Histopathological Changes in the Upper Respiratory Tract of F344 Rats Following Infection with a Rat-adapted Influenza Virus

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Abstract. The present study determined the morphogenesis of upper respiratory tract disease in rats following infection with a rat-adapted influenza virus. Sixty-eight 60-day-old, male F344 rats were infected by intranasal inoculation and necropsied at days 1, 2, 4, 7, 14, and 28 post-inoculation (PI). Responses to infection were studied by routine light microscopy for histopathologic changes and immunocytochemistry for localization of viral antigen. Severe infection-induced changes involved the respiratory epithelium and underlying lamina propria, and the nasal-associated lymphoid tissue, with minimal involvement of the transitional epithelium. The lesions were most severe on the septum and the medial aspect of the nasoturbinates. Viral antigen, located in the respiratory epithelium of affected regions at days 1 and 2 PI, was associated with neutrophilic infiltration and epithelial necrosis and erosion. At day 4 PI, an infiltrate of lymphocytes, macrophages, and fewer neutrophils was present, often accompanied by epithelial regeneration. Changes in the nasal-associated lymphoid tissue were evaluated using morphometric analysis and consisted of hyperplasia (days 4 to 7 PI) followed by progressive involution (days 14 to 28 PI). Mild lesions associated with foci of viral antigen were also observed in the nasal olfactory epithelium on days 1, 2, and 4 PI. The changes observed in the present study indicate the potential value of rat-adapted influenza virus infection as a model of human influenza.

Key words: Immunohistochemistry; influenza; nasal cavity; pathology; rat.

Numerous pathogenic microorganisms are transmitted via inhaled air and are capable of causing severe morbidity and mortality in individuals with altered respiratory immunity.[3] The economic impact of influenza virus infection is estimated to be between three and five billion US dollars direct cost per year in the United States alone.[19] Concurrent exposure to air pollutants may favor such disease processes, resulting in enhanced or prolonged viral infection, with attendant increases in morbidity, mortality, and socioeconomic impact.[3] Identification of components of the airway immune defenses and characterization of their responses to important air pollutants, in conjunction with relevant infectious disease processes, will help to determine the presence or absence of synergism between inhaled toxicants and infectious agents.

Influenza viruses are epitheliotropic and specifically target the airway lining of the upper and lower respiratory tracts.[20] Complete descriptions of pulmonary lesions induced in humans by these viruses have been published.[18] Qualitative and quantitative descriptions of nasal changes during rhinovirus infection have also been reported.[12,22] Lesions resulting from infection by a number of viruses in the nose of rats have been described in more detail, including Sendai virus[11] and sialodacryoadenitis virus.[4] However, little information is available on changes in the upper respiratory tract of humans or laboratory animals resulting from influenza virus infection. The virus used in the present study was adapted to rats by serial passage and developed as a model for the study of respiratory tract immune responses.[10] The present publication reports a sequential morphological description of the events that occur in the respiratory tract following rat-adapted influenza virus infection of rats, with special reference to the nasal mucosa and the nasal-associated lymphoid tissue including localization of the virus by immunohistochemistry.

Materials and Methods

Animals and husbandry

Sixty-eight male F344 rats (CDF [F344]/CrLBR, Charles River Laboratories, Inc., Wilmington, MA) were used at 8 weeks of age in all studies. The rats were housed in shoe-box cages with three rats per cage. Animals were maintained at

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22 ± 2 C and a relative humidity of 50 ± 10% with a 12-hour light–dark cycle. All rats received water and food (Purina rat chow) ad libitum. Additional animals were part of a sentinel program and were screened for specified murine pathogens, including endoparasites and ectoparasites, and fecal *Pseudomonas* spp. Nasopharyngeal, tracheal, and lung washing samples were cultured for murine respiratory tract pathogens. Tissues were collected for histopathologic evaluation, and serum samples were tested for antibody to Sendai virus, reovirus type 3, pneumonia virus of mice, encephalomyelitis virus, Toolan H-1 virus, Kilham rat virus, lymphocytic choriomeningitis virus, and *Mycoplasma pulmonis*. Results of all these tests were negative.

Rat-adapted influenza virus (RAIV)

A human influenza virus, adapted through 10 successive passages of infected lung homogenate in F344 rats, was used for all infectivity experiments. Influenza A/Port Chalmers/1/73 (H3N2) virus was purchased from the American Type Culture Association (ATCC VR-810) and used as the initial infectious agent in the adaptation procedure. Influenza A/Port Chalmers/1/73 virus propagated in the allantoic fluid of 10-day-old embryonated chicken eggs was used as a stock virus to be adapted to growth in the F344 rat lung. A 0.2-ml suspension of allantoic fluid containing the influenza virus was administered intranasally, using a 22-gauge 1-in. needle on a 1.0-cc Monoject syringe, to male F344 rats lightly anesthetized with ethyl ether (Mallinkrodt). Lungs were harvested 24 hours post-infection and scored for lung consolidation.[15] A 10% w/v lung homogenate in Eagle's minimum essential medium (E-MEM) was made on ice using a Polytron homogenizer (Kinematica GmbH, Switzerland) on setting 7 for 15 seconds. The homogenate was centrifuged at 1,000 × *g* for 30 minutes to remove cellular debris, and the supernatant aliquoted and stored at −70 C until analyzed for virus content by using the Madin–Darby canine kidney (MDCK) plaque assay.[13] The homogenate showing the highest viral titer and most advanced lung consolidation was chosen for the next passage in F344 rats. This process of passaging the virus in the rat lung was repeated until a plateau in the viral titer was observed. Infectious viral titers used in challenge studies were quantified utilizing MDCK cells[13] and an overlay medium.[1] This method has been described in detail.[9]

Histopathology

Fifty-eight 8-week-old rats were randomly assigned to two groups (control and infected group) of 29 animals per group. At day 0, the treated animals were intranasally inoculated with 0.2 ml of a 1 : 100 dilution of rat-adapted influenza virus (RAIV): 2.0×10^4 plaque-forming units /ml in Eagle's minimum essential medium with L-glutamine, non-essential amino acids, and penicillin/streptomycin (E-MEM, GIBCO). The control animals were inoculated in the same way with 0.2 ml of culture medium. Animals were euthanatized and necropsied on days 1, 2, 4, 7, 14, and 28 post-inoculation (PI). At each time point, five animals of each group (four animals per group at day 28) were anesthetized with an intraperitoneal injection of sodium pentobarbital, body weights were recorded, and the animals were euthanatized by exsanguination. A complete necropsy was performed. The nasal cavities were gently flushed with formalin via the nasopharyngeal meatus and then immersed in fresh fixative. The skulls were decalcified in a 5% formic acid solution with ion-exchange resin. Six 2–3-mm-thick transverse blocks of the nasal cavity[5] were placed rostral face down (with the exception of the nose tip) in cassettes. The tissues were then processed in a conventional manner, embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin (HE), and examined by light microscopy. The distribution of nasal lesions was recorded on diagrams of the nose of the F344 rat.[17] The severity of nasal lesions was scored as follows: 0 = no lesion observed, + = minimal lesion, ++ = moderate lesion, +++ = severe lesion. Lesion score was evaluated blindly with randomized slides and was based on both local severity and extent of tissue involvement.

Immunohistochemistry

A modification of the streptavidin–biotin immunohistochemical system, Histostain-SP (Zymed Laboratories, San Francisco, CA), was used to detect influenza virus antigen in the tissues. Deparaffinized tissue sections of the nose were incubated with 0.5% H_2O_2 in methanol for 30 minutes to inhibit endogenous peroxidase and then rinsed three times in phosphate-buffered saline (PBS). After treatment for 10 minutes in 0.1% pronase E (Sigma Chemical Co., St. Louis, MO) (pH 7.3) at room temperature and three washing steps, blocking of nonspecific staining was achieved with normal horse serum diluted 1 : 5 in PBS for 15 minutes. The blocking serum was drained and replaced with a murine monoclonal antibody (anti-influenza A virus antibody that recognizes the nucleoprotein; Biodesign International, Kennebunkport, ME) in a 1 : 250 dilution in PBS overnight at room temperature. After washing in PBS, sections were incubated with a biotin-labeled horse anti-mouse immunoglobulin antibody (Vector, Burlingame, CA), diluted 1 : 200 in PBS for 30 minutes at room temperature. After rinsing, sections were incubated with an HRP-Streptavidin (Zymed Laboratories) diluted 1 : 200 in PBS for 30 minutes at room temperature. Following three washings, the substrate and chromogen mixture (AEC red substrate kit, Zymed Laboratories) was prepared in distilled water and applied for 5 minutes. The sections were washed three times, counterstained with Harris' hematoxylin, and coverslipped. As a negative control, PBS was substituted for the primary antibody using selected tissues. The specificity of the viral stain was examined by using tissues from a control F344 rat, uninfected with RAIV. The tissues were processed as described above.

Morphometric analysis

For a morphometric study of reactivity of the nasal-associated lymphoid tissue (NALT), ten rats were randomly allocated into a control group and an infected group of five animals per group, and inoculated as described above. The animals were necropsied on day 7 PI, using procedures identical to those described above. After decalcification, about 1-mm-thick serial transverse blocks from the region of the nose containing the NALT (from the first palatal ridge to the posterior opening of the nasopharyngeal duct) were placed

Table 1. Progression of the lesions present in the nasal cavity following rat-adapted influenza virus infection. − = normal or negative, + = minimal, ++ = moderately severe, +++ = severe.

	Day After Inoculation					
	1	2	4	7	14	28
Respiratory epithelium						
Neutrophilic inflammation	++	+++	+	−	−	−
Epithelial necrosis	−	++	+	−	−	−
Mononuclear inflammation	−	+	++	+++	+	−
Regeneration	−	−	+	++	+	−
Detectable viral antigen	++	+++	−	−	−	−
Olfactory epithelium						
Sensory cell loss	−	+	+	−	−	−
Regeneration	−	−	−	+	+	−
Detectable viral antigen	+	++	++	−	−	−
Nasal-associated lymphoid tissue (lymphoepithelium)						
Hyperplasia	−	−	++	+++	+	−
Involution	−	−	−	−	+/++	+++
Detectable viral antigen	+	+	−	−	−	−

rostral face down in cassettes. The tissue blocks were trimmed in a manner that permitted determination of block number (five to seven per NALT) and orientation (left or right) on the basis of block shape. These tissues were then processed as described previously for the HE sections. The morphometric analysis was performed using an Image-1 Processing System (Universal Imaging Corporation, West Chester, PA). Prior to collection of data, the magnification was calibrated using a stage micrometer. The cross-sectional area of the NALT in histological sections (four for each animal) was determined for each cross section. The total area of the NALT was calculated by adding the values obtained for each section.

Statistical analysis

Statistical analysis of the measured NALT was performed. The data were tested for equality of group means by use of an unpaired student's *t*-test. The 5% significance level was used as a criterion for statistical significance.

Results

Clinical observations; body and organ weights

No clinical illness was observed in infected rats. A moderate and transient decrease in body weight was apparent at 7 days post-inoculation (PI). At other times, body and organ weights were within the normal range, with no statistically significant differences between groups (data not shown).

Gross pathology

No significant macroscopic changes were observed during necropsy. However, during trimming of the decalcified noses, small dark foci of apparent congestion were found in the nasal-associated lymphoid tissue (NALT) in animals necropsied at day 28 PI.

Histopathology

The lesions progressed and resolved in a characteristic fashion (Table 1). In rat-adapted influenza virus (RAIV)-infected rats, lesions in the nasal cavity primarily involved the respiratory epithelium of the nasal septum and medial aspect of the nasoturbinates (Fig. 1). At day 1 PI, moderate numbers of neutrophils were present within or beneath the nasal respiratory epithelium or both. Thereafter (day 2 through 7 PI), a mixed population of neutrophils, macrophages, and fewer lymphocytes was present throughout the lamina propria in affected areas, with variable degrees of epithelial cell deciliation and degeneration (Figs. 2, 3). Concurrently (day 2 PI), a mild to moderate, patchy to diffuse epithelial cell necrosis occurred in areas where neutrophils were more abundant. Sloughed necrotic epithelial cells, intermixed with debris and degenerating inflammatory cells, formed a serocellular exudate in the nasal lumen adjacent to the most severely affected areas of the nasal mucosa. Regenerative epithelial changes appeared at day 4 PI and increased in magnitude at day 7 PI. The regenerating respiratory epithelium was mainly located at the free margins of the naso- and maxilloturbinates and along the medial septum, and was characterized by densely packed polyhedral epithelial cells with hyperchromatic, basophilic cytoplasm. Respiratory epithelial cell hyperplasia, characterized by thickening of the epithelium with increased cellularity, was focal and transient and vanished by day 14 PI (Fig. 4). From day 7 to 14 PI, the inflammatory component of the lesion receded to become minimal and had disappeared at day 28 PI. The transitional epithelium of the lateral meatus was less

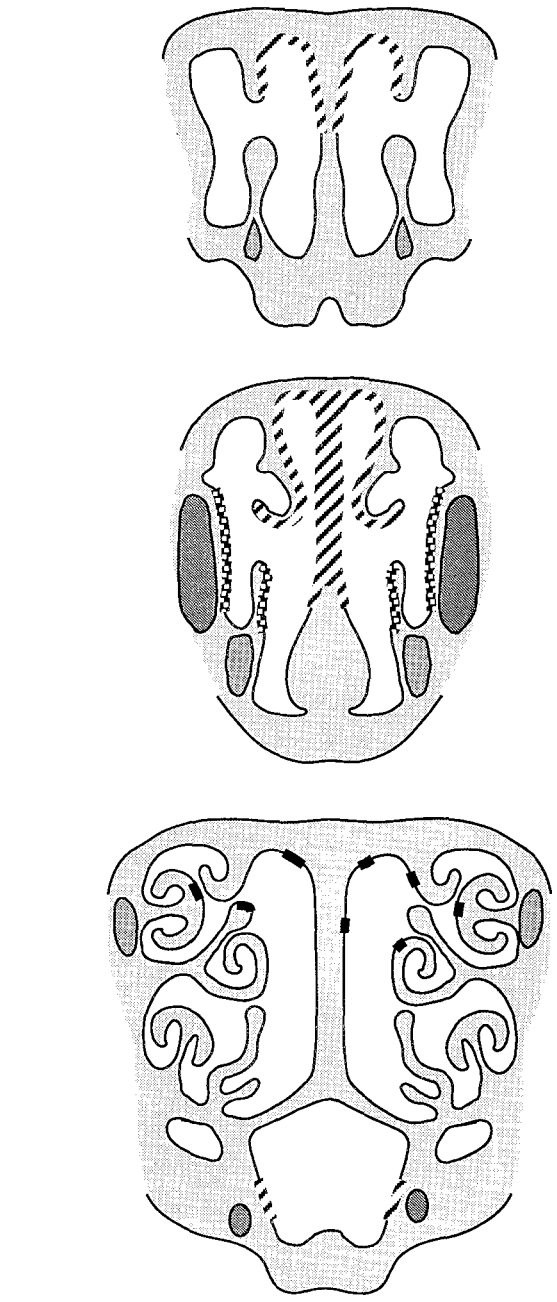

Fig. 1. Nasal cavity, rat. Map of nasal passages, modified from Mery et al.,[17] showing distribution of nasal lesions and influenza viral antigen.

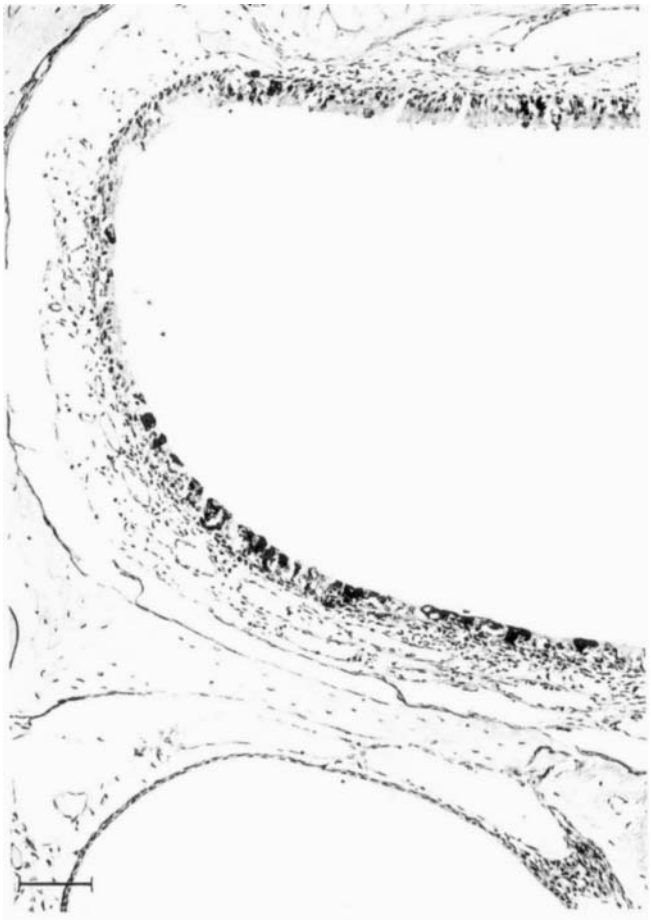

Fig. 2. Nasal cavity, rat, dorsal medial meatus, 2 days post-inoculation. The lesion is characterized by a mixed inflammatory cell infiltrate in the lamina propria subjacent to a region of epithelial degeneration. Numerous respiratory epithelial cells are immunoreactive for the viral antigen. Anti-influenza A virus stain using the streptavidin–biotin immunohistochemical system, hematoxylin counterstain. Bar = 180 μm.

severely affected, with lesions generally confined to the lateral wall (Fig. 1) and sparing the lateral aspect of the nasoturbinates, in spite of severe lesions in the respiratory epithelium of the medial aspect of this structure (Fig. 2).

Olfactory epithelial lesions were confined to small, apparently randomly scattered foci of sensory cell degeneration (Figs. 1, 8) at days 2 and 4 PI in areas exhibiting staining for viral antigen (see below). By days 7 and 14 PI, the olfactory lesions were confined to small foci of slight hypercellularity of the basal cell layer, indicating sensory epithelial regeneration.

Changes in the NALT were characterized by an enlargement of the lymphoid aggregates owing to the presence of increased numbers of small, well-differentiated lymphocytes (days 4 through 7 PI). These lym-

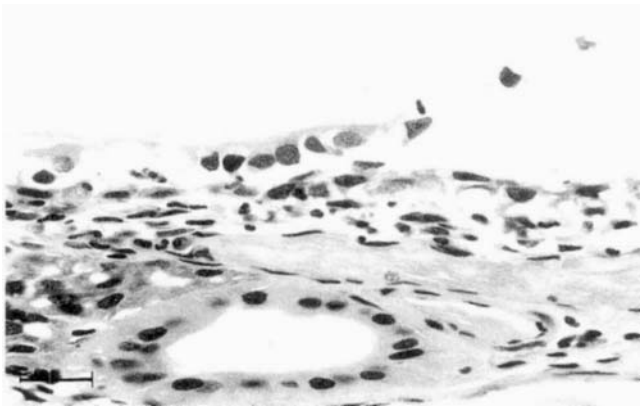

Fig. 3. Nasal cavity, rat, nasal septum, 2 days post-inoculation. The respiratory epithelium is deciliated and has numerous degenerate and/or necrotic epithelial cells. Intraepithelial and subepithelial neutrophils are present. HE. Bar = 70 μm.

Fig. 5. Nasal-associated lymphoid tissue, control animal, normal. HE. Bar = 70 μm.

phoid aggregates formed well-circumscribed follicles that often had germinal centers (Figs. 5[control], 6). Morphometric analysis revealed that the NALT of infected animals at day 7 PI had almost twice the cross-sectional area of the NALT in control animals (control mean ± SD, 0.43 ± 0.21 sq. mm; infected mean ± SD, 0.81 ± 0.12 sq. mm, $P < 0.05$). At day 14 PI, the lymphoid follicles were markedly reduced in size. The germinal centers had faded and widened and contained widely scattered lymphoid cells mixed with necrotic cellular debris. At day 28 PI, in two infected animals, lymphoid follicles were involuted with no evidence of germinal centers. The density of the lymphocyte population of the NALT was below that in controls and occasional hemorrhages were present (Fig. 7).

Viral antigen was confined to animals infected with RAIV. At day 1 PI, viral antigen was present along the nasal dorsal medial meatus, medial septum, and the medial aspect of the nasoturbinates (Fig. 1), as well as within the lymphoepithelium covering the NALT. The immunostained cells within the lymphoepithelium were mainly nonciliated with the morphological characteristics of M-cells. A few cells of the maxillary sinus epithelium also stained positively for viral antigen. At day 2 PI, viral antigen-positive cells were also present in the lateral meatus and less frequently on the maxilloturbinates. No respiratory epithelial cells stained for the virus on or after day 4 PI. Patches of olfactory epithelial cells that had the morphological characteristics of immature sensory cells, and adjacent sustentacular cells, were also positive on days 1, 2, and 4 PI (Fig. 8). The distribution of viral antigen staining in

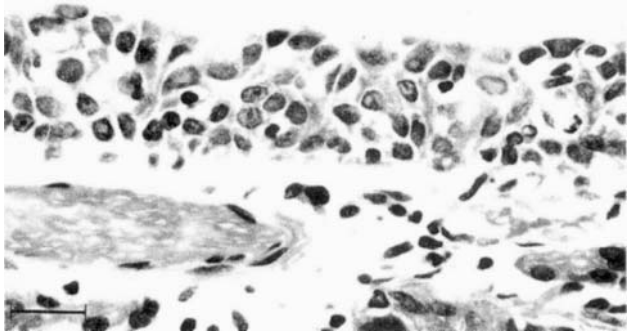

Fig. 4. Nasal cavity, rat, nasal septum, 7 days post-inoculation showing hyperplastic regenerating respiratory epithelium. Remnants of mononuclear inflammatory cell infiltrate are scattered throughout the lamina propria. HE. Bar = 70 μm.

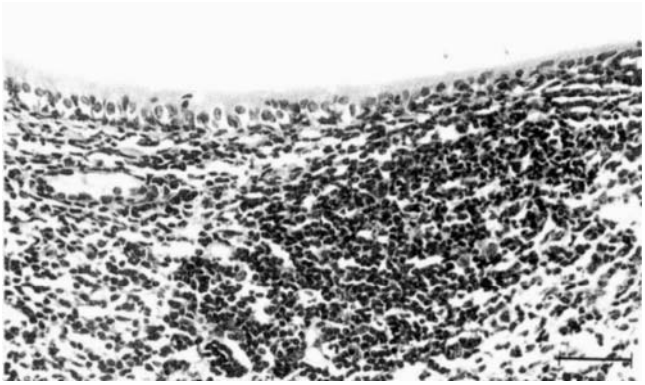

Fig. 6. Nasal-associated lymphoid tissue (NALT), rat, day 7 post-inoculation. The NALT is enlarged and contains well-differentiated lymphoid follicle. HE. Bar = 70 μm.

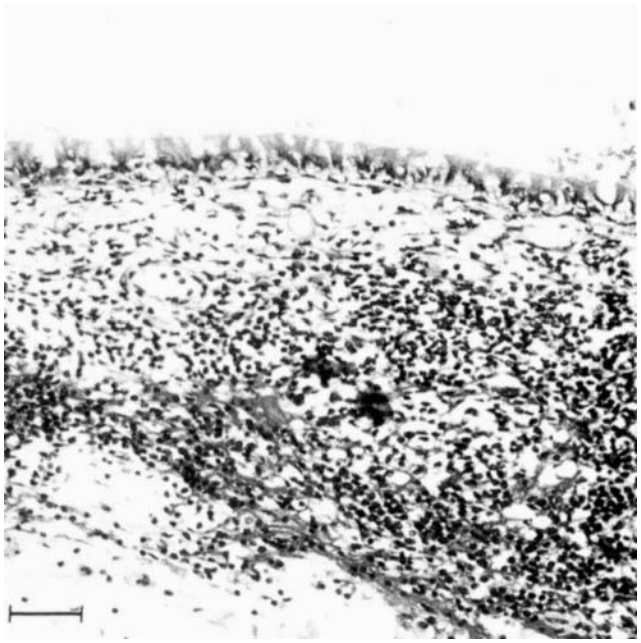

Fig. 7. Nasal-associated lymphoid tissue (NALT), rat, day 14 post-inoculation. The NALT has involuted and contains shrunken follicles. Congested vessels and hemorrhages are present. HE. Bar = 70 μm.

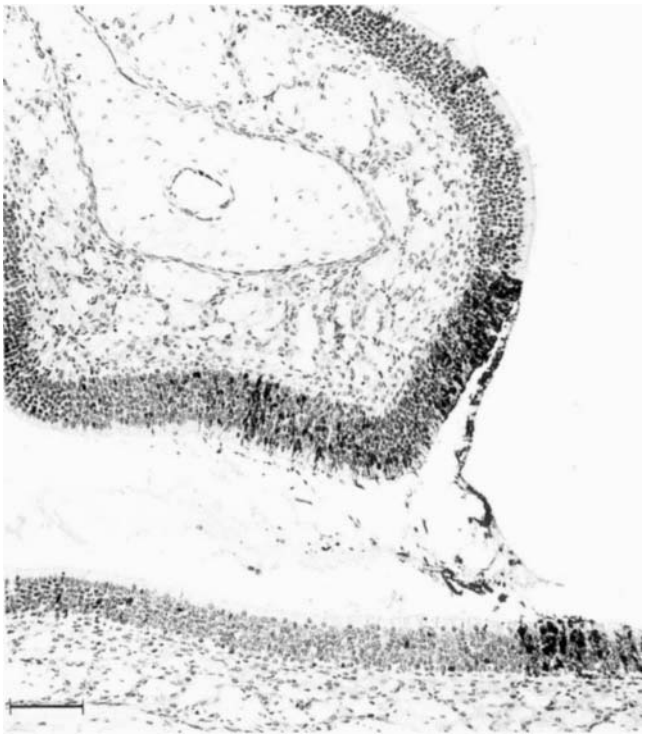

Fig. 8. Nasal cavity, rat, ethmoturbinate, olfactory epithelium, day 4 post-inoculation. Immunohistochemistry. Patches of olfactory cells are randomly positive for the viral antigen. This was the most severely affected area of olfactory epithelium observed in the present study. Anti-influenza A virus stain using the streptavidin–biotin immunohistochemical system, hematoxylin counterstain. Bar = 70 μm.

the olfactory epithelium appeared random, with some preponderance of staining in the medial airways.

Discussion

Numerous and extensive descriptions of the lesions induced by influenza viruses in humans have been reported,[7,14] but these descriptions are generally focused on intrathoracic lesions. Information on the nature of nasal lesions in humans was obtained following epithelial scrape biopsies collected within 24 to 32 hours of the onset of symptoms.[2,16] These observations, in spite of being very informative, did not provide detailed information on the distribution and morphogenesis of influenza virus-induced disease in the nose.

The present study of rat-adapted influenza virus (RAIV) infection in rats demonstrated that the rodent disease resembles human influenza infection, both with respect to time course and morphogenesis of the respiratory tract lesions. Furthermore, this model revealed the distribution of lesions and sites of viral antigen expression in the upper respiratory tract, including the presence and nature of lesions in the olfactory mucosa and nasal-associated lymphoid tissue (NALT). These results indicate that RAIV is a potentially valuable model for studies of influenza virus infections. Rat-adapted influenza virus (RAIV) exhibited a clear epitheliotropism directed towards the respiratory and, to a lesser extent, the transitional and olfactory epithelia, whereas the squamous epithelium was apparently resistant to infection. The significance of these differing epithelial susceptibilities to the influenza virus and the potential role of regional differences in viral exposure levels are unknown.

The observation that RAIV induced, albeit mild, olfactory epithelial damage may be relevant to the fact that upper respiratory tract infections, including those induced by influenza viruses, can permanently damage the sense of smell.[6] Interestingly, RAIV also induces olfactory epithelial lesions in neonatal Sprague-Dawley rats, including focal necrosis and inflammation.[8] The viral antigen observed in the olfactory epithelium in the present study was prominent in immature sensory cells and was not associated with an inflammatory response in this site. Furthermore, the viral antigen persisted in this location through day 4 PI when all immunocytochemical evidence of the virus was eliminated from the more severely affected respiratory epithelial regions. The prolonged presence of RAIV antigen in the olfactory mucosa, in contrast to the respiratory region, may be attributable to the absence of an inflammatory response in this site, presumably because the virus was not "visible" to the immune system in this location. The clinical significance of viral infection

of the olfactory epithelium merits further investigation.

The NALT is the only organized lymphoid structure in the nose and nasopharynx of rats. The NALT is situated in the posterior floor of the nose, adjacent to the septal window at the entrance of the nasopharyngeal duct.[21] In the present study, the NALT clearly responded to RAIV infection. The NALT is characterized by a specialized lining epithelium that contains a specific cell type: the M cell. When antigens adhere to this epithelium they are taken up by these specialized epithelial cells.[23] The positive staining of the M cells within the lymphoepithelium for the anti-influenza antibody is consistent with the proposed role of M cells in presenting antigens to the nasal immune system.

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References

1 Appleyard G, Mayer HB: Plaque formation by influenza viruses in the presence of trypsin. J Gen Virol **25:**351–357, 1974

2 Armstrong KL, Fraser DK, Faogoali H: Gastrointestinal bleeding with influenza virus. Med J Aust **154:**180–182, 1991

3 Bender BS, Small JPA: Influenza: pathogenesis and host defense. Semin Respir Infect 7(1):38–45, 1992

4 Bihun CGD, Percy DH: Morphologic changes in the nasal cavity associated with sialodacryoadenitis virus infection in the Wistar rat. Vet Pathol **32:**1–10, 1995

5 Cauna N, Hinderer KH, Wentges RT: Sensory receptor organs of the human nasal respiratory mucosa. Am J Anat **124:**187–192, 1969

6 Doty RL, Bartoshuk LM, Snow JBJ: Causes of olfactory and gustatory disorders. *In:* Smell and Taste in Health and Disease, ed. Getchell TV, Doty RL, Batoshuk LM, and Snow JBJ, pp. 449–462. Raven Press, New York, NY, 1991

7 Douglas RGJ: Influenza in man. *In:* The Influenza Viruses and Influenza, ed. Kilbourne ED, pp. 395–447. Academic Press, Inc, New York, NY, 1975

8 Dye JA, Morgan KT, Neldon DL, Tepper JS, Burleson GR, Costa DL: Characterization of upper respiratory disease in rats following neonatal inoculation with a rat-adapted influenza virus. Vet Pathol **33:**43–54, 1996

9 Ehrlich JP, Burleson GR: Enhanced and prolonged pulmonary influenza virus infection following phosgene inhalation. J Toxicol Environ Health **34:**259–273, 1991

10 Ehrlich JP, Gunnison AF, Burleson GR: Influenza virus-specific cytotoxic-T-lymphocyte activity in Fischer 344 rat lungs as a method to assess pulmonary immunocompetence: effect of phosgene inhalation. Inhal Toxicol **1:** 129–138, 1989

11 Everitt JI, Richter CB: Infectious diseases of the upper respiratory tract: implications for toxicologic studies. Environ Health Perspect **85:**239–247, 1990

12 Fraenkel DJ, Bardin PG, Sanderson G, Lampe F, Johnston SL, Holgate ST: Immunohistochemical analysis of nasal biopsies during rhinovirus experimental colds. Am J Respir Crit Care Med **150:**1130–1136, 1994

13 Gaush CR, Smith TF: Replication and plaque assay of influenza virus in an established line of canine kidney cells. Appl Microbiol **16:**588–594, 1968

14 Heilman C, LaMontagne JR: Influenza: status and prospects for its prevention, therapy, and control. Pediatr Clin N Am **37:**669–688, 1990

15 Horsfall FL: Neutralization of epidemic influenza virus. J Exp Med **70:**209–222, 1930

16 Loosli CG, Hamre D, Berlin BS: Airborne influenza A virus infections in immunized animals. Trans Assoc Am Physicians **66:**222–230, 1953

17 Mery S, Gross EA, Joyner DR, Godo M, Morgan KT: Nasal diagrams: a tool for recording the distribution of nasal lesions in rats and mice. Toxicol Pathol **22**(4):353–372, 1994

18 Miller RR: Viral infections of the respiratory tract. *In:* Pathology of the Lung ed. Thurlbeck WM, pp. 149–151. Thieme Medical Publishers, Inc, New York, NY, 1988

19 Schoenbaum SC: Economic impact of influenza: the individual's perspective. Am J Med **82**(Suppl. 6A):26–30, 1987

20 Soderberg M, Hellstrom S, Lundgren R: Bronchial epithelium in humans recently recovering from respiratory infections caused by influenza or mycoplasma. Eur Respir J **3:**1023–1028, 1990

21 Spit BJ, Hendriksen EGJ, Bruijntnes JP: Nasal lymphoid tissue in the rat. Cell Tissue Res **255:**193–198, 1989

22 Thomas LH, Fraenkel DJ, Bardin PG, Johnston SL, Holgate ST, Warner JA: Leukocyte responses to experimental infection with human rhinovirus. J Allergy Clin Immunol **94:**1255–1262, 1994

23 Von Poppel MNM, Van Der Berg TK, Von Rees EP: Reticulum cells in the ontogeny of nasal-associated lymphoid tissue (NALT) in the rat. Cell Tissue Res **273:**577–581, 1993

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