J. Hyg., Camb. (1977), **79**, 203
Printed in Great Britain

Protective efficacy of RIT 4025, a live attenuated influenza vaccine strain, and evaluation of heterotypic immunity to influenza A viruses in ferrets*

By A. DELEM

Virology Department, Recherche et Industrie Therapeutiques (RIT),
89 rue de l'Institut, B-1330 Rixensart, Belgium

(*Received* 10 *January* 1977)

SUMMARY

Ferrets immunized with an H3N2 recombinant of A/PR/8/34 and A/Scotland/840/74 (RIT 4025 vaccine strain) were almost completely protected against a challenge with the homologous strain A/Scotland/840/74. The protection was lower but highly significant when the challenge was performed with the heterologous A/Victoria/3/75 wild strain. The protection afforded by the vaccine strain was measured by three indicators: absence of temperature rise, absence or reduction of virus shedding and absence or reduction of nasal protein increase when compared with uninoculated controls.

Heterotypic immunity in this animal model was not significant when these three indicators were measured after a challenge inoculation performed 5 weeks after immunization.

INTRODUCTION

The ferret has been shown to be a suitable animal model in which to evaluate the degree of cross-reactive immunity conferred by a live attenuated virus vaccine (the 'Alice' strain) (Delem, 1976).

Using the same experimental design, ferrets were immunized with an H3N2 recombinant of A/PR/8/34 and A/Scotland/840/74 (RIT 4025 strain) (to be published) and the protection afforded by this vaccine was assessed against homologous and heterologous challenge viruses. Heterotypic immunity was also investigated by challenge of A/PR/8/34 infected ferrets with an H3N2 virulent strain. The protection against the wild strains was measured mainly by three factors: absence of pyrexia, absence or reduction of virus shedding, absence or reduction of nasal protein increase.

MATERIALS AND METHODS

Virus vaccine and viruses

All virus strains were inoculated intranasally in a 0·5 ml volume. A freeze-dried batch of the RIT 4025 strain was inoculated at a dose of $10^{7\cdot0}$ EID 50/ferret.

* Part of this study was supported by a grant from the Institut pour l'Encouragement de la Recherche Scientifique pour l'Industrie et l'Agriculture (I.R.S.I.A.).

The A/PR/8/34 (PR 8) strain was inoculated at a dose of $10^{7.5}$ EID 50/ferret, the A/Scotland/840/74 strain at a dose of $10^{7.5}$ EID 50/ferret and the A/Victoria/3/75 strain at a dose of $10^{6.8}$ EID 50/ferret.

Experimental design

Two trials were performed: in the first one, eight ferrets were immunized with the RIT 4025 strain and five ferrets were used as uninoculated controls. The homologous challenge with A/Scotland/840/74 strain was performed 5 weeks later.

In the second experiment, 19 ferrets were immunized with RIT 4025, 10 were infected with PR 8 and 15 uninoculated animals were kept as controls. All animals were challenged 5 weeks after inoculation with the A/Victoria/3/75 strain.

Temperature was measured twice daily from day 6 before inoculation to day 4 after inoculation. Only ferrets showing a mean temperature between 38 and 39 °C without any peak higher than two standard deviations of the mean were used in the test.

Nasal washings for virus isolation were collected on days 2, 3 and 4 after inoculation in Hanks' medium containing 5 % gelatine. Samples were stored at −70 °C before infectivity titration in eggs.

Nasal washings for protein content were collected before inoculation and on day 9, 11 and 14 after inoculation. They were concentrated exactly tenfold and the protein content was measured by Lowry's method (Lowry, Rosebrough, Farr & Randall, 1951).

Blood samples for antibody determination by HI test were collected before and 3 weeks after immunization or challenge.

RESULTS

As observed with the 'Alice' strain (Delem, 1976; Lobmann, Delem, Peetermans & Huygelen, 1976) inoculation of ferrets with RIT 4025 did not induce any temperature rise although the ferrets excreted the vaccine strain and responded with a high titre of HI antibodies (g.m.t. 21 days after inoculation = 1/2000) (Table 1).

Homologous challenge

As expected from previous results (Delem, 1976; Potter *et al.* 1972), all immunized ferrets were fully protected against the challenge virus infection with the exception of one animal that excreted the wild type virus on day 2 only (Table 1). In contrast, all control animals showed a febrile response; they all shed the virus for at least 4 days and the mean increase in nasal protein content was tenfold on day 9 ($P < 0{\cdot}001$, paired t test).

Heterologous and heterotypic challenges

Nineteen ferrets were immunized with the RIT 4025 strain and ten ferrets were inoculated with the A/PR/8/34 strain. They were challenged 5 weeks after immunization with the A/Victoria/3/75 wild type strain. The control group consisted of 15 animals that received the challenge virus only.

Table 1. *Challenge of ferrets vaccinated with RIT* 4025 (*PR*8 × *A/Scotland*/840/74) *with the homologous A/Scotland*/840/74 *strain*

	RIT 4025	Controls
Number of animals	8	5
Serum HI g.m.t. before inoculation	⩾2000	<8
Temperature rise	0/8	5/5
Virus shedding		
Day 2	1/8 (0·5)*	4/5 (0·9 ± 0·5)
Day 3	0/8	5/5 (2·4 ± 0·5)
Day 4	0/8	5/5 (⩾3·4 ± 0·5)
Nasal protein increase, day 9	NS	tenfold

NS = not significant.
* Titre of the positive isolate.
( ) = Geometric mean titre ± standard deviation.

Upper respiratory symptoms (rhinitis with nasal discharge, sneezing) and depression were present in the control group but not among ferrets previously immunized with either strain. Polypnoea was also recorded for three control animals. Lack of symptoms therefore could not be considered evidence of protection since the animals preinfected with an unrelated H0N1 strain (PR 8) did not exhibit any significant clinical reaction.

The geometric mean serum HI antibody titre among vaccinees with RIT 4025 was only 1/40 against A/Victoria/3/75, i.e. 25 times lower than against the homologous strain (Table 2).

Temperature rise and virus shedding are shown in Table 2. There is no significant difference between the PR 8 and the control groups in temperature rises or in the number of ferrets shedding the wild virus (χ^2 test) but the geometric mean infective titre of the virus isolates on day 4 is higher in the control group than in the PR 8 group (t test, $0{\cdot}01 < P < 0{\cdot}05$). However, the total excretion is not significantly different between those two groups (geometric mean titre of the isolates from day 2 to 4).

Among ferrets vaccinated with RIT 4025 only one had a temperature rise and the viral excretion of this group was significantly different from the control and PR 8 group on days 3 and 4 after challenge (χ^2; $P < 0{\cdot}001$ and $0{\cdot}01 < P < 0{\cdot}05$ respectively).

The nasal protein content increased significantly in every group on day 9 after infection (paired t test) (Table 2). However, in the RIT 4025 group the pre-inoculation nasal protein concentration was reached again by day 11 whereas a significantly higher concentration was still present in the PR 8 and control groups. Nasal protein increase pattern for the PR 8 group (Fig. 1) was intermediate

Table 2. *Challenge of ferrets immunized with RIT 4025 and A/PR/8/34 strains, with A/Victoria/3/75 strain (heterologous and heterotypic challenge)*

Group ...	RIT 4025	PR8	Controls
Number	19	10	15
Serum HI g.m.t. before challenge	40	< 8	< 8
$T°$ rise	1/16*	6/9*	10/14*
Virus shedding			
Day 2	15/19 (1·1 ± 1·0)†	10/10 (2·1 ± 0·7)	15/15 (1·5 ± 0·7)
Day 3	2/19 (0·5)	8/10 (0·8 ± 0·7)	15/15 (1·3 ± 0·7)
Day 4	*3/19* (0·5)	*6/10* (0·5 ± 0·4)	*12/15* (1·3 ± 0·8)
		†	
Nasal protein increase			
Day 9	3 ×	5 ×	7·9 ×
Day 11	NS	2 ×	4·5 ×

NS = not significant.
* Number with $T°$ ranging from 38 to 39 °C before inoculation.
( ) = Geometric mean titre ± standard deviation.
⌐ = Chi-square with Yate's correction: $P < 0{\cdot}01$, except †: $0{\cdot}02 < P < 0{\cdot}05$

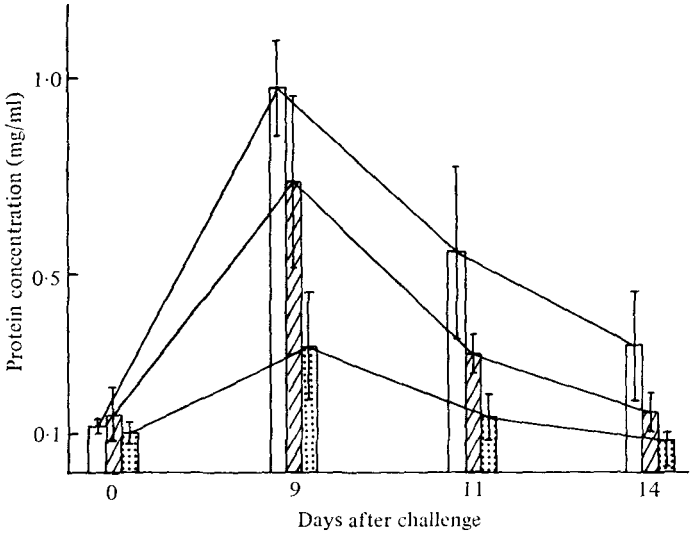

Fig. 1. Nasal protein increase in ferrets challenged with the A/Victoria/3/75 strain. □, Uninoculated controls; ▨, PR8 infected; ▦, RIT 4025 vaccinees.

https://doi.org/10.1017/S0022172400053006 Published online by Cambridge University Press

between that for the 4025 and control groups. It might be possible that the peak of protein increase was reached earlier for PR8 infected ferrets than for the uninoculated animals.

DISCUSSION

We have shown that ferrets immunized with the 'Alice' strain were completely protected against reinfection with the homologous strain and the A/Port Chalmers/1/73 heterologous strain (Delem, 1976): similar results were observed in human volunteers vaccinated with the 'Alice' strain and challenged with the A/England/42/72 and A/Port Chalmers/1/73 strains (Prinzie, Delem & Huygelen, 1976).

We therefore tried to extend this correlation between artificial challenge in man and ferrets to another vaccine strain: the RIT 4025 strain. In ferrets, the comparison between homologous and heterologous challenge tests showed that there was a reduction in the protective efficacy of the RIT 4025 strain against the heterologous A/Victoria/3/75 wild strain. However, a substantial protection was afforded as judged by the three factors measured.

In man, artificial challenge data (R. G. Douglas, unpublished) confirmed that a significant protection was afforded when volunteers vaccinated with RIT 4025 were challenged with an A/Victoria/3/75 wild strain.

In mice, heterotypic immunity has been described by Schulman & Kilbourne (1965). These mice were immunized with an influenza A subtype and challenged 4 weeks later with an unrelated strain. A significant protection was observed as measured by a decreased mortality rate, reduced titres of pulmonary virus and reduced lung lesions. However, this type of cross immunity between two unrelated viruses could not be demonstrated in hamsters (Potter & Jennings, 1976) and did not appear in ferrets when the challenge inoculation with a pathogenic strain was performed 10–12 weeks after immunization (McLaren & Potter, 1974).

We did not observe a significant immunity among the PR8-infected ferrets challenged with the A/Victoria/3/75 strain. Pyrexia was the same as in the control group and the total virus excretion (days 2–4) was not significantly different from the controls. The protein content of the nasal washings did not increase as much as among the uninoculated animals but the peak of protein increase for the PR8 immunized ferrets might have occurred earlier.

McLaren (1973) reported some non-specific effect or partial immunity in ferrets infected with PR8 and challenged 5 weeks later with an A_2/HK/68 virus. He recovered a lower amount of virus on day 3 after challenge in pre-infected ferrets than in uninoculated controls. In our experiment, the only striking difference between PR8 immunized and non-infected controls was the lack of symptoms in the former group. One should therefore disregard the absence of clinical symptoms as an indicator of protection unless the challenge is performed later. The reason for the discrepancy between our results and those of McLaren regarding the viral shedding is unknown. The absence of heterotypic protection reinforces the pertinence of the correlation between ferret and man, since heterotypic immunity has not been reported in man.

In conclusion, the ferret is a good animal model in which to assess cross-reactive

immunity for type A (H3N2) influenza viruses if the three indicators of protection described here are taken into account.

We fully acknowledge the technical assistance of Mrs C. de Marneffe and Mr B. Jambers.

REFERENCES

DELEM, A. (1976). Response of ferrets to infection with a live attenuated influenza virus and to subsequent heterologous challenge. *Developments in Biological Standardization* **33**, 226.

LOBMANN, M., DELEM, A., PEETERMANS, J. & HUYGELEN, C. (1976). Laboratory characteristics of an attenuated influenza type A (H3N2) virus ('Alice' strain). *Journal of Hygiene* **77**, 181–8.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, P. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**, 265–75.

MCLAREN, C. (1973). General discussion. *Developments in Biological Standardization* **20**, 240.

MCLAREN, C. & POTTER, C. W. (1974). Immunity to influenza in ferrets. VII. Effect of previous infection with heterotypic and heterologous influenza viruses on the response of ferrets to inactivated influenza virus vaccines. *Journal of Hygiene* **72**, 91–100.

POTTER, C. W., OXFORD, J. S., SHONE, S. L., MCLAREN, C. & STUART-HARRIS, C. (1972). Immunity to influenza in ferrets. 1. Response to live and killed virus. *British Journal of Experimental Pathology* **53**, 153–67.

POTTER, C. W. & JENNINGS, R. (1976). The hamster as a model system for the study of influenza vaccines. *Postgraduate Medical Journal* **52**, 345–51.

PRINZIE, A., DELEM, A. & HUYGELEN, C. (1976). Protective efficacy of a live, attenuated, influenza virus vaccine ('Alice' strain). *Postgraduate Medical Journal* **52**, 395–8.

SCHULMAN, J. L. & KILBOURNE, E. D. (1965). Induction of partial specific heterotypic immunity in mice by a single infection with influenza A virus. *Journal of Bacteriology* **89**, 170–4.