Immunology 1987 **60** 83–89

Production of passive immunity in neonatal ferrets following maternal vaccination with killed influenza A virus vaccines

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Accepted for publication 8 September 1986

SUMMARY

Neonatal ferrets may be passively immunized following maternal vaccination with formalin-inactivated influenza A virus vaccine, but the level of protection from partial to complete depends upon the number of doses used to vaccinate the mother, the presence or absence of aluminium hydroxide adjuvant, whether or not the mothers were 'primed' by prior infection with a serologically heterologous type A virus, and the age of the neonate at challenge. Neonates were completely protected up to 2 weeks of age, but susceptibility returned to nasal epithelium at 5 weeks and to lung at 7 weeks. Mothers immunized up to 9 months previously also partially or completely protected their offspring, this correlating with the maternal serum haemagglutination-inhibition (HI) antibody titre at the time of neonatal challenge, not the duration of immunity.

INTRODUCTION

Influenza is a common respiratory infection of young children, and it has been implicated in bronchiolitis, croup, pneumonia, febrile convulsions and the sudden infant death syndrome (SIDS) (Stuart-Harris & Schild, 1976; Laraya-Cuasay *et al.*, 1977; Paisley *et al.*, 1978; Kim *et al.*, 1979; Glezen, 1980; Murphy *et al.*, 1981). As a possible model for infant human infection, 1-day-old (newborn) ferrets were infected intranasally with clone 7a (H3N2) of the reassortant influenza virus A/Puerto Rico/8/34-A/England/939/69 (Collie *et al.*, 1980). This infection proved to be invariably fatal. All influenza virus-infected newborn ferrets showed severe involvement of the upper respiratory tract, and some died, apparently from obstruction of the airways (Collie *et al.*, 1980). A significant proportion, however, appeared to have died of uncomplicated influenzal pneumonia (Collie *et al.*, 1980).

Previously we have shown that 1-day-old newborn ferrets can be protected from infection with clone 7a by immune components acquired from the mother immunized 3 weeks previously by live infection with the same or serologically related strains (Husseini *et al.*, 1984). Protection appeared to be antibody-mediated since it was subtype-specific, and milk-derived since newborn ferrets born to non-immune mothers but fostered onto immune mothers exhibited a similar level of protection to neonates born to and suckled by immune mothers (Husseini *et al.*, 1984).

Since maternal immunization is probably the most effective way of preventing perinatal and/or postnatal infection with most viral pathogens including influenza, and as no live attenuated influenza virus is yet acceptable as a vaccine, it was of interest to determine whether protection of newborn ferrets could also be achieved using inactivated virus vaccines. In the present communication, the ability of formalin-inactivated virus, used with and without aluminium hydroxide as adjuvant and given with or without prior infection (priming) with a different subtype of influenza A virus (Potter *et al.*, 1973a, b), has been examined. Aluminium hydroxide was used as adjuvant since it is acceptable for human use and has been used with killed whole or subunit influenza virus vaccines shown to protect adult humans and ferrets against live challenge (Jennings, Potter & McLaren, 1975; Potter *et al.*, 1975, 1977). The results indicate that killed vaccines can be equally as effective as live virus for passive immunization of newborn ferrets.

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MATERIALS AND METHODS

Influenza viruses and their assay

Clone 7a (H3N2; virulent) of the reassortant influenza virus A/Puerto Rico/8/34-A/England/939/69 and the attenuated parent strain A/Puerto Rico/8/34 (H1N1) were prepared as described previously (Matsuyama *et al.*, 1980; Sweet, Stephen & Smith, 1974a, b) and assayed using the egg-bit or egg techniques (Sweet *et al.*, 1974a). Titres are expressed in 50% egg-bit infectious doses ($EBID_{50}$) or 50% egg infectious doses (EID_{50}).

The reassortant virus A/Puerto Rico/8/34-A/Hong Kong/68. (H3N2) (strain X-31 of Kilbourne, 1969) was supplied by Dr G. Appleyard of the Wellcome Research Laboratories, Beckenham, Kent, as formalin-inactivated preparations containing 2·7–3 mg protein/ml. X-31 is antigenically indistinguishable from clone 7a as determined by haemegglutination-inhibition

and neutralizing antibody tests (G. Appleyard, personal communication).

Ferrets and their inoculation

Adult ferrets were obtained from A. S. Roe, Little Fakenham, Norfolk. They were mated as described by Sweet, Toms & Smith (1977). Adult female ferrets were immunized according to one of several protocols.

(i) Non-immune pregnant ferrets were inoculated subcutaneously with 0·2 ml of phosphate-buffered saline (Dulbecco A; PBS) containing X-31 virus together with an equal volume of a 50% suspension of aluminium hydroxide gel (Fisons, Loughborough, Leicester) in PBS. One group was immunized at Week 4 of gestation (i.e. 2 weeks before parturition: see Sweet *et al.*, 1977) with 100 μg of X-31 vaccine; a second group with 100 μg and 25 μg X-31 vaccine at Weeks 3 and 5 of gestation, respectively; while two further groups were immunized at Weeks 3, 4 and 5 of gestation with 100 μg, 100 μg and 25 μg X-31 vaccine, respectively. Two remaining groups were non-immunized controls (see Table 1).

(ii) Non-immune ferrets were inoculated intranasally (under ether anaesthesia) with 0·5 ml (0·25 ml per nostril) of PBS containing 10^6 $EBID_{50}$ of A/Puerto Rico/8/34 (H1N1) within 1–2 days of mating. Pregnant animals were subsequently immunized subcutaneously at Weeks 3 and 5 of gestation with 100 μg and 25 μg respectively, of unadjuvanted X-31 vaccine, or at Weeks 3, 4 and 5 of gestation with 100 μg, 100 μg and 25 μg respectively of unadjuvanted X-31 vaccine. Animals were also immunized at Week 4 of gestation with 100 μg of X-31 with aluminium hydroxide adjuvant; another group was immunized at Weeks 3 and 5 of gestation with 100 μg and 25 μg, respectively, of adjuvanted X-31, while a third group was immunized with 100 μg, 100 μg and 25 μg of adjuvanted X-31 at Weeks 3, 4 and 5 of gestation. A remaining group was a non-immunized control (see Table 2).

(iii) Non-immune pregnant ferrets were inoculated intranasally under ether anaesthesia with 10^6 $EBID_{50}$ clone 7a (at Week 3 of gestation).

Challenge of newborn ferrets and collection of respiratory tissues

After most immunizing protocols described above, 1-day-old ferrets were inoculated intranasally (without anaesthesia) with 50 μl of PBS containing 1·0 $\log_{10}$ $EBID_{50}$ clone 7a. At 4 and/or 6 days post-infection, the newborn ferrets were killed by intraperitoneal (i.p.) injection of 0·1 ml Sagatal (May and Baker Ltd, Dagenham, Kent). The lungs and nasal turbinates were removed and homogenized in 3 ml of Hanks' balanced salt solution (Gibco, Paisley, Renfrewshire), supplemented with 0·01 g/ml bovine serum albumin, 100 U/ml penicillin G (Sigma, Poole, Dorset) and 100 μg/ml Streptomycin (Sigma) using a Sorvall Omnimixer as described previously (Sweet *et al.*, 1977).

Following some immunizing protocols, the neonates were challenged with 1·0 $\log_{10}$ $EBID_{50}$ clone 7a at 3–4 days of age or with 6·0 $\log_{10}$ $EBID_{50}$ clone 7a when 2–7 weeks old.

Haemagglutination-inhibition (HI) tests

Sera collected from female ferrets by cardiac puncture before infection, immunization and/or challenge of newborn ferrets with X-31 and/or clone 7a were tested for anti-influenza antibodies using the HI test as described previously (Basarab & Smith, 1969).

RESULTS

Virus titres in nasal turbinates and lungs of newborn ferrets born to mothers immunized with formalin-inactivated X-31 vaccine and adjuvant (aluminium hydroxide)

As described previously (Husseini *et al.*, 1983, 1984; Coates *et al.*, 1984) 1-day-old (newborn) ferrets born to unimmunized mothers were completely susceptible and produced high virus titres in both the lungs and nasal turbinates at 4 and 6 days p.i.; generally, titres were about 10-fold higher in the lungs than in nasal turbinates (Table (1). Mothers immunized with a single dose of formalin-inactivated virus with adjuvant 2 weeks before parturition produced good serum HI antibody levels (320–640), and newborn ferrets born to such mothers showed some degree

Table 1. Mean total virus titres in nasal turbinates and lungs at 4 and 6 days post-intranasal challenge (d.p.i.) with 1·0 $\log_{10}$ $EBID_{50}$ clone 7a (H3N2) of newborn ferrets born to and suckling on mothers immunized subcutaneously with formalin-inactivated X-31 *(H3N2) virus and adjuvant (aluminium hydroxide)

Immunizing protocol			Immune status of mothers		Mean total virus titre ($\log_{10}$ $EBID_{50}$) in:			
					Nasal turbinates (SEM) (d.p.i.)		Lungs (SEM) (d.p.i.)	
Dose (μg)	Time before challenge (weeks)	Age of neonate at challenge (days)	HI titres in serum to† clone 7a (H3N2)	Total number of newborn ferrets (no. of litters)	4	6	4	6
Nil	—	1	<10	11 (4)	6·25 (0·19)	6·20 (0·41)	6·88 (0·13)	6·91 (0·36)
100	2	1	320–640	17 (3)	5·46 (0·49)	4·65 (0·37)	3·34 (0·45)	2·60 (0·40)
100+25	3 and 1	1	ND‡	10 (2)	5·17 (0·24)	4·98 (0·20)	3·16 (0·66)	2·70 (0·41)
100+100+25	3,2 and 1	1	2560–10,240	15 (3)	3·59 (0·56)	3·04 (0·64)	2·14 (0·14)	2·20 (0·20)
Nil	—	3–4	<10	16 (3)	5·43 (0·15)	ND	5·78 (0·15)	ND
100+100+25	3,2 and 1	3–4	640–10,240	13 (3)	<2·00 (0·0)	<2·00 (0·0)	<2·00 (0·0)	<2·00 (0·0)

* The reassortant virus A/Puerto Rico/8/34 - A/Hong Kong/68 (strain X-31, H3N2) is antigenically indistinguishable from clone 7a of the reassortant virus A/Puerto Rico/8/34-A/England/939/69 (H3N2).

† The titres stated are prior to neonatal challenge; animals were seronegative to clone 7a at the onset of the experiment.

‡ ND not done.

Table 2. Mean total virus titres in nasal turbinates and lungs at 4 and 6 days post-intranasal challenge (d.p.i.) with 1·0 $\log_{10}$ $EBID_{50}$ clone 7a (H3N2) of newborn ferrets born to and suckling on mothers 'primed' by infection with an H1N1 virus (A/Puerto Rico/8/34) and subsequently immunized subcutaneously with formalin-inactivated X-31 (H3N2) virus with or without adjuvant (aluminium hydroxide)

Immunizing protocol after priming infection				Immune status of mothers		Total no. of newborn ferrets (no. of litters)	Mean total virus titre ($\log_{10}$ $EBID_{50}$) in:			
				HI titres in serum to:*			Nasal turbinates (SEM) (d.p.i.)		Lungs (SEM) (d.p.i.)	
Dose (μg)	Adjuvant	Time before challenge (weeks)	Age of neonate at challenge	Clone 7a (H3N2)	A/PR/8 (H1N1)		4	6	4	6
Nil	—	—	1	<10	320	3 (1)	5·35 (0·35)	6·90 (0·0)	5·40 (0·5)	5·80 (0·0)
100+25	—	3 and 1	1	640–2560	640–1280	20 (4)	4·62 (0·52)	4·22 (0·35)	2·47 (0·24)	2·25 (0·17)
100+100+25	—	3,2 and 1	1	1280–2560	640–1280	28 (5)	2·16 (0·13)	2·35 (0·26)	2·08 (0·08)	<2·00 (0·0)
100	+	2	1	640	1280–2560	8 (2)	<2·00 (0·0)	<2·00 (0·0)	<2·00 (0·0)	<2·00 (0·0)
100+25	+	3 and 1	1	320–20,480	1280–2560	28 (5)	<2·00 (0·0)	<2·00 (0·0)	<2·00 (0·0)	<2·00 (0·0)
100+100+25	+	3, 2 and 1	1	10,240–20,480	640–1280	9 (3)	<2·00 (0·0)	<2·00 (0·0)	<2·00 (0·0)	<2·00 (0·0)

* The titres stated are prior to neonatal challenge; ferrets were seronegative to both clone 7a and A/PR/8/34 at the onset of the experiment, and they were seronegative to clone 7a after A/PR/8/34 infection but before immunization with X-31.

of protection (Table 1). Titres in nasal turbinates were reduced about 10-fold on Day 4 and about 35-fold on Day 6 in comparison with titres in neonates born to unimmunized mothers; the lungs were better protected with virus titres reduced by 3·5 $\log_{10}$ $EBID_{50}$ on Day 4 and 4·3 $\log_{10}$ $EBID_{50}$ on Day 6 (Table 1). Two inoculations of killed vaccine and adjuvant 3 weeks and 1 week before birth were no more protective (Table 1); however, three inoculations at 3 weeks, 2 weeks and 1 week before birth produced considerable protection of the upper respiratory tract and almost complete protection of the lung (Table 1). In agreement with the increased protection, maternal serum HI antibody levels were considerably higher in this group (2560–10,240).

None of these immunization procedures produced complete protection of either the lower or upper respiratory tracts when neonates were challenged at 1 day of age. However, if the neonates were allowed to suckle for 3–4 days prior to challenge, then three inoculations of killed virus and adjuvant produced complete protection unlike similarly challenged neonates from non-immune mothers (Table 1).

Virus titres in nasal turbinates and lungs of newborn ferrets born to mothers 'primed' by infection with A/Puerto Rico/8/34 (H1N1) and subsequently immunized with formalin-inactivated X-31 with or without adjuvant (aluminium hydroxide)

Adult ferrets are difficult to immunize with killed vaccines in the absence of adjuvant (Potter *et al.*, 1972a, b; Sweet, Stephen & Smith, 1974c), but such vaccines are effective if ferrets are 'primed' by prior infection with a heterotypic virus (Potter *et al.*, 1973a, b; Sweet *et al.*, 1974c). Priming alone produces no protection (Table 2; see also Husseini *et al.*, 1984), but mothers primed by infection with A/Puerto Rico/8/34 (H1N1) and then given two doses of formalin-inactivated X-31 vaccine (H3N2) without adjuvant (Table 2) produced similar, although slightly greater, protection in their offspring than mothers immunized with two doses of adjuvanted vaccine without priming (cf. Table 1). Similarly, three doses of unadjuvanted vaccine following priming induced HI titres (1280–2560) comparable to mothers given similar doses of adjuvanted vaccine (2560–10,240), but again protection was slightly better, the lungs and nasal turbinates being almost completely protected (Table 2). Thus, immunization with unadjuvanted vaccine following priming produces better (but still incomplete) protection to similar protocols using adjuvanted vaccine without priming.

Complete protection of both the upper and lower respiratory tracts was produced in neonates following immunization of mothers with adjuvanted vaccine following priming. Increasing the number of doses from one to three produced a corresponding increase in the serum HI titre in the mothers (Table 2), but even a single dose of killed adjuvanted vaccine produced complete protection despite a relatively low serum HI antibody titre (640; Table 2).

Duration of immunity

The duration of protection afforded by the maternal immune components was relatively short-lived. Previously, we have shown that virus levels in the lungs and nasal turbinates of 15-day-old neonates born to non-immune mothers were $10^{7.0}$ and $10^{6.2}$ EID_{50}, respectively, 4 days after challenge with 10^6 $EBID_{50}$ (Coates *et al.*, 1984); these titres being similar to those of non-immune adult animals (Sweet *et al.*, 1981). When 2-week-old suckling neonates from mothers immunized by infection with clone 7a were challenged with 10^6 $EBID_{50}$, no replication occurred in either the lung or nasal turbinates (Table 3). By 3 weeks of age, protection was beginning to wane, but virus levels were considerably reduced compared to neonates nursed by non-immune mothers. By 5 weeks of age, when ferrets are about to be weaned, the nasal turbinates were now fully susceptible. Full susceptibility of the lung, however, did not occur until 2 or more weeks after weaning (Table 3).

In contrast, the ability of mothers to protect their offspring was relatively long lasting. When mothers used in the previous experiments were subsequently remated and the progeny challenged 3–9 months after the original maternal immunization, they showed partial or complete protection correlating with the maternal serum HI titre, not the time since previous immunization. Thus, neonates born to mothers immunized 3–9 months previously, but possessing HI titres $\leqslant$160, showed very little

Table 3. Mean total virus titres in nasal turbinates and lungs 4 days post-intranasal inoculation (d.p.i.) with 6·0 $\log_{10}$ $EBID_{50}$ clone (H3N2) of 2-, 3-, 5-, 6- and 7-week-old ferrets born to and suckling on mothers immunized by intranasal infection with 6·0 $\log_{10}$ $EBID_{50}$ of clone 7a

Immune status of mothers			Mean total virus titre ($\log_{10}$ EID_{50}) in:	
HI titres in serum to clone 7a (H3N2)	Total number of newborn ferrets (no. of litters)	Age of newborn ferrets (weeks)	Nasal turbinates (SEM) (d.p.i.)	Lungs (SEM) (d.p.i.)
			4	4
320–1280	6 (3)	2	< 2·0 (0·0)	< 2·0 (0·0)
2560–5120	9 (2)	3	3·81 (0·26)	2·11 (0·11)
5120	5 (2)	5	7·00 (0·28)	3·00 (1·00)
5120	6 (2)	6	5·90 (0·13)	5·49 (0·72)
5120	6 (2)	7	6·43 (0·15)	5·99 (0·06)

protection as compared with those born to unimmunized animals (see Table 1) in either their nasal turbinates or their lungs (Table 4). Greater protection was afforded by maternal HI titres of 320–1280; nasal turbinates exhibited some protection, and lungs almost complete protection (Table 4). When 3-day-old neonates were challenged, they showed greater protection than 1-day-old neonates, but again the lungs were better protected; indeed, with maternal HI titres of 320–640, the lungs were completely protected (Table 4).

DISCUSSION

As shown previously for neonates born to mothers immunized by infection, the formalin-inactivated H3N2 virus used in the present study also immunized mothers, and this immunity was passively transmitted to their offspring. The level of protection induced by killed vaccine depended upon four factors: (i) the number of doses of killed vaccine; (ii) the presence of adjuvant; (iii) whether the mothers were primed by prior infection with a serologically different subtype (H1N1); and (iv) the age of the neonate at challenge. As expected, when the number of doses given with adjuvant increased from 1 to 3, both the level of serum HI antibody and neonatal protection increased (Table 1). Nevertheless, even three doses of adjuvanted vaccine did not completely protect neonates challenged at 1 day of age. An incomplete level of protection, similar to that observed in neonates with adjuvanted vaccine, was also obtained in 1-day-old neonates with unadjuvanted vaccine when the mothers were first primed with A/Puerto Rico/8/34 (H1N1). Complete protection occurred if mothers were either primed and then given adjuvanted vaccine (Table 2) or immunized with three doses of adjuvanted vaccine without priming if the neonates were allowed to nurse for 3 days before challenge (Table 1).

Maternal immunity was relatively long-lived, neonates born to and suckled by mothers immunized up to 9 months previously being partially or completely protected depending upon the level of maternal serum HI antibody at birth. Again, neonates allowed to suckle for 3 days before challenge were better protected (Table 4). In contrast, the ability of neonates to absorb colostrally or milk-derived antibody was relatively short-lived. While 2-week-old neonatal ferrets born to and suckled by immune mothers were completely protected from a high challenge dose of influenza virus, by 3 weeks neonates were beginning to lose this protection. Nasal turbinates were completely susceptible to infection by 5 weeks, and lungs by 7 weeks (Table 3). This agrees with the studies on respiratory syncytial virus (RSV) of Suffin *et al.* (1979) who showed that transmucosal uptake of ferret anti-RSV IgG by the infant ferret occurred

Table 4. Mean total virus titres in nasal turbinates and lungs at 4 and 6 days post-intranasal challenge (d.p.i.) with 1·0 $\log_{10}$ $EBID_{50}$ clone 7a (H3N2) of newborn ferrets born to and suckling on mothers immunized 3 – 9 months previously by various protocols

Immune status of mothers			Mean total virus titre ($\log_{10}$ $EBID_{50}$) in:			
			Nasal turbinates (SEM) (d.p.i.)		Lungs (SEM (d.p.i.)	
HI titres in serum to clone 7a (H3N2)*	Total number of newborn ferrets (no. of litters)	Age of neonates at challenge (days)	4	6	4	6
⩽160	27 (5)	1	4·65 (0·31)	4·02 (0·61)	3·59 (0·32)	3·89 (0·62)
320–1280	45 (9)	1	4·16 (0·28)	3·04 (0·33)	2·29 (0·15)	2·30 (0·23)
⩽160	28 (6)	3	4·18 (0·33)	3·27 (0·60)	2·32 (0·25)	< 2·00 (0·0)
320–640	41 (6)	3	2·94 (0·29)	2·47 (0·25)	< 2·00 (0·0)	< 2·00 (0·0)

* The titres stated are prior to neonatal challenge.

Table 5. Immunization of pregnant mothers by intranasal infection with live vaccine or subcutaneously with various doses of killed vaccine with adjuvant (alhydrogel) and neonatal challenge at 1 day of age

Immune status of mothers		Neonatal infection (number infected/number inoculated) in:	
HI titre in serum to clone 7a (H3N2)	Immunization protocol	Nasal turbinates	Lung
⩾2560	Infection*	1/16 (6†)	0/16 (0)
320–1280		7/32 (22)	0/32 (0)
80		11/13 (85)	0/13 (0)
⩾5120	Vaccination	4/11 (36)	0/11 (0)
640–2560		21/23 (91)	10/23 (44)
⩽320		3/4 (75)	3/4 (75)

* Data from Husseini *et al.* (1984).
† No. infected/no. inoculated as a percentage.

only over the first 30 days of life. Similarly, there was no difference in replication of RSV in the lungs of 2-month-old cotton rats delivered to and nursed by immune or non-immune mothers (Wong & Ogra, 1986).

The present results, demonstrating that 3-day-old neonates are better protected than 1-day-old neonates, and that virus titres in both the upper and lower respiratory tracts of neonates born to and nursed by immune mothers decrease from 4 to 6 days post-infection, confirm the previous suggestion (Husseini *et al.*, 1984) that immunity is largely or exclusively colostrally derived. However, while the general correlation between maternal HI antibody levels and neonatal protection still holds, other factors may also be involved. For killed vaccines, higher maternal HI antibody levels are required to induce levels of protection in neonates similar to those obtained when live infection is used to immunize mothers (Table 5). Thus, the lungs of 1-day-old neonates born to mothers immunized by live infection were completely protected with maternal antibody levels as low as 80 (Husseini *et al.*, 1984), while titres of 640–2560 did not completely protect neonates nursed by vaccinated mothers (Table 5). Although less marked, the nasal turbinates of neonates born to infected mothers are similarly better protected than those suckled on vaccinated mothers. Similar suggestions that factors in addition to antibody contribute to protection come from studies with RSV in both neonatal cotton rats and ferrets (Prince *et al.*, 1983; Suffin *et al.*, 1979). Whether antibody to virion components other than the haemagglutinin or is cell-mediated immunity is important is as yet unknown. In adult mice, antibody to the neuraminidase protein provides limited protection, whereas passive transfer of large amounts of either anti-matrix or anti-nucleoprotein provides no protection (Virelizier, Allison & Schild, 1979). Passive transfer of influenza virus-specific cytotoxic T cells is also protective (Yap, Ada & McKenzie, 1978; Lin & Askonas, 1981; Lukacher, Braciale & Braciale, 1984), but these are generally cross-reactive and recognize the nucleoprotein (Taylor & Askonas, 1986). Factors in maternal milk other than immune components may also play a role in the recovery of suckling ferrets, e.g. as for human milk, macrophages, lymphocytes and neutrophils (Pittard, 1979). However, whether such factors play a role in the protection of infected neonates must remain conjectural, since migration of milk leucocytes through the wall of the gastrointestinal tract of newborn animals or human infants has never been reported (Pittard, 1979).

Another interesting but as yet unexplained observation is the differential protection of the upper and lower respiratory tract. Previously, immunization of mothers by live infection did not always fully protect the nasal turbinates of newborn ferrets, whereas the lung was invariably protected by maternal transfer of immunity (Husseini *et al.*, 1984). The same pattern was observed here with killed vaccine. This phenomenon is not peculiar to influenza virus in neonatal ferrets, since the lungs of neonatal cotton rats were better protected than nasal epithelium against RSV after maternal passive immunization (Prince *et al.*, 1983) or after intraperitoneal inoculation of antiserum (Prince, Horswood & Chanock, 1985; Wong & Ogra, 1986), and similar observations were made following influenza virus infection of infant (6-day-old) mice (Reuman *et al.*, 1983). This differential protection may be due to different components of the immune response being effective at different sites, e.g. IgA in nasal epithelium and IgG in the lung, or to differential transport of immunoglobulins or immune components to the lung. Serum antibody prevented viral pneumonia (Loosli, Hamre & Berlin, 1953) but not tracheitis (Ramphal *et al.*, 1979) or rhinitis (Kris *et al.*, 1985) in adult mice. IgG in passively administered immune maternal serum abolished RSV replication in the lungs of neonatal cotton rats but not in nasal epithelium (Wong & Ogra, 1986). Also, prevention of upper respiratory tract infection has been shown to be a function of local immunity in the ferret (Barber & Small, 1978). In addition, the most likely mediator of resistance to human influenza is IgG, derived entirely or partly from serum (Couch *et al.*, 1984), and the lower respiratory tract contains a higher proportion of IgG than does the nasopharynx (Reynolds *et al.*, 1978). The role of serum IgG in the differential protection of upper and lower respiratory tracts of neonatal ferrets is currently under investigation.

Our results using ferrets, and others from experiments with mice (Reuman *et al.*, 1983), allow two main conclusions of relevance to human neonatal infection. Firstly, infection of prospective human mothers with influenza virus prior to or during pregnancy followed by breast feeding, especially in the first few months of life, may be beneficial to their offspring. In support of this, passive transfer of maternal antibody to influenza virus occurs in humans (Masural *et al.*, 1978; Sumaya & Gibbs, 1979), and transplacentally acquired anti-influenza IgG has been associated with less severe influenzal disease in the human infant (Puck *et al.*, 1980). Breast feeding may also protect as shown with RSV (Downham *et al.*, 1976). Secondly, vaccination with killed virus may be equally effective. Immunization of pregnant women with influenza vaccines is safe and effective in terms of antibody response (Sumaya & Gibbs, 1979; Murray *et al.*, 1979), but it remains to be seen whether antibodies stimulated by vaccination of pregnant mothers can provide protection in infants, and such studies should be initiated.

ACKNOWLEDGMENTS

This work was supported by a grant from The Foundation for the Study of Infant Deaths. We gratefully acknowledge the technical assistance of Mr J. Atkinson, Mrs J. Kitt and Mr J. Martin.

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