral approved for treatment of human respiratory syncytial virus (*Pneumovirinae*), which is responsible for the highest level of hospitalisation of infants worldwide, is ribavirin. However, ribavirin is not in general use clinically, and high-risk children are treated prophylactically with the monoclonal antibody palivizumab [6].

We have developed a new approach that harnesses defective-interfering (DI) viruses as antivirals. The active principle is the DI genome which arises spontaneously from the genome of infectious virus and contains one or more major deletions. This results in elimination of at least one essential gene and renders it defective for replication. DI viruses are thus dependent on coinfection with the virus that gave rise to the DI genome or one with which it is genetically compatible to provide missing function(s) in trans. Current thinking is that DI viruses reduce production of helper virus in cell culture probably as a result of the

smaller DI genome having a competitive edge [7-11]. The DI virus particles retain receptor specificity and hence are targeted to the natural sites of infection in vivo. DI viruses are produced with varying facility by nearly all virus families. Influenza DI viruses were the first to be recognised [12-14], and while their molecular biology has been studied extensively [10, 15, 16] analysis has been difficult as a single DI preparation can contain many different DI RNA sequences, which can arise from any of the 8 viral RNAs each with variably located central deletions [17, 18], although most arise from the three largest viral RNAs. We have solved this heterogeneity problem by producing cloned viruses that contain one major species of DI RNA [1, 18-21], and have characterized 244 DI RNA, that is particularly active in protecting mice from a variety of different influenza A subtypes [1]. We have designated DI viruses that have demonstrable, reproducible, in vivo activity as 'protecting viruses' [22]. The observed cross-subtype antiviral activity of DI virus 244/PR8 (244 RNA packaged by A/PR8), coupled with its replication dependence on infectious virus and the common genetic system of all influenza A viruses, suggest that it acts at the level of genome competition [8]. Overall, however, the use of DI viruses to protect from infection in vivo has been disappointing, with very limited data showing that a few DI viruses can protect from clinical disease caused by their 'parental' virus [23-26].

It is well known that double stranded RNAs are efficient stimulators of type I interferon and, consistent with this, DI RNAs which have an extensively double stranded structure (such as the copyback and snapback DI RNAs of vesicular stomatitis virus and Sendai virus) also stimulate interferon type I [27-31]. However, there are no reports that the interferon stimulated by copyback or snapback DI RNAs can protect in vivo against genetically unrelated virus infections. Influenza viruses do not produce copyback or snapback DI RNAs and influenza DI RNAs in general, and 244/PR8 DI RNA in particular, have no major regions of double-stranded sequence that would be expected to induce interferon. However, it was

recently reported that during influenza infection of cells in vitro, the smaller influenza genomic RNAs and various DI RNAs become associated with the retinoic acid inducible gene 1 (RIG-1) that contributes to interferon type I induction [32], although it was not possible to say which of the RNAs (genomic or DI) was responsible for activation of an interferon-stimulated promoter and the luciferase gene under its control. These data raise the possibility that influenza virus DI RNAs may be able to induce interferon in vivo and that this may contribute to protection from infection by other non-influenza A viruses. In this way, DI RNAs might represent an additional weapon in the armoury for combatting virus infections. In this report we demonstrate that influenza DI virus 244/PR8 protects mice from respiratory infection caused by an unrelated paramyxovirus, pneumonia virus of mice (PVM). PVM is a member of the genus *Pneumovirus* that includes human respiratory syncytial virus (HRSV), a particular problem to infants and the elderly, and is the recognised animal model for HRSV [33]. PVM is known to be a murine virus but is antigenically related to viruses that also occur in humans [34].

2. Materials and methods

2.1. Viruses

Defective interfering (DI) influenza A virus 244/PR8 arose spontaneously during generation of cloned virus by transfection of 293T cells with a set of influenza A/PR/8/34 (H1N1) plasmids [1]. The protecting 244/PR8 DI virus was amplified in MDCK cells and then in embryonated chicken's eggs, and purified by centrifugation through sucrose. Preparations were standardized to 2×10^5 haemagglutination units (HAU)/ml and stored in liquid nitrogen. DI RNA 244 is 395 nucleotides in length and is derived from segment 1 RNA of influenza

strain A/PR/8/34 apparently by having undergone a single deletion. It comprises nt 1-244 and 2191-2341 of the A/PR8 minus-sense segment 1 RNA, and retains the original termini and sequences essential for replication and encapsidation. Analysis by RT-PCR with primers specific for genome segment 1 showed that the 244 RNA was the major defective RNA present. Helper virus infectivity was eliminated by irradiating with UV for 40 seconds at 253.7 nm. This has little effect on the DI RNA because of the small UV-target size of the protecting RNA (395 nucleotides) compared with the infectious viral genome (13,600 nucleotides). Prolonged UV irradiation (8 minutes) destroys the mouse-protecting activity but does not affect viral HA or neuraminidase (NA) activities [1], and provides a control for immune system-stimulating or receptor-blocking effects. Influenza virus A/WSN/40 virus (H1N1) was also prepared in embryonated eggs. Pneumonia virus of mice (PVM, *Paramyxoviridae*), strain J3666, was grown and assayed in BSC-1 cells.

2.2. Animal studies

C3H/He-mg mice (4-5 weeks-old, 16-20 g, bred in house) were inoculated intranasally under light ether anaesthesia with protecting 244/PR8 virus [35, 36]. Mice were infected intranasally with 10 LD_{50} PVM (250 infectious units) after anaesthesia with ketamine and xylazine. PVM is highly pathogenic and its effects were monitored [37] by group weight and individual clinical disease according to the scheme: healthy mice score 1, ruffled fur with some deep breathing, and looking less alert score 2, obvious weight loss, with laboured breathing, and possible tremors score 3, abnormal gait, difficulty with walking, frequently emaciated, and may show cyanosis of tails and/or ears score 4, and death scores 5. The percentage change in mouse group weight, normalized against the weight prior to treatment, was also recorded. All animal experiments were carried out in accordance with the relevant

UK regulations and mice were culled if judged in severe distress in accordance with the requirements of the UK Home Office licence under which the work was performed. Interferon type I receptor-null mice (129Sv/Ev IFNα/βR$^{-/-}$;Banting and Kingman Ltd., UK) and wild type mice (129Sv/Ev; Charles River) were used as indicated.

2.3. Assay of PVM lung infectivity in vitro

Lungs from infected mice were stored at -70°C. The right-hand lung of each subject was thawed, homogenised with sand and PBS containing 0.1% w/v BSA, and then centrifuged to remove debris. Infectivity was titrated in BSC-1 cells using a focus-forming assay in 96-well plates. Triplicate assays were incubated at 31°C for 72 hours. The cells were then fixed in 1:1 (v/v) methanol: acetone, and blocked with 5% w/v milk powder in PBS. Virus-positive cells were detected using a mouse monoclonal antibody specific to the PVM P protein [38], and goat anti-mouse IgG-alkaline phosphatase conjugate (Sigma), both in buffered saline containing 0.1% v/v Tween 20, and finally alkaline phosphatase substrate (NBT/BCIP in TMN buffer; Sigma). At least 50 positively stained cells (foci) at an appropriate dilution were counted in each of three wells and averaged to give a titre in focus-forming units (FFU)/lung.

2.4. Assay for interferon antiviral activity

Lung samples were assayed for interferon type I activity in mouse L929 cells by challenge with Chandipura virus (CV; *Rhabdoviridae*) or Semliki Forest virus (SFV; *Togaviridae*). The readout was reduction in virus-induced cytopathology. Lungs were homogenized as above and clarified supernatant stored at -70°C. For the assay of interferon, supernatant was serially 2-fold diluted from 1/10 and added to cells for 24 hours at 37°C to allow for induction of the

antiviral state. Cells were infected next day with challenge virus and incubated at 33°C. Putative virus-inhibitory interferon activity was recorded by staining surviving cells with crystal violet, and quantitating extracted stain on an ELISA reader. Each plate contained a virus control, a cell control and an interferon type I standard. The standards were recombinant mouse type 1 α and β interferons sourced from PBL Biomedical Laboratories. The interferon was identified using the same assay in which cells were first incubated with antibody (10µg/well for 1 hour at 37°C) to the external domain of the mouse interferon type I receptor (R&D Systems Inc., #WXB01).

3. Results

3.1. A single dose of DI influenza virus 244/PR8 protects mice from lethal infection with the paramyxovirus PVM

We initially investigated the ability of a single dose of intranasally administered 244/PR8 (1.2 µg per mouse) to protect C3H/He-mg mice against an intranasal lethal challenge with PVM, given 1 day later. Control groups which received UV-inactivated, non-protective 244/PR8, followed by PVM, developed severe clinical signs and weight loss similar to those in the PVM-infected control group, and all died (Fig. 1a). In contrast, protecting virus 244/PR8 protected all animals against PVM-induced clinical disease (Fig. 1a) apart from a small reduction in weight on day 8 (Fig. 1c). The remaining control groups, treated with 244/PR8 alone, or mock-inoculated mice (not shown) displayed steady weight gain and no clinical disease (Fig. 1a, c). A 10-fold lower dose of 244/PR8 (0.12 µg) gave some protection, delayed and ameliorated disease in some mice and enabled 40% to survive with subsequent weight gain. The lowest dose tested (0.012 µg) was not protective (Fig. 1a, d). These data

demonstrate that 244/PR8 is highly effective at protecting mice from an unrelated viral infection in a dose-responsive manner, and that the active principle central to protection is abrogated by prolonged UV irradiation, as we showed earlier with an influenza A virus challenge [1]. This is the first report demonstrating the ability of an influenza virus-derived DI virus to protect from disease caused by a completely unrelated virus in vivo. Further analysis showed that multiplication of PVM in the lung was greatly reduced in infected mice treated with 244/PR8: infectivity was detected 2 days later in protected mice than in those treated with inactivated 244/PR8 or saline before PVM infection, and after 5 days PVM infectivity was reduced by 98.8% (Fig. 1b). By 9 days after infection, PVM infectivity was undetectable in the protected group. Mice in the PVM-infected control groups were all dead by day 7.

3.2. Duration of prophylaxis afforded by DI influenza virus 244/PR8 on PVM infection

Mice were treated with a single dose of 244/PR8 at 1, 3 and 7 days before challenge with a lethal dose of PVM strain J3666. With treatment at 1 day before infection there was strong protection with only mild symptoms occurring during days 7-11 and some weight loss over days 8-10 (Fig. 2a, b). Mice then recovered completely. Treatment with 244/PR8 given 3 days before infection with PVM gave significant protection with 40% of mice surviving (Fig. 2c, d). Treatment with 244/PR8 given 7 days before PVM challenge afforded no protection (data not shown). Control mice challenged with PVM alone all died.

3.3. Immune status of mice protected from PVM by a single dose of DI influenza virus 244/PR8

The detection of low levels of replication of the highly pathogenic PVM challenge raised the possibility that mice which had been successfully protected against PVM disease by treatment with 244/PR8 may have established a PVM-specific immune response. The 244/PR8-protected mice were rechallenged with intranasal PVM at 3 weeks after the first PVM infection, proved to be solidly immune and showed no clinical disease or weight loss, whereas all age-matched control mice died (data not shown). Thus 244/PR8 converts the original PVM lethal challenge into a low level infection that induces a fully protective immune response.

3.4. Therapeutic efficacy of a single dose of DI influenza virus 244/PR8 against PVM in vivo

Therapeutic efficacy of 244/PR8 against PVM infection was tested by treating with a single dose of 244/PR8 at 0, 24, 48, and 72 hours after infection (Fig. 3a, b). Inoculation of protecting virus at the same time as PVM challenge completely abrogated clinical disease and weight loss. Mice given therapy at 24 hours after PVM infection showed a mild and short-lived clinical disease and some weight loss over days 7-8, but made a 100% recovery. Therapy at 48 hours significantly reduced clinical disease and gave 50% survival. Therapy at later times was not effective.

3.5. A functional type I interferon response is required for DI influenza virus 244/PR8-mediated protection against PVM in vivo

There is no report in the literature for genetic interaction between the *Orthomyxoviridae* and *Paramyxoviridae*, and influenza protecting virus 244/PR8 does not interfere with the multiplication of PVM in vitro (data not shown). A possible mechanism for the protection in

vivo is induction of type I interferon in vivo. To investigate this we compared 244/PR8-mediated protection in mice that lacked the common interferon type I receptor (129Sv/Ev IFNα/βR$^{-/-}$) using the relevant *wt* mice (129Sv/Ev) as control. Mice were inoculated with a single dose of either active or UV-inactivated 244/PR8 followed by PVM one day later. Data show that *wt* (Fig. 4a, b) and interferon receptor knock-out mice (Fig. 4c, d) given inactivated 244/PR8 followed by PVM all became ill by day 5 and were dead by day 9. Treatment of *wt* mice with 244/PR8 delayed the onset and reduced the severity of clinical disease and weight loss. All these mice resumed weight gain and recovered completely (Fig. 4a, b). In comparison, 83% of type I interferon receptor knock-out mice treated with 244/PR8 died, although illness and weight loss were delayed by a few days (Fig. 4c, d). These data indicate that type I interferon plays a key role in 244/PR8-mediated protection against PVM infection in vivo and demonstrate for the first time that interferon induction by an influenza virus-derived DI stimulates sufficient levels of interferon in vivo to abrogate infection. However, the 4-day delay in symptom onset caused by 244/PR8 in knock-out mice also suggests that protecting virus stimulates antiviral factor(s) other than interferon type I. In contrast to the PVM scenario, 244/PR8 protected both *wt* (Fig. 4e, f) and interferon receptor knock-out mice (Fig. 4g, h) from an influenza A virus challenge (A/WSN), despite mice being treated and challenged simultaneously, giving less time for stimulation of interferon. A/WSN-infected interferon receptor knock-out mice treated with 244/PR8 showed only a trace of clinical disease and a dip in weight gain that rapidly resolved. Those given inactivated protecting virus all died (Fig. 4g, h). These data clearly show that 244/PR8 protection against influenza virus is not reliant on the interferon response.

3.6. Protecting virus 244/PR8, in the absence of challenge virus, induces type I interferon in the lungs of intranasally inoculated mice

Mice were inoculated intranasally with 12 μg of active 244/PR8 or UV-inactivated 244/PR8. None received any infectious challenge virus. After 1 day, lungs were processed for an assay that measures the ability of interferon type I to protect L929 cells from a cytopathic virus challenge. Lung extracts of mice inoculated with active 244/PR8 were positive for putative interferon activity (Table 1). There was no evidence of antiviral activity in mice inoculated with 1.2 μg 244/PR8 (data not shown), or with UV inactivated 244/PR8. In order to determine the duration of the antiviral activity mice were inoculated intranasally with active 244/PR8 or UV inactivated 244/PR8 (12 μg per mouse) and lungs were removed 1, 2 and 4 days later. Again no challenge virus was given. There was clear putative type I interferon activity in lung extracts taken on day 1 after inoculation, lower activity on day 2, and none on day 4 (≤1/10) (Table 2). Again no detectable antiviral activity was stimulated by inactivated 244/PR8 (data not shown).

All type I α-interferons and β-interferon share the same cell surface transmembrane protein receptor, and interferon activity can be identified by blocking the receptor with antibody specific for the external binding domain. Using the assay for antiviral activity described above, we incubated L929 cells with antibody specific for mouse interferon type I α/β receptor. A lung extract containing previously identified antiviral activity stimulated in mice by administration of 244/PR8 (12 μg) was then added to the cells, and incubated overnight to induce an antiviral state. Challenge virus was then added. Each plate contained a virus control, cell control and a positive interferon control. Fig. 5 shows that 244/PR8-induced antiviral activity in the lung completely inhibited the extensive cytopathology caused by the challenge virus. However, when antibody to the interferon type I α/β receptor was present the antiviral activity was inhibited, confirming that protection was due to type I interferon. Virus and cell controls without antibody performed as expected.

4. Discussion

The conventional view of the mechanism of action of defective interfering viruses is that they act against genetically compatible viruses by competing at the level of genome replication. While that appears to be true in vitro, the data presented here show that this is not the full explanation for the mode of action of protection by DI viruses.

Here we have shown that a DI influenza A virus, 244/PR8, can stimulate type I interferon in vivo in mice. We have also demonstrated for the first time that DI 244/PR8 virus, can induce sufficient levels of interferon to confer protection in vivo against infection with an unrelated paramyxovirus, PVM. Further, treatment and challenge of mice lacking the type I interferon receptor (and thus an interferon response) showed that 244/PR8-mediated protection against PVM requires a functional interferon system. This is in marked contrast to the situation where wild type mice and mice lacking the type I interferon receptor were challenged with influenza A virus, and both were completely protected. Our data demonstrate that the mechanism of protection from homologous and unrelated viruses differs, and that interferon type I is an essential component only of protection against genetically unrelated viruses. Consistent with this, 244/PR8 did not interfere with PVM multiplication in vitro (data not shown). A further difference is that homologous protection also lasts longer, with a single dose providing protection for up to 6 weeks [1].

244/PR8 given 1 day before infection protects more efficiently from PVM-induced disease than when given simultaneously with PVM, which is consistent with the requirement for induction of interferon and the anti-viral state (data not shown), the duration of 244/PR8-mediated protection against PVM, and effective life of 244/PR8-induced interferon in the lungs were similar (approximately 2-3 days). The fact that 10-fold less intranasal 244/PR8

protects mice but does not induce detectable interferon suggests that the in vitro assay is a less efficient measure of antiviral activity than protection in vivo and that the local concentration of interferon at the site of infection is sufficiently high to provide protection. A striking feature of the pathogenesis of the PVM infection in interferon receptor knockout mice was a consistent 4-day delay in the onset of disease, which suggests that the protecting 244/PR8 is also stimulating additional unknown factor(s) that contribute to protection (Fig. 4c, d).

In support of the PVM data we found that 244/PR8 also protects against the genetically unrelated influenza B virus in vivo through stimulation of type I interferon (Fig. 6 and unpublished data). Thus a single dose of 244/PR8 protects in vivo from infection with two genetically unrelated viruses by stimulating interferon type I, and possibly other elements of innate immunity. Direct administration of type I interferon α has been shown to protect mice against a lethal challenge with influenza A virus [39, 40]. Protection against disease has also been demonstrated in ferrets treated with multiple doses of interferon α administered before and during infection with seasonal human influenza viruses but not with highly pathogenic H5N1 avian influenza [41]. With the seasonal influenza strains, virus titres in the lung were not altered by the treatment. A recent human trial of intranasal treatment with low doses of interferon α2B showed evidence of protection against infection caused by influenza A virus, influenza B virus, parainfluenza viruses 1-3 and species B adenovirus but there was no effect on respiratory syncytial virus infection [42].

Despite the significant protection afforded against PVM disease by 244/PR8, replication of PVM was not completely eliminated. There was evidently sufficient replication to induce a protective immune response to rechallenge with PVM. Thus, in effect, DI virus treatment converts the lethal PVM disease into an avirulent, immunizing infection.

The ability of interferon type I to protect mice from PVM is not surprising as type I interferons, defined by their use of a common cell surface receptor, are active in combatting

virus infections [43-45]. While stimulation of interferon type I by DI viruses has been reported to date it has been seen only in cultured cells with the double-stranded copyback and snapback DI RNAs of vesicular stomatitis virus and Sendai virus [27-31]. No animal protection studies have been published with copyback and snapback DI viruses, and the role that their double stranded DI RNAs and interferon may play in ameliorating disease is not known. It is important to note that influenza DI RNAs do not possess large regions of double-stranded sequence, and influenza viruses do not produce copyback or snapback DI RNAs that would be anticipated to induce high levels of interferon.

Which of the components of the 244/PR8 particle is responsible for the interferon induction can only be surmised at present. UV-inactivated 244/PR8 retains virion structure, haemagglutination and neuraminidase activities, and does not protect mice or stimulate interferon, suggesting that none of these factors are involved. We are currently investigating the ability of other DI preparations to assess whether the level of interferon induction correlates with protection from infection. The 244 RNA has approximately 80% of the central coding region of virion RNA segment 1 deleted and is unable to make PB2, an essential protein component of the viral RNA-dependent RNA polymerase. Hence, there can be no *de novo* production of negative sense 244 RNA or any of the full-length RNAs present in the 244/PR8 virion in the absence of helper virus.

In conclusion, we have shown that the influenza virus-derived, molecularly cloned 244/PR8 is capable of inducing type I interferon in vivo and that this can protect against disease from a genetically unrelated virus. However, interferon induction is not required for protection against a homologous influenza A virus infection. The protection afforded by 244/PR8 has clinical benefit against a lethal infection within a window of 3 days before infection to 2 days after infection. Its advantages as an antiviral are its efficacy, single dosage, low amount of material, immediate effect, and protection regardless of antigenicity of the infecting virus. DI

244/PR8 may therefore be of benefit in the prophylaxis and treatment of other respiratory viruses that are sensitive to type I interferon, in addition to providing protection from type A influenza viruses. These observations demonstrate that DI RNAs may provide an additional and novel approach to antiviral therapy with little opportunity for the development of resistance in the target viruses.

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Figure legends

Fig. 1. A single dose of influenza A 244/PR8 protects mice from PVM infection. C3H/He-mg mice were anaesthetized and inoculated intranasally with 244/PR8 or inactivated 244/PR8 (day -1, solid arrow), and with PVM challenge virus (day 0, open arrow). Panel (a) shows the clinical score, (b) PVM lung infectivity, and (c, d) percentage group weight change. The clinical score is the averaged clinical evaluation for an experimental group as described in Methods. In panel (a) the 244/PR8 alone control has a score of 1 and is hidden under the solid square symbols. In panels (a) and (d) the inactivated 244/PR8 + PVM group followed the same clinical course as a PVM control, and a control group given diluent only exhibited no adverse affect (not shown). In panels (c) and (d) group weights are presented as a percentage weight change normalised to the group weight on day 0 and are recorded up to the first death. In panel (b) error bars show the standard deviation from the mean of PVM lung infectivity; those not apparent fall within the symbol. Arrows indicate that infectivity was not detected. No infectivity was detected in mice given 244/PR8 alone. ■, 1.2 μg 244/PR8 + PVM; ~, 0.12 μg 244/PR8 + PVM; -, 0.012 μg 244/PR8 + PVM; ▲, 1.2 μg inactivated 244/PR8 + PVM; ♦, PVM alone; ●, 1.2 μg 244/PR8 alone. There were 5 mice per infected group. The percentage of mice surviving each treatment is in parenthesis. Data are representative of two experiments.

Fig. 2. Duration of protection afforded by influenza A 244/PR8 against PVM infection. C3H/He-mg mice were inoculated with 244/PR8 (solid arrow) 1 day (a, b) or 3 days (c, d) before challenge with PVM (open arrow). Mice received 1.2 μg 244/PR8. (a, c), clinical score, (b, d) percentage weight change. ■, PVM + 244/PR8; ♦, PVM alone; ●, 244/PR8 alone. Data are representative of two experiments.

Fig. 3. Therapeutic efficacy of influenza A 244/PR8 against PVM infection. C3H/He-mg mice were infected with PVM at time 0 (open arrow). A single dose of 244/PR8 (1.2 µg) was administered at 0, 1, 2 or 3 days after infection (solid arrows) with PVM. Panel (a) shows the clinical score, and panel (b) the percentage weight change. ■, PVM + 244/PR8 at time 0; ♠, PVM + 244/PR8 at 24 h after infection; ♦, PVM + 244/PR8 at 48 h after infection; ◊, PVM + 244/PR8 at 72 h after infection; ~, 244/PR8 only at time 0. Control groups given diluent exhibited no weight loss or adverse clinical signs (not shown). Other information as in Fig. 1.

Fig. 4. Mice lacking the type I interferon receptor were protected poorly by influenza A 244/PR8 against PVM infection, but protected strongly by 244/PR8 against influenza A/WSN infection. PVM data are shown in panels (a) to (d), and A/WSN data in panels (e) to (h); 129Sv/Ev wild type mice: panels (a), (b), (e) and (f), and knock-out 129Sv/Ev IFNα/β$R^{-/-}$ mice: panels (c), (d), (g) and (h). Mice were treated with 1.2 µg of either 244/PR8 or UV inactivated 244/PR8 (solid arrow) 1 day before challenge with PVM or simultaneously with A/WSN (both open arrows). All inoculations were intranasal. Scores for clinical disease are shown in panels (a), (c), (e) and (g) and for percentage weight change in panels (b), (d), (f) and (h). ■, 244/PR8 + PVM or A/WSN; ▲, inactivated 244/PR8 + PVM or A/WSN; ♦, PVM or A/WSN alone; ●, 244/PR8 alone. A control group given only diluent had no adverse clinical signs or weight loss (not shown). Diluent alone gave a similar result to 244/PR8, and A/WSN alone gave a similar result to A/WSN + inactivated 244/PR8 (not shown). Other information as in Fig. 1.

Fig. 5. Identification of the virus-inhibiting activity stimulated in mice by 244/PR8 as interferon type I. L929 cell monolayers were incubated with antibody (Ab, 10µg/well) to the

mouse interferon type I-α/β receptor for 1 hour at 37°C, and then with the appropriate lung tissue extract (1/10 dilution) or an interferon-α standard preparation (10 units/well) for 24 hours. Cells were then inoculated with Chandipura virus (CV) and incubated for a 48 hours at 33°C. Survival of cells was determined by staining with crystal violet and measuring absorbance at 590 nm. Each error bar represents the standard deviation from the mean.

Fig. 6. Summary of heterologous and homologous protection exerted by influenza A virus 244/PR8.