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Influenza virus carrying neuraminidase with reduced sensitivity to oseltamivir carboxylate has altered properties in vitro and is compromised for infectivity and replicative ability in vivo

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Abstract

Oseltamivir phosphate (Tamiflu, Ro 64-0796) is the first orally administered neuraminidase (NA) inhibitor approved for use in treatment and prevention of influenza virus infection in man. Oseltamivir phosphate is the pro-drug of the active metabolite oseltamivir carboxylate (Ro 64-0802). Extensive monitoring throughout the oseltamivir development programme has identified a very low incidence of patients who have carried drug-resistant virus. The predominant mutation seen is the substitution of arginine for lysine at position 292 of the viral NA. The fitness of clinically isolated influenza virus A/Sydney/5/97 (H3N2) carrying this mutation was markedly reduced in animal models of influenza virus infection. The infectivity and replicative abilities of R292K mutant virus were reduced by at least 2 logs in a mouse model of influenza infection and by 2 and 4 logs, respectively, in the ferret model. Pathogenicity of R292K influenza virus A/Sydney/5/97 was reduced in ferrets as measured by inflammatory and febrile responses at least in parallel to the decrease in replicative ability. The data indicate that the R292K NA mutation compromises viral fitness such that virus carrying this mutation is unlikely to be of significant clinical consequence in man.

Keywords: Tamiflu; Oseltamivir phosphate; Influenza; Neuraminidase; Resistance

1. Introduction

In most years epidemics of influenza cause a significant increase in morbidity and mortality world-wide (Hughes, 2000). The neuraminidase

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(NA) inhibitors zanamivir (Relenza) and oseltamivir phosphate (Tamiflu) are a new class of antiviral agent licensed for use in treatment of influenza virus infection. Oseltamivir is also approved in the USA for the prevention of influenza virus infection in adults, and for the treatment of influenza illness in children. NA inhibitors are potent and selective drugs that inhibit influenza NA, an enzyme that promotes the release of influenza virus from infected cells and facilitates virus spread within the respiratory tract. Oseltamivir phosphate, unlike zanamivir, which is administered by inhalation, has antiviral activity following oral administration, which makes this drug easier to use in the patient groups most at risk i.e. very young and frail elderly patients.

As for all antiviral agents, there exists the potential during treatment with oseltamivir phosphate for the emergence of virus with decreased sensitivity to the treatment drug. Drug resistance is known to be pertinent to influenza virus through previous clinical experience with amantadine and rimantadine, for which the risk of resistance emergence to these drugs is high and estimated at between 25 and 38% (Hayden, 1996; Daly et al., 2000). Emergence of resistant virus with use of these drugs is rapid, often within 48 h of start of treatment, and virus remains fully functional, transmissible and pathogenic (Hayden, 1996).

In contrast, emergence of resistance to oseltamivir carboxylate through mutations in the NA would be disadvantageous to the virus and hence self-limited in incidence.

In vitro studies have shown that influenza virus with decreased sensitivity to oseltamivir carboxylate due to mutations in the viral NA was only generated with difficulty. Many more passages of virus in increasing drug concentrations were required to generate oseltamivir carboxylate resistant NA than were required to generate M2 mutations resistant to amantadine/rimantadine (Tai et al., 1998).

The most common mutation in the NA gene selected by exposure to NA inhibitors in vitro was an amino acid substitution of a lysine (K) instead of a conserved arginine (R) at position 292, in influenza N2 NA. The R292K mutation has been selected by oseltamivir carboxylate, zanamivir and BCX 1812, a NA inhibitor currently in clinical development (Tai et al., 1998; Gubareva et al., 1997; Bantia et al., 2000). An H274Y mutation in N1 has also been selected in vitro by oseltamivir carboxylate (Wang et al., 2000) and in vivo in an H1N1 influenza virus challenge study with oseltamivir phosphate in healthy volunteers (Gubareva et al., 2001). E119V is a novel mutation that arose clinically during oseltamivir phosphate treatment in just two patients infected with influenza A virus of the H3N2 subtype (Covington et al., 2000; Whitley et al., 2001), and had not previously been seen in vitro. No NA mutations giving rise to resistance have yet been generated by oseltamivir in influenza B NA in vitro or in vivo, although there is some evidence that zanamivir in vivo has induced development of a resistant mutation in influenza B NA in an immunocompromised child (Gubareva et al., 1998).

R292K NA influenza viruses have been studied in vitro and in vivo (Tai et al., 1998; Gubareva et al., 1997), and were shown to be resistant to inhibitor. Catalytic activity of the NA was decreased to about 2% of that of wild-type. The R292K NA mutation caused the virus to fail to grow in eggs and caused a reduction in plaque size and virus yield when grown on MDCK cells.

This mutation had previously been reported in an A/N2 background in a mutant virus with zanamivir (Gubareva et al., 1997) and in an A/N9 background in the presence of a zanamivir derivative which forms hydrophobic interactions in the neuraminidase enzyme active site similar to those of the 3-pentyloxy group of oseltamivir carboxylate (McKimm-Breschkin et al., 1998). These mutant viruses were tested for virulence and both were at least 1000-fold less infectious in mice than the parent virus from which they were derived. Thus the R292K NA mutation has been shown by independent groups to severely compromise the virus, although the degree of loss of infectivity may depending upon the background of the virus (Tai et al., 1998). Current data on resistance emergence compiled from NA inhibitor clinical trials are reassuring, and predict that the frequency of resistance emergence with NA inhibitor usage will be much lower than that resulting from use of the older anti-influenza agents (Jackson et al., 2000). In particular, the characteristics of

emerging influenza viruses resistant to oseltamivir carboxylate during the treatment and prophylaxis of adults and the treatment of children with naturally acquired infection have been studied extensively (Carr et al., 2000; Ives et al., 2000a,b). The incidence of resistant virus has been shown to be low, and resistant virus where it does occur is carried only transiently, arising late in infection and being cleared normally (Jackson et al., 2000; Whitley et al., 2001). In one clinical study in which 385 patients were treated for naturally acquired influenza infection (Treanor et al., 2000) only one patient was found to shed virus which carried NA with reduced sensitivity to oseltamivir carboxylate (Covington et al., 2000). This patient received 75 mg/bid oseltamivir phosphate. The virus isolated from a post-treatment nasal swab carried NA with the R292K mutation. Here we describe the characterisation in vitro and in vivo of this R292K NA influenza virus which arose in the clinical setting as a consequence of oseltamivir treatment of naturally acquired influenza infection.

2. Materials and methods

2.1. Identification and isolation of virus with reduced sensitivity to oseltamivir carboxylate

An A/Sydney/5/97-like influenza virus (described hereafter as A/Sydney/5/97) which had been identified as carrying NA with reduced sensitivity to oseltamivir carboxylate was isolated from a patient on the active treatment arm of a clinical study of oseltamivir phosphate efficacy. The viral NA in the expanded sample derived from the pre-treatment nasal swab from this patient retained wild-type sensitivity to oseltamivir carboxylate, whereas the last virus-positive sample post-treatment (day 4) gave an IC_{50} value indicative of a reduced sensitivity of enzyme to active drug. Genotypic analysis of the NA sequences from these pre- and post-treatment isolates revealed that the post-treatment isolate carried R292K NA. Genotypic analysis of virus HA1 sequence confirmed there were no changes in the viral HA1, between pre- and post-treatment virus in the swab samples. Two further rounds of expansion and plaque purification of the pre- and post-treatment isolates were carried out on MDCK cells to produce stocks of virus for further characterisation. The pre- and post-treatment stocks were checked for NA gene sequence and were confirmed as wild-type and R292K, respectively, following the expansion steps. The MDCK cells used were originally obtained from Dr Alan Hay at the National Institute of Medical Research (London, UK) and passaged in Dr Frederick Hayden's Laboratory at the University of Virginia Health Sciences Centre, using standard laboratory procedures prior to distribution to Roche Discovery Welwyn.

2.2. Influenza virus NA gene sequence analysis

A novel method for the sequencing of NA genes (residues 100 to the C terminus) was developed and carried out by Professional Genetics Laboratory, Sweden. In brief, the methods used were: viral RNA isolation from ferret nasal wash followed by RT-PCR to obtain cDNA. The NA gene is then amplified by nested PCR as two overlapping fragments, using a specific primer set designed for the NA subtype N2. The DNA-sequencing reactions use T7 DNA-polymerase and solid phase technique in a Pharmacia ALF Express autosequencer.

2.3. Characterisation of influenza virus NA enzyme properties

IC_{50}, K_m and K_i values for influenza virus NA from patients were derived according to a modification of a previously described method (Potier et al., 1979) using 2′-(4-methylumbelliferyl)-D-*N*-acetyl-neuraminic acid (MUNANA) as substrate. The rationale for using enzyme sensitivity rather than antiviral sensitivity to test phenotypically for emergence of resistance is published elsewhere (Zambon and Hayden, 2001).

2.4. Virus growth in MDCK cells

Both pre- and post-treatment isolates at equivalent particle number as determined by HA reactiv-

ity with human erythrocytes ($\log_{10}$ $TCID_{50}$ of 3.3 and 3.6, respectively) were used to infect confluent MDCK cells in a 96-well format. Briefly, the cells were rinsed free of culture media containing 10% FCS with PBS, and the culture media was replaced with trypsin-containing media to support virus replication (MEM containing 25 mM Hepes, 200 mM penicillin/streptomycin and 1.25 μg/ml TPCK trypsin). Serial 10-fold dilutions were made from each well containing virus across the plate, and adsorption took place over a 2 h incubation at 34 °C/5% CO_2. Excess virus was removed from the plate and fresh media was reapplied. The plates were incubated for 10 days at 34 °C/5% CO_2, and aliquots of the culture supernatants were tested for HA positivity daily. Virus titres for each time point were calculated using the Spearman–Karbar equation.

2.5. Sensitivity of R292K NA influenza virus to oseltamivir carboxylate in vitro

Oseltamivir carboxylate (concentration ranging from 100 μM to 3 nM) was applied to confluent MDCK cells in quadruplicate (96-well format) for 1 h prior to infection of the cell layer with either influenza virus A/Sydney/5/97 carrying 292R NA (wild-type) or A/Sydney/5/97 carrying 292K NA (mutant), at $10 \times 50\%$ tissue culture infectious dose ($TCID_{50}$). Cell cultures were incubated with virus inoculum for 2 h at 34 °C after which time the inoculum was removed and replaced with fresh media containing oseltamivir carboxylate. Infected cultures were incubated for a further 7 days and virus growth assayed after this time by HA positivity. IC_{50} values for oseltamivir carboxylate against both wild-type and mutant influenza virus A/Sydney/5/97 were determined.

2.6. Infectivity of R292K NA influenza virus in mice

Influenza virus A/Sydney/5/97 carrying either wild-type or mutant NA were used to infect female pathogen free BALB/c mice 6–8 weeks in age (supplied by Charles River). Groups of mice ($n = 3$) were infected intranasally under light anaesthesia (isofluorane) with a challenge dose based on range equal virus particle numbers, with the highest challenge dose given equating to 2.7 $\log_{10}$ $TCID_{50}$/ml per mouse for wild-type virus, and to 3.5 $\log_{10}$ $TCID_{50}$/ml per mouse for mutant virus. Lungs were removed from the mice on day 3 post-infection, and clarified lung homogenates were assayed on MDCK cells for the presence of infectious virus. The method for determining virus titres was exactly as described for virus growth in MDCK cells (described in Section 2) with the substitution of lung homogenate for virus stock as inoculation material.

2.7. Infectivity of R292K NA influenza virus in ferrets

Infectivity and pathogenicity of influenza virus A/Sydney/5/97 carrying the R292K NA was assessed in ferrets. Female ferrets approximately one year of age were obtained from Foxfield Farms UK Ltd. A range of infectious doses of influenza virus carrying either wild-type or mutant NA were used to infect groups of ferrets ($n = 4$). Serial dilutions ranging from 10-1 down to 10-4 were prepared from stocks of wild-type and mutant virus ($\log_{10}$ $TCID_{50}$ of 3.3 and 3.6, respectively), and 0.5 ml were applied to the nasal cavity of the anaesthetised ferret. The relative infectivities of wild-type and mutant virus were compared by titration on MDCK cells of infectious virus recoverable from nasal wash samples taken daily from days 1 through 6 post-infection. The method for determining virus titres from ferret nasal wash was exactly as described for virus growth in MDCK cells (described in the Section 2) with the substitution of nasal wash for virus stock as inoculation material.

The relative pathogenicities of wild-type and mutant virus were compared by measurement of the following parameters: inflammatory cell counts in nasal washes made on each day post-infection, and rectal temperature recorded daily post-infection.

Table 1
Comparison of NA enzyme properties from wild-type influenza virus and from R292K NA influenza viruses selected by oseltamivir carboxylate in vivo and in vitro

		K_m (μM)	K_i (nM)
Clinical isolate A/Sydney/5/97	Wild type	23.5	0.44
	Mutant	41	4300
In vitro selected virus A/Victoria/3/75[a]	Wild type	84	0.13
	Mutant	337	3600

[a] Data from Tai et al., 1998.

3. Results

3.1. Comparison of wild-type and R292K NA in vitro

The effects of the clinically selected R292K mutation on the properties of the NA enzyme were consistent with those previously reported for NA carrying the same mutation derived by in vitro selection (Table 1). The R292K mutation in A/Sydney/5/97 NA reduced NA affinity for synthetic substrate by approximately the same degree to that reported for the mutated enzyme of A/Victoria/3/75 generated by in vitro selection (2- and 4-fold reductions, respectively). The R292K mutation selected in vivo also effected an approximate 10 000-fold reduction in the affinity of NA for oseltamivir carboxylate, which was less than, but of the same order as the approximate 30 000-fold reduction in inhibitor affinity reported for the enzyme selected in vitro.

3.2. Comparison of virus carrying wild-type or R292K mutated NA in vitro

The R292K mutation did not appear to impair the replicative ability of clinically selected virus in MDCK cells since the growth curves for both wild-type virus and mutant virus are closely similar (Fig. 1). This finding was in contrast to a previously reported reduction in replication in MDCK cells for R292K mutant virus selected by oseltamivir in vitro (Tai et al., 1998), but consistent with the growth characteristics of zanamivir-selected R292K in an H4N2 influenza virus (Gubareva et al., 1997). The tendency for influenza virus culture in MDCK cells to lead to artefactual virus adaptations is reported elsewhere (Gubareva et al., 1998; Tisdale, 2000; McKimm-Breschkin, 2000; Zambon and Hayden, 2001) and, therefore, virus replication in MDCK cells does not closely represent replication in human or animal respiratory epithelial cells.

The effect of the R292K mutation was to reduce whole virus sensitivity to oseltamivir carboxylate in vitro by 1000-fold. Oseltamivir carboxylate had IC_{50} values of 100 nM and 100 μM against wild-type and mutant viruses, respectively. The reduction in sensitivity of virus to inhibition by drug caused by the R292K mutation parallels, but is less than the reduction (around 3500-fold from IC_{50} values) in sensitivity of the isolated NA enzyme to inhibition by drug. However, drug-sensitivity data derived from antiviral assay in MDCK cell culture is difficult to interpret since it is becoming increasingly apparent antiviral sensitivity of influenza virus to NA inhibitors in cell culture may not reflect NA sensi-

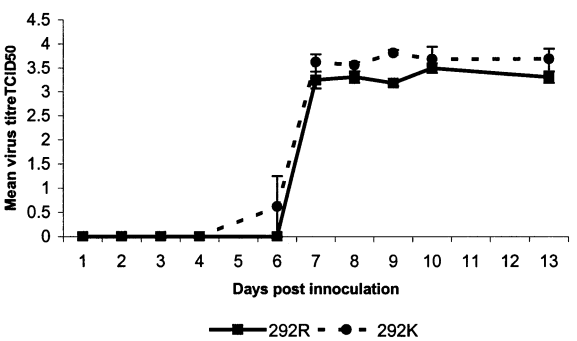

Fig. 1. The growth profiles of wild-type and R292K influenza virus A/Sydney/5/97 in vitro. Mean virus titres were calculated from four separate $TCID_{50}$ determinations per time point for each virus.

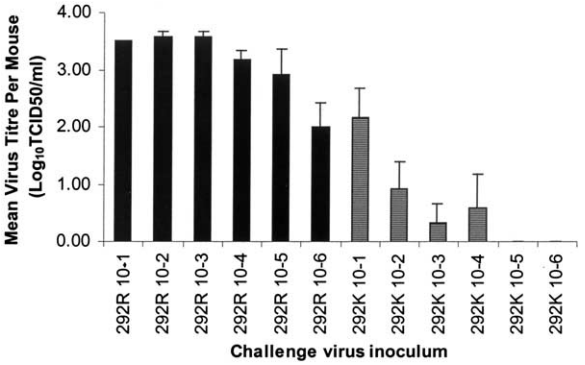

Fig. 2. Mean virus titre recovered from the lungs of wild-type (292R) or mutant (292 K) influenza virus A/N2 infected mice ($n = 3$).

tivity. This is due to the functional interplay between HA and NA and the artefactual adaptations induced by the sub-optimal nature of virus binding to MDCK cell entry receptors.

3.3. Comparison of influenza virus carrying wild-type or R292K NA in mice

R292K NA A/Sydney/5/97 had an approximate 2 log reduction in infectivity in mice (Fig. 2). Additionally, the titres of virus recovered from lungs of mice infected with virus carrying mutant NA were reduced by between 1 and 3 logs on day 3 post-infection, in comparison to the titres of virus recovered from mice infected with wild-type virus, at equivalent challenge doses. Virus was recovered from the lungs of all three mice in each challenge dose group of wild-type A/Sydney/5/97 virus infected mice. Virus was recovered from only 2/3 mice infected with R292K A/Sydney/5/97 virus at the two highest challenge doses and was recovered from just 1/3 mice at challenge doses at dilutions of 10^{-3} and 10^{-4}. No virus was recovered from the lungs of mice infected with the two lowest challenge doses of R292K A/Sydney/5/97 virus. It was not possible to recover viral RNA from frozen lung homogenate samples sufficient to determine the neuraminidase gene sequence by PCR.

3.4. Comparison of influenza virus carrying wild-type or R292K NA in ferrets

3.4.1. Virological analysis of ferret nasal washes

At the highest challenge dose (10^{-1}), wild-type virus was recovered from ferret nasal washes at higher titre than was recovered from ferrets infected with R292K influenza virus (Fig. 3). The difference in titre recovered between wild-type and R292K virus infected animals was even more marked at lower challenge doses (10^{-2} and 10^{-3}). At the lowest challenge dose of 10^{-4}, neither wild-type nor R292K virus were recovered in any significant quantity. Therefore, the replicative ability of mutant virus in the ferret respiratory tract was reduced compared with that of the wild-type counterpart. For challenge doses of 10^{-1} through 10^{-3}, significantly lower titres of virus were recovered from nasal wash samples taken from ferrets infected with R292K virus compared with the titres recovered from ferrets infected with wild-type virus (AUC compared by Student's t-test).

The infectivity of R292K virus in ferrets is reduced by between 2 and 3 logs in comparison to wild-type virus. Wild-type virus was recovered in high titre from ferrets infected with a challenge dose of 10^{-3}, yet none was recovered from the ferrets infected with the next lower challenge dose of 10^{-4}. Thus it can be assumed that the wild-type virus recovery profile most similar to the best R292K virus recovery profile (from ferrets infected with a challenge dose of 10^{-1}) lies between the challenge doses of 10^{-3} and 10^{-4}.

NA sequence analysis of the day 6 samples from ferrets infected with both wild-type and R292K virus at a challenge dose of 10^{-3} confirmed that both wild-type and mutant genotypes were stable throughout the 6 day passage in ferrets. Only one from four ferrets infected with R292K virus at this challenge dose yielded infectious virus on day 6, and sample from this same animal proved to be the only NA positive sample in the NA sequencing assay. The virus contained in this single positive sample carried a NA gene that had the lysine for arginine substitution as the only species at position 292. The corresponding samples from ferrets infected with an equivalent

challenge dose of wild-type virus were all culture positive for infectious virus, and consistent with this were also positive by NA detection, and had retained arginine at position 292 on the NA gene.

The lowest challenge dose given, in the case of both wild-type and R292K influenza virus A/Sydney/5/97 was insufficient to permit virus recovery from nasal washes at any time post-infection.

3.4.2. Inflammatory cell count analysis in ferret nasal washes

The inflammatory response induced by R292K influenza virus A/Sydney/5/97 was significantly reduced compared with that induced by the wild-type virus, at the two highest challenge doses ($P < 0.05$ compared by Student's t-test) (Fig. 4). At the lower challenge doses, there were no differ-

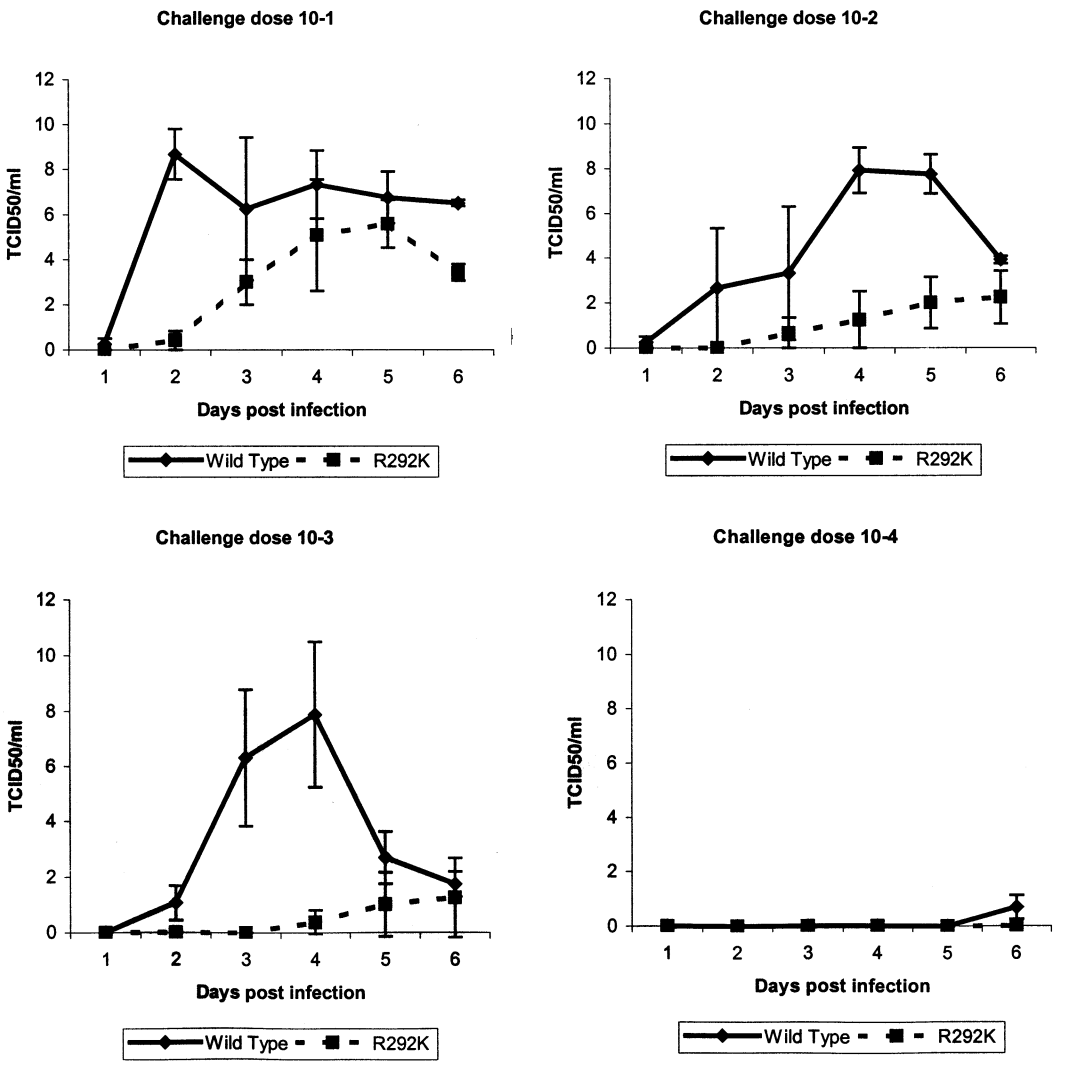

Fig. 3. Mean virus titres recovered from the nasal washes of ferrets infected with different dilutions of influenza A/Sydney/5/97 virus carrying either wild-type or R292K NA. The mean titres were calculated from groups of three infected ferrets for the two highest challenge doses, and from groups of four ferrets for the remaining four challenge doses.

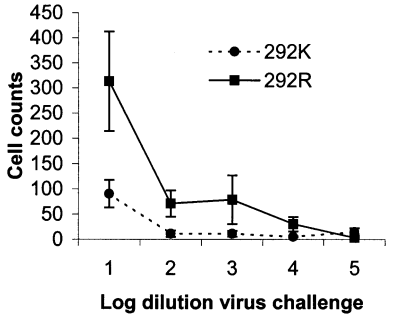

Fig. 4. The induction of an inflammatory response, as measured by inflammatory cell counts in nasal wash samples measured over the duration of infection, by wild-type and R292K influenza virus A/Sydney/5/97.

ences in the inflammatory responses elicited by the two viruses. The profile of inflammatory cell counts in nasal wash samples, therefore, correlated with virus titres recovered from the samples (Fig. 3).

3.4.3. Febrile response analysis in ferrets

R292K influenza virus A/Sydney/5/97 induced a significantly lower febrile response than did wild-type virus, at the lower challenge doses ($P < 0.01$ compared by Student's *t*-test) (Fig. 5). At the two highest challenge doses of virus (10^{-1} and 10^{-2}), there was no significant difference in the body temperatures between ferrets infected with R292K or wild-type virus. The differences between the febrile responses that occurred at the lower end of the range of challenge doses may be attributable to

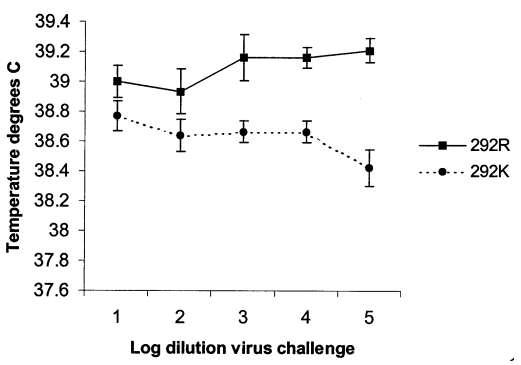

Fig. 5. The febrile response induced in ferrets by infection with wild-type and R292K influenza virus A/Sydney/5/97, over the duration of infection.

the mutant virus having lost the ability to effect a febrile response at the low challenge doses, whilst the wild-type virus had not.

4. Discussion

The introduction of a new antiviral treatment for influenza illness carries with it the risk of generating resistant virus, which may retain pathogenicity and transmissability and may, therefore, with time, become the predominant circulating strain. An extensive assessment of the likelihood and consequence of emergence of resistant influenza viruses was incorporated as an integral part of the development programme for oseltamivir phosphate. The findings presented here provide reassurance that the most commonly occurring oseltamivir-resistant virus, carrying R292K NA, is severely compromised in infectivity, and pathogenicity is reduced at least in parallel with the reduced infectivity. Influenza virus with R292K NA is, therefore, unlikely to be transmitted in man. Here we have described the characterisation of virus carrying an R292K NA gene, which arose as a consequence of oseltamivir treatment of naturally acquired influenza infection. This approach was considered to provide the most relevant data with which to predict the likely outcome of occurrence of R292K NA virus in man.

The overall fitness (infectivity, replicative ability and pathogenicity) of the virus carrying the R292K mutation in the neuraminidase gene was reduced in both mouse and ferret models of influenza infection. This finding was consistent with the reduction in fitness in vivo reported for a laboratory strain of influenza virus with the same mutation selected with oseltamivir carboxylate in vitro (Tai et al., 1998), and was also consistent with previously reported attenuation of virus carrying this mutation, selected by zanamivir in vitro (Gubareva et al., 1997). Arginine at position 292 in the NA active site is highly conserved across all viral and bacterial NAs, and participates in the conformational change of sialic acid moiety necessary for substrate catalysis (Colman, 1994). Therefore, the reduced fitness of R292K virus can be attributed to this single amino acid substitution

impacting the essential function of neuraminidase in the virus lifecycle.

The compromised nature of R292K NA influenza virus means that this virus is unlikely to be transmitted in a clinical setting. We have conducted separate studies reported elsewhere in which this same influenza virus carrying R292K mutation failed to be transmitted from infected to naïve ferrets under conditions in which wild-type virus was transmitted readily (Carr et al., 2001).

As more data emerge from clinical experience of treatment of patients with oseltamivir phosphate, an important correlation between the features of influenza virus drug resistance in vitro and in man has been found. The in vitro experiments preceding clinical assessment of resistance were good predictors of eventual resistance patterns in man. Particularly, the difficulty experienced in generation of resistance mutations in vitro was predictive for the low incidence ultimately recorded in patient trials. Specifically, R292K was the most commonly selected mutation in N2 in vitro, and this was the predominant genotypic change seen clinically, in the few patients that carried resistant virus. Similarly, the subtype specificity of resistance mutations described in the early in vitro studies proved to hold true in the clinic, since R292K only arose in the N2 subtype in vitro and the few patients on oseltamivir treatment found to be carrying influenza virus with R292K were all infected with influenza virus H3N2 as the only species. Furthermore, R292K in the viral NA has been selected in vitro without selection of a predisposing HA mutation, and the clinical isolate described here also carried R292K NA independently of any predisposing HA changes. Therefore, there is no evidence that NA mutation arising in a clinical setting will follow on from a preceding HA mutation. Finally, the compromised nature of the virus with R292K NA described previously for in vitro selected isolates and in this paper for a clinically derived isolate, is consistent with the clinical course of influenza illness in the patients found to carry R292K influenza virus. These patients' symptom scores were generally indistinguishable from those with wild-type virus and the patients continue to recover normally, following emergence of resistant virus at time points late in infection. Thus there was no evidence for enhanced pathogenicity of the resistant viruses in humans, and no disease consequence to the patient of viral R292K NA mutation occurring during the course of infection (Jackson et al., 2000; Whitley et al., 2001).

The other NA mutations that have arisen with oseltamivir treatment of naturally acquired influenza infection, again with low frequency, are H274Y (one patient) and E119V (two patients) (Whitley et al., 2001; Covington et al., 2000). As for patients carrying R292K virus, there was no change in symptom score and recovery for the patients where H274Y and E119V occurred in the viral neuraminidase. Similar in vitro and in vivo characterisation experiments to those reported here, using the actual clinical isolates carrying these NA mutations, have determined that influenza virus carrying H274Y and E119V NA are also compromised in infectivity and pathogenicity (Carr et al., 2000; Ives et al., 2000b). Further support for the view that the oseltamivir carboxylate resistant viruses are of no consequence clinically and will likely not be transmitted in man, comes from a mathematical model developed to predict the incidence and transmission of wild type and drug resistant influenza virus during community use of oseltamivir phosphate (Ferguson and Mallett, 2001).

Overall, the compromised nature of all three NA mutated viruses combined with the low frequency with which these viruses arise provides evidence that generation of resistance will not limit the clinical usefulness of oseltamivir carboxylate in the treatment and prevention of influenza infection.

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