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Influenza Virus Infection of Tracheal Gland Cells in Culture

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Influenza virus-induced tracheobronchitis causes limited epithelial deciliation but markedly decreased mucociliary transport. This suggests that virus-induced alterations in airway mucus play a role in decreased mucociliary transport. Airway submucosal glands are a primary source of mucus. Therefore, we examined virus-gland cell interactions by exposing primary cultures of isolated feline tracheal gland cells to influenza A/Scotland/840/74 H3N2 virus for 1 h at a multiplicity of infection of 0.1. Virus production and release into the culture medium first occurred between 8 and 12 h postinfection and eventually reached a steady state that continued for at least 8 days. Virus which was produced and released by infected cells infected other monolayers, resulting in viral production similar to that after infection with stock virus. Hemadsorption assays conducted 24 h after infection demonstrated that most of the cells in a monolayer became infected. The infection was nonlytic according to cell morphology, trypan blue dye exclusion, and release of lactate dehydrogenase. Because lysis of a cell subpopulation could have been masked by subsequent cell division, we compared the uptake of [^{3}H]thymidine by infected and control monolayers. There was no increase in uptake by infected monolayers. These results demonstrate that feline tracheal gland cells in primary culture undergo productive and nonlytic infection with influenza A virus. This model provides a unique system for the study of virus-gland interactions isolated from the influence of other tissues.

Influenza A virus infection results in substantial morbidity and, for patients with chronic cardiovascular and pulmonary disease, increased mortality (7, 28). Infection with the virus usually causes tracheobronchitis characterized clinically by fever, myalgia, substernal discomfort, and cough (7, 28). Pathologically, there is patchy necrosis of the surface epithelium which leaves large areas of ciliated epithelium intact (20). Infection with the virus has also been shown to decrease tracheal mucociliary transport (17), an important defense mechanism of the lungs (13). The presence of large areas of intact ciliated epithelium during infection suggests that factors other than necrosis of ciliated cells are responsible in part for decreased mucociliary transport. Such factors may include reduced ciliary beating or an alteration in the quantity or rheological quality of the mucus. Most airway mucus is derived from submucosal glands (25). During infection, relatively little glandular necrosis has been observed histologically in airway tissue taken at autopsy (20, 22). However, influenza virus infection might alter glandular secretory function by mechanisms other than cellular lysis. For example, inflammatory mediators released from other cell types during infection of the airway may affect glandular function (16). Alternatively, a direct nonlytic infection of the gland cells could occur. The importance of nonfatal virus-induced changes in infected-cell function is an emerging concept in the study of the pathogenesis of viral infection. Evidence from in vitro experiments suggests that influenza virus can decrease oxidative metabolism and bacterial killing by monocytes and polymorphonuclear leukocytes without causing cell lysis (1). Furthermore, explant cultures of whole airway tissue undergo viral infection in which progeny virus is produced persistently without histological damage to the host cells (24, 32).

Because of the structural and cellular complexity of tracheobronchial tissue, it is difficult to determine the cellular specificity of viral infection and to analyze glandular secretory function without interference from surface epithelial constituents. For this reason, we have used primary cultures of feline tracheal submucosal gland cells to study the direct interactions of airway glands with influenza virus. The purpose of the present study was to determine whether gland cells could indeed serve as targets for influenza virus infection. If infection occurred, we wished to determine the characteristics of viral production and cell viability.

MATERIALS AND METHODS

Cell isolation and culture conditions. Gland cells were isolated from cat tracheas by a modification of the method of Culp et al. (6). Perfused and excised tracheas from three to five cats were used for each experiment. Each trachea was opened along its posterior aspect, and the luminal surface was brushed with a nylon test tube brush to remove the surface epithelium. The gland-containing submucosal layer was scraped away from underlying cartilage with a glass slide. The submucosal tissue was minced and subjected to a series of separate enzymatic digestions in 50 ml of incubation medium (6) in a 37°C shaker bath under an atmosphere of 100% O_2. The submucosal tissue was incubated for 30 min with 900 to 1,500 U of collagenase (type VII; Sigma Chemical Co.) and then for 30 min in Ca^{2+}- and Mg^{2+}-free incubation medium containing 1.5 mM EDTA (6). The tissue was then incubated for 30 to 60 min with 500 to 1,000 U of elastase (type I; Sigma) and then for 50 to 100 min in medium containing 900 to 1,000 U of collagenase type VII and 9 to 10 mg of collagenase type IA. The elastase and final collagenase digestions were monitored by phase-contrast microscopy. The elastase digestion was terminated when connective tissue fibers formed a loose network surrounding intact gland acini. The final collagenase digestion was terminated when individual cells could be released from acini by agitation of the tissue. Individual gland cells were separated from residual connective tissue by repeated aspiration in a 5-ml pipette. The resultant cell suspension was then filtered through

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125-μm nylon mesh to remove debris, pelleted, and resuspended in culture medium. Cells were counted with a hemacytometer, and viability was assessed by trypan blue dye exclusion (15).

The cells were suspended in culture medium (described below) and plated onto gels on rat tail collagen (1.3% solution) prepared in 24-well culture dishes (500 μl per well) by the method of Elsdale and Bard (10). The gels were fixed with 2.5% glutaraldehyde and rinsed copiously with 0.9% NaCl before the cells were plated.

Isolated cells were cultured initially at a density of 2.5×10^5 cells per cm^2 in a mixture of 50% Ham F12 medium and 50% Dulbecco modified Eagle medium (GIBCO Laboratories) supplemented with insulin (10 μg/ml; Sigma), β-retinyl acetate (10^{-7} M; Sigma), gentamicin (50 μg/ml; Sigma), and fetal bovine serum (10%; GIBCO) in a humidified atmosphere of 95% air–5% CO_2 at 37°C. This medium was changed daily.

Histological examination. Cell cultures were fixed in 10% buffered Formalin (pH 7.5), dehydrated in serial ethanol solutions, and embedded in HistoResin (LKB Instruments, Inc.). Sections (4 μm) were digested in 0.1% diastase to remove glycogen and stained with periodic acid-Schiff stain by standard histochemical procedures.

Virus infection of cell monolayers. Influenza A/Scotland/840/74 H3N2 virus stock was grown in allantoic cavities of 10-day-old embryonated chicken eggs and stored at −70°C. Virus titer was adjusted to $10^{8.0}$ to $10^{8.2}$ 50% tissue culture infective doses per milliliter in Madin-Darby canine kidney (MDCK) cells (31) as described below. Samples of this viral stock were stored at −70°C and were thawed immediately prior to use. Once the submucosal gland cells had formed confluent monolayers (7 to 10 days), they were incubated in a serum-free culture medium composed of 50% Ham F12 medium and 50% Dulbecco modified Eagle medium supplemented with bovine serum albumin (1.0 mg/ml), insulin (10 μg/ml), hydrocortisone (0.5 μg/ml), epithelial growth factor (25 ng/ml), fetuin (0.25 mg/ml), selenic acid (2 ng/ml), triiodothyronine (5×10^{-11} M), β-retinyl acetate (10^{-7} M), and gentamicin (50 μg/ml), all from Sigma. This medium was used in all subsequent steps because it maintained cells without the presence of serum, which can interfere with viral infection of cells (8). After 24 h, spent culture medium was removed from the monolayers and replaced with 500 μl of fresh virus-containing medium. The cells were incubated with virus for 1 h and washed four times with fresh medium. (Unless otherwise indicated, cells were exposed to the A/Scotland/840/74 H3N2 strain of influenza virus.) Another quantity (1.5 ml) of medium, termed the time zero sample, was then added to the monolayer, agitated, immediately removed, and frozen at −70°C. This sample was used to assess the adequacy of washing. Fresh medium was then added and changed at daily intervals. At the same time, separate portions (500 μl) of the stock solution of virus-containing medium used to infect the cells were incubated in a 24-well plate without cells. The contents of a single well were collected at daily intervals. These samples served to correlate inactivation with time in culture. All samples were frozen and stored immediately at −70°C after collection.

In a separate experiment, human serum which yielded a titer 1:128 in a hemagglutination inhibition assay with the A/Scotland/840/74 H3N2 virus was obtained. Six monolayers were exposed to influenza virus and washed as described above. Three of the monolayers were treated for 30 min at 37°C with 0.5 ml of anti-influenza serum, and the other three monolayers were incubated with medium for 30 min. All six monolayers were then washed five times with medium, and a time zero sample was taken. The medium was changed at 2, 5, 8, 12, 16, and 24 h postinfection. Medium samples from monolayers within each group were pooled at each time point for determination of viral titers.

Determination of viral titers. Viral titers were determined by the method of Tobita et al. (31). Confluent monolayers of MDCK cells (Flow Laboratories, Inc.) were grown in 24-well plates in minimal essential medium (M.A. Bioproducts) with 10% fetal bovine serum (HyClone Laboratories, Inc.), penicillin (100 U/ml; Sigma), streptomycin (50 μg/ml; Sigma), and amphotericin (2.5 μg/ml; Sigma). Cultures were maintained at 37°C in a humidified atmosphere consisting of 95% air and 5% CO_2. The cell monolayers were washed twice with phosphate-buffered saline (PBS). Samples of gland cell culture medium were serially diluted (10-fold) in Earle balanced salt solution (M.A. Bioproducts) and assayed for virus. MDCK cell monolayers were inoculated in quadruplicate with 0.2 ml of the fluid samples per well and incubated for 1 h. The culture plates were agitated every 15 min to ensure an even distribution of inoculum and to maintain moisture over the cell surfaces. Following the infection period, the sample was removed and the cells were washed with PBS. The cell monolayers were then overlaid with agarose containing serum-free minimal essential medium, antibiotics, and tolylsulfonyl phenylalanyl chloromethyl ketone (1 μg/ml; Cooper Biomedical). After 3 more days of incubation, the cells were fixed with 10% Formalin for at least 1 h, the agarose layer was removed, and the wells were washed with water. A 0.3% methylene blue stain was added for 15 min, and the cultures were washed and air dried. Plaques were counted, and the results were recorded as the means of the quadruplicate determinations for each assay. Titers were expressed in PFU per milliliter.

Hemadsorption assay. Hemadsorption assays for the cellular expression of viral antigens were performed by the method of Bell et al. (4). Gland cell monolayers (2 cm^2) were washed with cold PBS, 1 ml of a 0.5% (vol/vol) suspension of guinea pig red blood cells was added, and the monolayers were incubated for 30 min at 4°C. Following incubation, the monolayers were washed vigorously with cold PBS, fixed for 30 min in cold 2.5% glutaraldehyde ([vol/vol] in PBS), and then examined by phase-contrast microscopy.

Trypan blue staining. Viability of cell monolayers was assessed by trypan blue dye exclusion (15). The monolayers were washed three times with PBS and then incubated with 0.08% trypan blue dye in PBS. After 5 min the dye solution was removed, and the monolayers were washed three times with PBS and then fixed in 10% Formalin for observation with a dissecting microscope.

LDH. Lactate dehydrogenase (LDH) activity in spent media collected from gland cell monolayers at daily intervals was measured by the spectrophotometric method of Wroblewski and LaDue (33), with minor modifications. Prior to the assay, however, samples were incubated for 30 min at room temperature in 1% Triton X-100 to release membrane-enclosed enzyme. At the end of each experiment, each monolayer was homogenized in 10 mM phosphate buffer (pH 5.0) with 50 strokes of a ground-glass tissue grinder. LDH activity in the homogenate was then measured, and total cellular LDH was calculated. LDH activity released daily into the culture medium was normalized as a percentage of the total activity in the homogenized monolayer.

Thymidine uptake. Gland cell monolayers were incubated for 24 h in medium containing 50 μCi of [^{3}H]thymidine per ml. The monolayers were then washed with medium to

remove unattached cells and were homogenized as described above. Incorporation of radiolabeled thymidine into DNA extracted from the homogenate was measured by the trichloroacetic acid precipitation method of Yager and Miller (34). Cells cultured in hydroxyurea (10 mM) were used to determine nonspecific incorporation of [^{3}H]thymidine into the acid-precipitable fraction (18).

RESULTS

Infection of cell monolayers. The average yield from three separate cell preparations was (45.9 ± 7.84) × 10^6 (mean ± standard error) live gland cells. Viability was 80 to 95% by trypan blue exclusion. Once confluent and in serum-free medium, the cells had a cobblestone appearance when observed by phase-contrast microscopy (Fig. 1). Cells prepared for histology at this time were cuboidal and contained large nuclei, prominent nucleoli, and periodic acid-Schiff stain-positive secretion granules. Measurement of the DNA content of confluent monolayers compared with that of freshly isolated cells revealed a cell density of approximately 9 × 10^6 cells per cm^2 (data not shown). Cell monolayers were infected with influenza A/Scotland/840/74 H3N2 virus sufficient to achieve a calculated multiplicity of infection (MOI) of 0.1. Monolayers infected at this MOI maintained a steady state of viral production for a prolonged period. Viral production remained constant for at least 8 days (Fig. 2). In preliminary experiments we used higher MOIs (of 1 and 10) but found that the resultant viral titers (measured at daily intervals for 8 days) were not significantly different from those of cells infected at an MOI of 0.1 (data not shown). We therefore used this lower MOI for all subsequent experiments. It can also be seen that the original input virus was inactivated within 2 days when incubated without cells (Fig. 2). At day 1 postinfection, the titer of input virus was still high, though substantially below the titer obtained from exposed cell cultures. These data suggested that the virus observed in the cell cultures on day 1 was due in part to input virus adsorbed to the monolayers and subsequently released. As a result, we conducted an experiment in which one group of virus-exposed monolayer was treated with anti-influenza virus antibody immediately after infection and one group was not. The antibody served to inactivate adsorbed but not infecting virus. The time courses of virus titers determined in the two groups of monolayers were compared (Fig. 3). In antibody-treated cultures, no virus was detected until 8 h after infection, after which time virus production progressively rose. High titers of virus were observed at just 2 h postinfection in the untreated cultures.

The virulence of the virus produced during gland cell infection was demonstrated by its ability to produce lytic plaques in MDCK cells during viral titration. To assess its virulence in tracheal gland cells, we first determined the

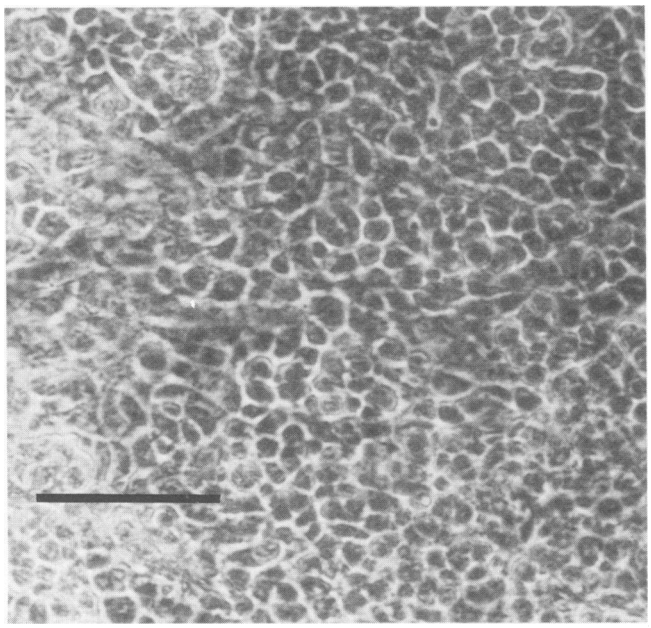

FIG. 1. Gland cell monolayer after 20 days in culture. Phase-contrast photomicrograph. Bar = 100 μm.

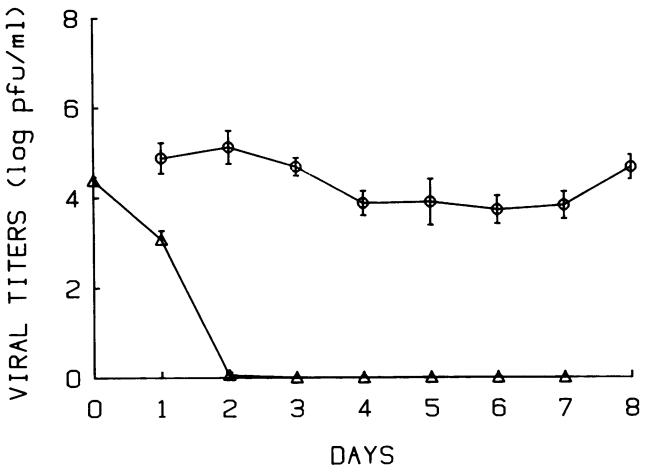

FIG. 2. Daily production of virus by tracheal gland cells. Confluent monolayers were infected with virus as described in the text (MOI, 0.1). Spent medium was replaced daily, and virus titers were determined (○). At the same time, a sample of the original input virus was incubated without cells. Samples were collected at daily intervals, and virus titers were determined (△). Results are the mean ± standard error from three experiments.

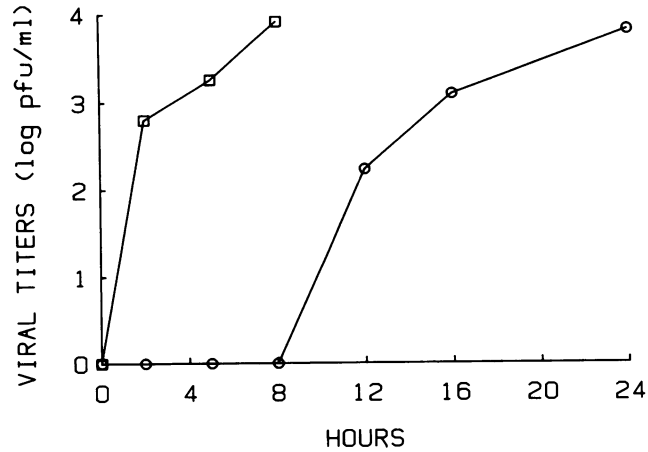

FIG. 3. Viral titers in medium from infected monolayers at intervals postinfection. Immediately after infection, monolayers were incubated either with (○) or without (□) anti-influenza serum as described in the text. The medium was changed at the indicated intervals, and virus titers were determined. Results are from a single experiment.

virus titer in pooled medium taken from cells infected with influenza A/Scotland/840/74 H3N2 virus. Four cell monolayers then were infected with 1 ml of this pooled medium containing virus (1.2×10^4 PFU/ml) which had been passaged through gland cell monolayers. Four other monolayers from the same cell culture preparation were infected under the same conditions with an identical amount of stock virus (MOI, 0.006). Medium samples from monolayers within each group were pooled for titer determination. The passaged virus caused a productive infection in the gland cell monolayers. The log viral titers of passaged virus at days 1, 2, and 3 postinfection were 4.08, 4.05, and 3.05 PFU/ml, respectively. These were not substantially different from the log viral titers of medium samples from monolayers infected with stock virus, which were 4.96, 4.96, and 3.05 PFU/ml at days 1, 2, and 3 postinfection.

In additional experiments, two groups of three monolayers were exposed at an MOI of 2×10^{-3} to 3×10^{-3} to one of two clinical isolates of influenza A/Taiwan/1/86 H1N1 virus, which had been isolated from clinical specimens with permissive mammalian cells and was never cultured in eggs. These strains also resulted in productive infections (Table 1), although the onset of detectable virus production was delayed for 4 to 5 days after exposure. The titers eventually reached levels similar to those seen with the A/Scotland/840/74 H3N2 strain, even though the initial infecting titers of the clinical specimens were much lower.

Hemadsorption assays. To assess the proportion of cells infected, we performed hemadsorption assays on confluent monolayers 24 h after they were exposed to influenza A/Scotland/840/74 H3N2 virus. Adherent erythrocytes covered 80 to 100% of the monolayer surface. Similar results were obtained when the MOI was varied over a range from 0.01 to 10.0. At an MOI of 0.001, however, 48 h were required for the adherence to reach a maximum. An example of erythrocyte adherence is shown in Fig. 4.

Cell viability with infection. The preceding results indicated that feline tracheal gland cells in primary culture underwent a productive infection with influenza A virus. We next evaluated whether the viral infection killed the host cells. Infected monolayers were observed daily for 8 days postinfection by phase-contrast microscopy. The infected monolayers remained intact and were identical in appearance to control monolayers. A more sensitive test of cell viability, however, is trypan blue dye exclusion. We divided 12 monolayers from a single cell preparation into two groups of 6 infected and 6 control monolayers. Three monolayers from each group were incubated with trypan blue at day 1 postinfection, and the remaining three monolayers were incubated with trypan blue day 5 postinfection. After incubation with trypan blue, each monolayer was washed, fixed in Formalin, and examined under a dissecting microscope at a magnification of ×42. In all cases, only a few cells were stained with the dye, and no differences were seen between infected and control monolayers. Typical monolayers are shown in Fig. 5. A control monolayer which was killed by exposure to NaCN (1% solution in PBS) for 20 min prior to staining is also shown for comparison.

Cell death in gland cell monolayers was also assessed by measuring LDH activity in medium removed from cell monolayers. Daily LDH activity was measured and normalized to the total LDH activity in the homogenized monolay-

TABLE 1. Virus production by monolayers infected with clinical isolates of A/Taiwan/1/86 H1N1

A/Taiwan/1/86 H1N1 isolate[a]	Log viral titer (in PFU/ml) on postinfection day:								
	1	2	3	4	5	6	7	8	9
159	0	0	0	2.87	5.23	6.23	5.73	5.60	5.27
31	0	0	0	0	4.50	5.37	6.00	5.80	5.17

[a] These isolates were passaged only in cynomolgus monkey kidney cells (M.A. Bioproducts), and titers were less than 10^3 50% tissue culture infective doses in MDCK cells. Medium from each group was collected and pooled at daily intervals postinfection for determination of viral titers.

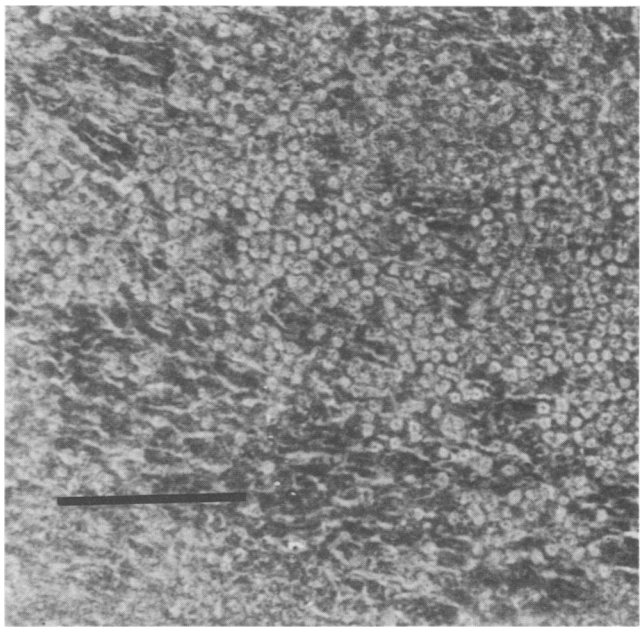

FIG. 4. Adsorption of guinea pig erythrocytes to a gland cell monolayer 24 h after infection (MOI, 0.1). Phase-contrast photomicrograph. Bar = 100 μm.

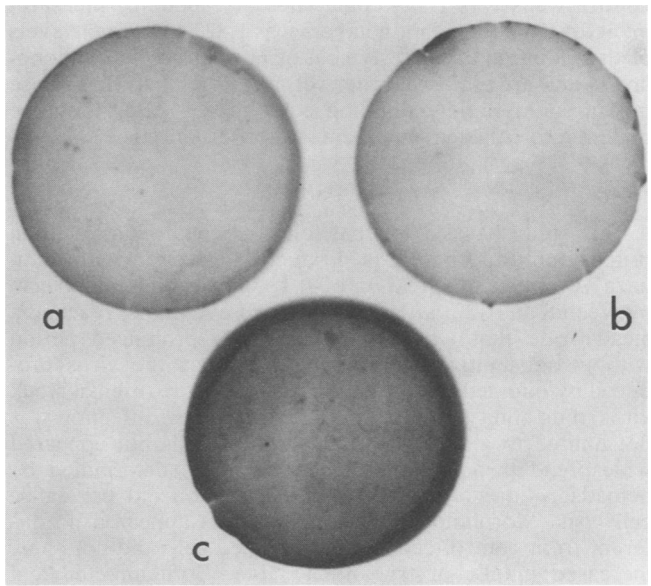

FIG. 5. Gland cell monolayers stained with trypan blue (Magnification, ×3.5). (a) Uninfected monolayer; (b) monolayer 1 day after viral infection; (c) monolayer stained after the addition of cyanide (1% solution of NaCN in PBS for 20 min).

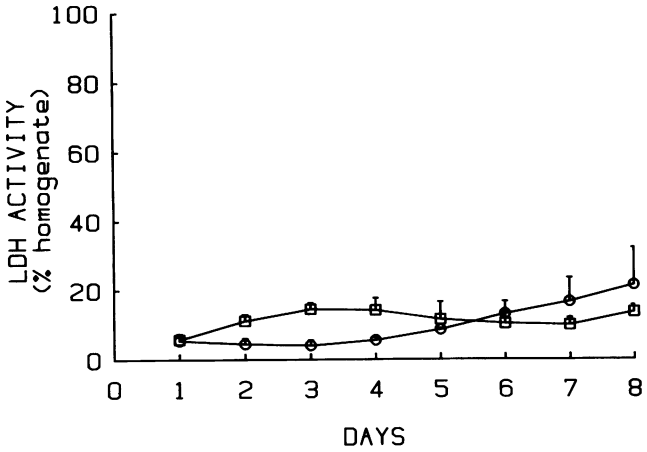

FIG. 6. Daily release of LDH by infected (○) and control (□) monolayers. Monolayers were infected as described in the text (MOI, 0.1). Spent medium was replaced daily and assayed for LDH activity. Activity was normalized to the total cellular LDH activity in each monolayer as determined at day 8. Each point is the mean ± standard error from three experiments.

ers (Fig. 6). There were no substantial differences in LDH release by infected and control cells, and in both instances the LDH released was a small percentage of the total activity in the cell homogenate. A similar low level of daily release of LDH was measured in the monolayers infected with the A/Taiwan/1/86 H1N1 clinical isolate (data not shown).

One possible explanation for the apparent lack of cell death with viral infection was that cells dying from infection were being replaced rapidly by cell division. This would be manifested by an increased rate of DNA synthesis in infected cells. To examine this possibility, the uptake of [^{3}H]thymidine into cellular DNA was measured in four infected and four control monolayers. [^{3}H]thymidine was added to the infected monolayers at 24 h postinfection. The mean activity of [^{3}H]thymidine incorporated over 24 h by the control wells was 1,759 cpm. The corresponding measurement for the infected monolayers was 1,823 cpm. Such levels of thymidine uptake are typical of normal confluent monolayers and are almost 2 orders of magnitude less than those measured in rapidly dividing cells shortly after they are plated onto collagen gels (personal observation).

DISCUSSION

This study has shown that feline tracheal gland cells in primary culture undergo a productive infection with influenza A virus. At an MOI of 0.1, viral production began between 8 and 12 h after infection and eventually reached a steady rate that was maintained for a prolonged period without substantial diminution. Moreover, the virus produced by infected cells was comparable to the original stock virus in its ability to infect gland cell cultures. Infection was not limited to a small subpopulation of cells but appeared widespread throughout the monolayer, as determined by hemadsorption. Nevertheless, the infection did not cause cell lysis. Morphologically, infected cells appeared no different from control cells. Also, infected cells did not show increased uptake of trypan blue dye. Both infected and control cells released a small but constant amount of LDH daily. This may have reflected cell turnover, as indicated by a small incorporation of [^{3}H]thymidine (14), or release of cytoplasm during discharge of secretion granules (A. Gashi, J. A. Nadel, and C. Basbaum, Clin. Res. 32:429A, 1984). Finally, infection did not lead to increased cell division, as indicated by [^{3}H]thymidine incorporation into cellular DNA.

The influenza virus strain, A/Scotland/840/74 H3N2, used in most of these studies was passaged in eggs, which conceivably could have caused attenuation of pathogenicity. In previous studies, however, we have shown that this strain can infect both human monocytes and lymphocytes, with new synthesis and surface expression of virus-encoded proteins occurring within 6 h postinfection (19, 26, 27). Infection of the leukocytes is abortive, and viral progeny are not released. Nevertheless, the virus infection commonly results in depressed mitogen- or antigen-stimulated proliferative responses due to altered monocyte-macrophage accessory cell function, with lymphocyte responsiveness preserved (26). The monocyte-macrophages also actively respond to the virus with synthesis of interferon (27). Thus, the virus strain retains many aspects of in vitro pathogenicity representative of in vivo events, and its use in the current studies is reasonable. Furthermore, we demonstrated that exposure to other strains of influenza A virus, clinical isolates 31 and 159, which were not passaged in eggs, resulted in productive infection of gland cell monolayers. We also found that infection with these clinical isolates failed to increase the daily release of LDH, even after levels of virus production comparable to those of the stock virus were reached. Because the clinical isolates were inoculated at such low MOIs, we could not directly compare their lytic activity with that of the stock A/Scotland/840/74 H3N2 virus. Nevertheless, exposure to the clinical isolates resulted in substantial virus production with no measurable increase in cell death.

The establishment of a productive and nonlytic infection in primary cultures of submucosal gland cells by the A/Scotland/840/74 H3N2 virus suggests that these cultures may serve as a model to study the pathogenesis of influenza virus infection. A number of previous reports have described viral infection in tissue cultures (2, 5, 9, 11, 21, 24, 32). Virtually all of these studies examined the interaction of virus with pieces of whole tissue which contained many different cell types. In such complex systems, it is difficult to distinguish the response of a single cell type. This aspect assumes even greater importance in light of the many neurologic and nonneurologic mediators which normally affect tracheal gland secretion (3). Our model eliminates much of this complexity. These primary cultures do have some inherent disadvantages. For example, the isolation process may select for a particular type of cell. Also, the culture conditions may lead to a loss of other cellular differentiated functions. Finally, species differences may play a role. For example, cats did not exhibit clinical signs when exposed to a clinical strain of influenza virus (23). On the other hand, infection in ferrets has been characterized by upper-airway symptoms and in mice by pulmonary parenchymal inflammation (29). Therefore, results from experiments with cultured animal cells ultimately require correlation with both in vivo and in vitro studies using more complex systems and different species.

Although our data suggest that influenza virus infection is nonlytic in gland cells, infection may cause alterations in the secretory properties of these cells. Submucosal glands secrete a number of substances, including mucous glycoproteins, proteoglycans, lactoferrin, lysozyme, and secretory immunoglobulin A (3). Alterations in these secretions could have serious implications. For example, secretory immunoglobulin A is an important element in the defense of the lungs

(13). A diminution in its action could weaken the ability of the host to respond to a pathogenic stimulus. Also, mucous glycoproteins have been shown to interact with viruses and bacteria (30). A decrease in such interactions may result in a higher risk or morbidity. Such decreased interaction would naturally occur if mucous secretion decreased. However, the biochemical characteristics of the secreted mucous glycoproteins are also important. For example, bacterial interaction with salivary mucous glycoproteins is dependent on specific carbohydrate side chains of the glycoproteins (30). If these side chains are altered, this interaction may change, irrespective of the quantity of mucous glycoprotein present. In previous studies we have shown that gland cell cultures secrete high-molecular-weight sulfated macromolecules suggestive of mucous glycoproteins and that secretion is responsive to cholinergic stimulation (D. K. P. Lee, D. J. Culp, D. P. Penney, and G. M. Marin, Physiologist **28**:372, 1985). Therefore, our cultured gland cell monolayers offer a unique opportunity to study the effects of infection on cell secretion in a highly controlled environment.

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