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Passive serum antibody causes temporary recovery from influenza virus infection of the nose, trachea and lung of nude mice

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SUMMARY

BALB/c normal and nude mice were infected with a non-lethal mouse-passaged A/PC/1/73 (H3N2) influenza virus in order to assess the role of T cells on the course of disease of the nose, trachea and lung. The tracheal epithelium of both mouse strains was desquamated by 3 days after infection. Although normal regeneration began, nude mice never completed that regeneration whereas normal mice had fully regenerated tracheas by Day 14. This failure to complete the recovery was also evident from the continued virus shedding by the nude mouse. In order to assess the role of serum antibody on recovery from infection, ferret, goat or mouse antibody to H3N2 influenza virus was passively administered to nude mice after infection. It resulted in a transient decrease in virus shedding from the nose, trachea and lung, and complete but temporary regeneration of the tracheal epithelium. However, later in the course of the infection, when serum antibody levels were no longer detectable, the tracheal epithelium of these animals redesquamated and large amounts of virus were again shed from nose, trachea and lungs. We conclude that: (i) desquamation of the ciliated epithelium of the trachea is not T-cell dependent; and (ii) serum antibody can contribute to temporary recovery from infection, but by itself is insufficient for permanent recovery of the nose, trachea or lung.

INTRODUCTION

Host defence against influenza virus can be clarified by recognizing two important distinctions; first, the difference between prevention and recovery from infection, and second, the difference between disease of the alveoli and that of the ciliated epithelium (trachea and nose). Although influenza virus infection of man usually does not lead to viral pneumonia, most previous animal studies have dealt with pulmonary virus infection.

The role of serum antibody in recovery of the lung from influenza virus infection is controversial. Serum antibody has been implicated in recovery from pulmonary influenza virus infection of mice by some authors (Loosli, Hamre & Berlin, 1953; Schulman, Khakpour & Kilbourne, 1968; Virelizier, 1975; Ramphal *et al.*, 1979a) and shown to be non-essential by others (Wells, Ennis & Albrecht, 1981; Kris *et al.*, 1985). In contrast, there is a general agreement that T-lymphocytes play a role both in recovery from lethal pulmonary influenza virus infection of mice (Suziki, Ohya & Ishisa, 1974; Sullivan *et al.*, 1976; Wyde *et al.*, 1977; Iwasaki & Nozima, 1977; Yap, Ada & McKenzie, 1978; Lin & Askonas, 1981; Lukacher, Braciale & Braciale, 1984) and in the pathogenesis of viral pneumonia in mice (Suziki *et al.*, 1974; Sullivan *et al.*, 1976; Wyde *et al.*, 1977). All of these studies have concentrated on alveolar disease measured by isolation of virus from the lung, by observation of lung consolidation and by death of infected animals.

The immune mechanisms operating in recovery of the trachea and nose, however, have not been studied as extensively. There have been no studies on the role of T cells in recovery of the trachea or nose, although the data dealing with recovery from pulmonary influenza virus infections (Wells *et al.*, 1981; Yap *et al.*, 1978) suggest by analogy that cytotoxic T cells probably play a role. Recovery of the nose has been shown to occur in the absence of detectable serum antibody, but to be delayed a few days (Kris *et al.*, 1985). In some experiments, normal mice, given passive serum antibody, seem to regenerate their ciliated epithelium somewhat more rapidly after influenza infection (Ramphal *et al.*, 1979a). Because these mice are immunocompetent, the role of the passive serum antibody could have been obscured by the animals' own immune system. Thus, it is of interest to determine the effect of serum antibody on recovery in nude mice that lack T-cell function.

Abbreviations: EID_{50}, 50% egg infectious dose; HA, haemagglutination; HI, haemagglutination inhibition; i.p. intraperitoneal; NMS, normal mouse serum; PBS, phosphate-buffered saline; RDE, receptor-destroying enzyme; URT, upper respiratory tract.

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The purposes of this study were: (i) to determine whether serum antibody, although not required for recovery, nevertheless helps in the process; and (ii) to study influenza infection of the nose, and trachea in nude mice, since previous studies (Sullivan *et al.*, 1976; Wyde *et al.*, 1977) have only evaluated lung disease.

MATERIALS AND METHODS

Animals

Seven to eight-week-old BALB/c mice were obtained from Charles River Breeding Laboratories, Williamstown, MA and BALB/c nude mice from Life Sciences, Tampa, FL. At the time of infection, mice were housed in an infection isolation unit. Mature ferrets were obtained from Marshall Research Animals Inc., North Rose, NY. The goat was obtained from Animal Resources, Gainesville, FL.

Virus

An egg-grown non-lethal strain of A/Port Chalmers/1/73 (H3N2) influenza virus was mouse adapted by nine passages in mouse lungs. The virus pool was stored at −70° in 1-ml aliquots. The A/Port Chalmers/1/73/MRC-11 vaccine was a gift from Lilly Laboratories, Indianapolis, IN.

Infection

Mice were infected intranasally with 0·05 ml of A/Port Chalmers/1/73 influenza virus while under anaesthesia induced with sodium pentobarbital (0.06 mg/gm body weight). This route of infection produces an infection of the nose, trachea and lung (Yetter *et al.*, 1980).

Serum pools

Normal serum pools and high-titre immune pools were prepared from ferrets infected as described previously (Ramphal *et al.*, 1979a). Goats were immunized with 8×10^4 haemagglutinating (HA) units of A/Port Chalmers vaccine virus intramuscular weekly for 3 weeks. The sera were subjected to three successive precipitations with 33% saturated ammonium sulphate, heat inactivated and sterilized by filtration. The resulting antibody preparation had a haemagglutination-inhibition titre of 16,000 HI units. The mouse serum was obtained from BALB/c mice that were infected with A/Port Chalmers/1/73 virus and given an intraperitoneal (i.p.) injection of 4×10^4 HA units of A/Port Chalmers vaccine 3 weeks later. One week after the second immunization, the mice were exsanguinated. The sera were pooled and filter sterilized. The final titre was 512 HI units.

Virus isolation

The method for isolating virus from the lung, trachea and nose have been described previously (Yetter *et al.*, 1980).

Antibody titration

Serum antibody was measured using the HI test. Serum was treated with receptor-destroying enzyme (RDE) and absorbed chicken red blood cells, and heated at 56°. HA and HI tests were made using disposable microtitre plates (Cooke Engineering Co., Alexandria, VA) as described previously (Allan, Madeley & Kendal, 1971; Sever, 1962).

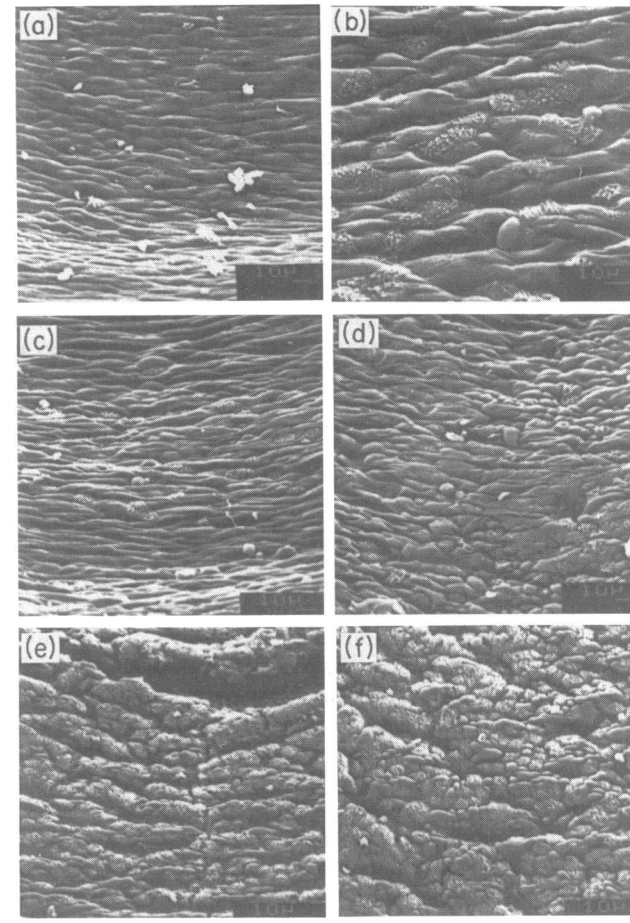

Figure 1. Scanning-electron micrographs of mouse tracheas at varying times following infection with influenza. Tracheas of nude mice on Day (a) 3, (b) 10, (c) 14 and (d) 24 of influenza infection. (e) Trachea of normal BALB/c mouse 14 days after infection with influenza. (f) Trachea of a nude mouse 14 days after infection with influenza virus and 1 week after treatment with passive antibody to influenza virus.

Statistical analysis

Viral and antibody titres were compared by the Student's *t*-test (Mendenhall, 1975) and infection ratio by Fisher's exact test (Siegel, 1956).

Scanning electron microscopy

Scanning electron microscopic studies were performed as described previously (Ramphal *et al.*, 1979b).

RESULTS

Influenza infection in normal and nude BALB/c mice

The course of influenza infection with A/PC/1/73 (H3N2) virus in BALB/c mice was similar to that seen previously in A/J mice (Ramphal *et al.*, 1979a). The tracheal epithelium was completely desquamated 3 days after infection, regeneration had begun at 5 days and was complete at 2 weeks (Fig. 1e). In nude mice the course of the infection was quite different. At 3 days post-

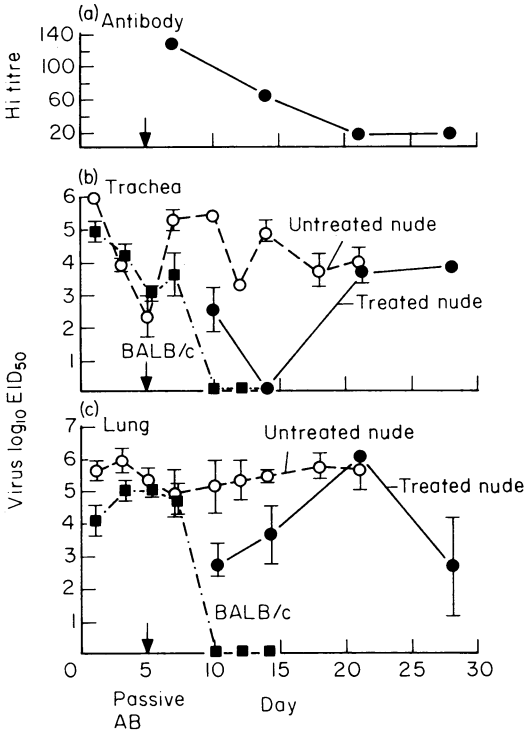

Figure 2. (a) Shows the average HI serum titres (three mice/point) of animals given passive ferret anti-influenza virus antiserum at Day 5 (arrow); (b) and (c) show virus shedding from tracheas and lungs of nude mice (dashed line, open circles) and normal BALB/c mice (broken lines, closed squares) following influenza virus infection, and of nude mice following treatment with passive antibody (solid lines, closed circles). Virus titres are presented as geometric means of groups of three mice ± SE. If no bar is shown, the SE is contained within the point.

infection there was complete desquamation of the trachea (Fig. 1a) and regeneration was in progress by 10 days (Fig. 1b); however, regeneration was not complete 14 or 24 days after infection (Fig. 1c, d). The occurrence of tracheal disease in nude mice was in marked contrast to the lack of macroscopic disease in their lungs. Although it was common to find signs of consolidation on gross examination of the lungs of BALB/c mice, such signs were observed less frequently with the nude mouse lungs.

Parallel studies of the virus shedding patterns of influenza virus-infected BALB/c normal and nude mice are depicted in Fig. 2. The inability of nude mice to complete regeneration of the tracheal epithelium is mirrored in their virus-shedding pattern. The results presented are those of a typical experiment that has been repeated several times with similar results. Initial virus titres in the tracheas of the nude mice were similar to those of the normal BALB/c mice (Fig. 2b). By Day 10 no virus was detectable from the tracheas of normal BALB/c mice while the nude mice continued to shed virus for at least 24 days in this experiment and 42 days in a subsequent experiment (Table 1). A similar pattern of virus shedding was seen in the lung (Fig. 2c). Virus titres rose and then fell to undetectable levels in the lungs of normal BALB/c mice, whereas there were high levels of virus shedding from nude mice for at least 42 days.

Administration of ferret and goat antibody to influenza virus-infected nude mice

Nude mice were infected with a non-lethal A/PC/1/73/ (H3N2) influenza virus under anaesthesia. Five days later they received an i.p. injection of ferret anti-H3N2 influenza virus serum. On Day 7, they had a serum HI titre of 128 (Fig. 2a). Mice treated with passive antiserum had completely regenerated tracheas by Day 14 (Fig. 1f). However, by Day 21, two of three mice had totally desquamated tracheas. This transient effect on tracheal desquamation had its counterpart in the pattern of virus shedding from both trachea and lung (Fig. 2b, c). Although virus titres in the trachea fell to undetectable levels by Day 14, on Day 21 they had risen once again to levels comparable to those of nude mice that had received no treatment. The effect in the lung was not as great, the depression of virus shedding was only a 100-fold decrease, and it was also transient. In most mice, virus titres in the lung returned to control levels by Day 21.

Goat antibody produced very similar results in influenza-infected nude mice (data not shown). One injection 5 days after infection led to a transient reduction or absence of virus shedding that lasted 1–3 weeks, whereas injections on Days 5 and 10 post-infection led to complete ablation of virus shedding from nose, trachea and lung at 21 and 28 days, but by 42 days virus shedding had returned to levels similar to that of infected untreated nude mice.

Administration of mouse antibody to influenza virus-infected nude mice

Having established that heterologous antisera could lead to temporary recovery, the next question was to determine the effect of the less readily available mouse serum antibody. Would homologous antibody be more effective? Mouse anti-H3N2 influenza virus antiserum or normal mouse serum (NMS) was injected i.p. on Days 5 and 10 after infection with H3N2 influenza virus in order to produce serum antibody titres equal to titres in convalescent mice (HI from 32 to 128). Fourteen days post-infection, virus shedding was undetectable from the lungs of all five passively immunized mice ($P=0{\cdot}008$) and from the trachea of four of five passively immunized mice ($P=0{\cdot}04$), but the decrease in nasal virus shedding was not statistically significant (Table 1). The tracheas from all the mice receiving immune serum were normal, whereas the tracheas of untreated infected mice or infected mice injected with NMS were fully desquamated (Table 1). By Day 42, serum antibody was no longer detectable in the immune serum-treated group and all experimental and control mice were shedding virus from the nose and lungs. The tracheas of two out of five immune serum-treated mice were desquamated, and virus shedding was detected from two of the five tracheas. All of the five NMS-treated control tracheas were desquamated and virus was detected in three of the five tracheas. Thus the effect of mouse antisera was similar to that of goat and ferret antisera.

Post-facto neutralization of virus by antibody during homogenization of respiratory tissue

Could the decrease in virus shedding in mice given passive antibody be attributed to an artifact, i.e. post-facto viral neutralization by antibody released from the tissue during homogenization? In order to examine this, six mice were

Table 1. Effect of mouse anti-influenza virus antisera on recovery of athymic nu/nu mice from influenza virus infection

Treatment	Day of killing post-infection*	No. mice	No. mice positive for virus			Amount of virus†			Serum HI antibody titre	Tracheal pathology	
			Nose	Trachea	Lung	Nose	Trachea	Lung		No. desquamated	No. normal
Immune mouse serum‡	14	5	3§	1¶	0**	3·4 (0·9)	1·0	UV††	368	0	5**
	42	5	5	2	5	4·8 (0·6)	2·2 (0·7)	3·7 (0·5)	<10	2	3§
Normal mouse serum‡‡	14	4	3	4	4	4·3 (0·4)	1·2 (0·1)	5·5 (0·6)	<10	4	0
	42	5	5	3	5	4·9 (0·5)	0	4·6 (0·4)	<10	5	0
None	14	5	5	5	5	3·3 (0·2)	1·1 (0·4)	4·0 (0·5)	<10	5	0

* NU/NU mice infected with 0·05 ml H3N2 influenza virus under anaesthesia.
† Geometric mean of virus titres from mice shedding virus, expressed as LOG_{10} EID_{50}/ml (SE) (Reed & Muench, 1938).
‡ NU/NU mice injected i.p. with 0·2 ml high-titre mouse anti-H3N2 serum (HI = 1024) 5 and 10 days post-infection.
§ Not significant compared with normal mouse serum controls (Fischer's Exact Test).
¶ $P = 0·04$ compared with normal mouse serum controls.
** $P = 0·008$ compared with normal mouse serum controls.
†† UV, undetectable virus.
‡‡ NU/NU mice injected with 0·2 ml normal mouse serum (HI < 10) 5 and 10 days post-infection.

infected with 0·05 ml H3N2 influenza virus under anaesthesia, thereby infecting the whole respiratory tract (Yetter *et al.*, 1980). Eighteen hours later, before desquamation of tracheal epithelium had occurred (Wells *et al.*, 1981), infected mice were injected with 0·2 ml high-titre mouse serum (HI = 1024) or normal mouse serum, and killed 30 min later. The animals' passive HI titre was 160. There was no significant difference in the amount of virus recovered from the nose (Exp., $10^{3·4}$; control, $10^{3·7}$), trachea (Exp., $10^{2·5}$; control, $10^{2·2}$) or lung (Exp., $10^{4·7}$; control, $10^{4·7}$) in the presence or absence of serum antibody. Therefore, virus neutralization by antibody released from the tissue during grinding does not seem to be an adequate explanation of the decrease in virus shedding.

DISCUSSION

Our studies show that the tracheal epithelium of nude mice desquamate following influenza infections. After the initial desquamation, the tracheas of nude mice began to regenerate but regeneration was never completed. Administration of passive antibody to nude mice resulted in complete regeneration of the tracheal epithelium, even though their tracheas subsequently desquamated again when antibody titres fell to undetectable levels. Thus, serum antibody allows regeneration of the tracheas of influenza virus-infected mice, and can produce a transient decrease of virus shedding from the nose, trachea and lung. When given sufficient passive antibody, virus shedding could often be completely suppressed, although the nasal shedding was most variable and persistent. Studies (Kris *et al.*, 1985) of recovery from influenza virus infection of anti-IgM-suppressed mice have demonstrated that mice can recover in the absence of detectable serum antibody. This recovery takes a few days longer than in normal mice. Therefore, although serum antibody may not be essential for recovery, if present, it helps the process.

As discussed earlier, this decrease in virus shedding could be attributed to post-facto viral neutralization by antibody released from the tissue during homogenization. Wells *et al.* (1981) have reported that homogenization of infected lungs from nude mice in the presence of antibody in the diluent, lowered virus titres from $10^{6·5}$ EID_{50} U/ml to undetectable levels. However, a more appropriate control addresses the question of whether antibody in the animal's tissue is released during homogenization in sufficient amounts to lower the virus titres. Homogenization of nasal and lung tissue in the presence of high-titre goat serum antibody did not affect nasal or pulmonary virus titres (Reuman *et al.*, 1983b). Similar results in this paper demonstrate that mouse serum antibody also does not affect nasal or pulmonary virus titres. Therefore, *in vitro* neutralization of virus does not account for the observed results. Even more compelling data are the observations showing that regeneration of the trachea occurs in mice passively immunized with anti-influenza virus serum antibody. This indicates serum antibody can help in recovery of the trachea from influenza virus infection *in vivo*.

The role of T cells in initiating tracheal desquamation has not been analysed previously. Because the trachea of influenza virus-infected nude mice desquamate, our results demonstrate that T cells are not required for tracheal desquamation. NK cells are present in nude mice (Hererbman, Nunn & Laurin, 1975) and theoretically could be involved in the desquamation. However, NK cells are undetectable in mice less than 3 weeks old yet trachea desquamation of young mice following influenza infection is very similar to that of adult mice (Reuman, Ayoub & Small, 1983a). Therefore, NK cells seem unlikely to be involved. These findings suggest that the mechanisms responsible for the pathological changes of influenza infection are not the same for the trachea and the lung. Direct viral injury appears to be the pathogenic mechanisms for the ciliated epithelium, whereas immune mechanisms may enhance damage to the alveoli (Suziki *et al.*, 1974; Sullivan *et al.*, 1976; Wyde *et al.*, 1977).

We have shown that serum antibody can lead to a temporary

recovery from influenza virus infection of both the upper and lower respiratory tract in the absence of mature T cells. This is only temporary because when the passive antibody is no longer detectable influenza virus infection is once more evident in both the upper and lower respiratory tracts. Others have shown that the cytotoxic T cell is responsible for stopping virus production in the lung. Therefore, it seems likely that the cytotoxic T cell is also responsible for permanent regeneration of the trachea. Serum antibody may speed recovery by leaking into respiratory secretions following desquamation of the ciliated epithelium and neutralizing the influenza virus. This could speed recovery by preventing infection of the remaining susceptible cells.

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