JOURNAL OF VIROLOGY, Jan. 1997, p. 226–236 Vol. 71, No. 1
0022-538X/97/$04.00+0


# Inflammatory Infiltration of the Upper Airway Epithelium during Sendai Virus Infection: Involvement of Epithelial Dendritic Cells

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Received 10 June 1996/Accepted 27 September 1996

**We undertook the present study to determine the nature of the cellular inflammatory response within the epithelial lining of the rat trachea during a Sendai virus infection. In particular, we aimed to investigate changes in the resident population of epithelial dendritic cells. Rats were infected with Sendai virus, and tracheal tissue was examined immunohistochemically at various times with a panel of cell-specific monoclonal antibodies. We found that Sendai virus infection was restricted to only the lumenal layer of epithelial cells and that virus nucleoprotein was present from days 2 to 5 postinfection. Starting around day 2 or 3, there was a large cellular influx consisting of macrophages, neutrophils, NK cells, and T cells; this coincided with expression of high levels of ICAM-1 on the basal (uninfected) layers of the epithelium. The T cells were mostly αβ T-cell receptor positive; however, a smaller influx of γδ T cells also took place. The number of resident dendritic cells increased markedly during infection, with numbers peaking around day 5 and remaining elevated 14 days later. The peak of the inflammatory response occurred on day 5 and declined thereafter, with the exception of dendritic cell and αβ T-cell numbers, which remained elevated. Starting around day 3, the tracheal epithelial cells expressed increasing levels of major histocompatibility complex class II antigen. This expression was maximal at day 5 and declined rapidly thereafter. In vitro culture of tracheal segments demonstrated that viral infection was not per se responsible for the upregulation of class II expression and that when cultured in the presence of gamma interferon, class II antigen was induced on epithelial cells.**

Viral infections of the upper respiratory tract remain a major cause of morbidity and mortality, especially in young children (9).

Antiviral immunity in the upper respiratory tract is dependent on the local production and release of secretory immunoglobulin A (IgA) onto the mucosal surface of the lumen. This is in contrast to the lung, where serum-derived IgG protects against lethal viral pneumonitis but does not protect against infection of ciliated epithelium within the trachea (41). Clearly, there are different mechanisms driving the generation of protective immunity at the mucosal surfaces of the upper and lower respiratory tracts. At present, most vaccines are administered parenterally; hence, they stimulate production of serum antibodies but fail to induce good local mucosal immunity. Although we are beginning to understand the nature of the immune response required to provide protective immunity in the upper airways, the main impediment appears to lie in our lack of knowledge of how to achieve this immunity.

Sendai virus is a parainfluenza type 1 virus which is exclusively pneumotropic in rodents and is structurally and serologically related to human parainfluenza type 1 virus; as such, Sendai virus has been used extensively as a model of viral respiratory tract infection. After inhalation, the virus is activated by a Clara cell-produced tryptase and infects only ciliated and secretory cells within the trachea and bronchi (30) and type II epithelial cells within the lung (7). Only the outer ciliated cells of the tracheal epithelium are infected; the basal cells remain uninfected.

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A number of studies have examined the nature of the lesions within the lungs of Sendai virus-infected rats, both histologically and by examining the cells obtained by bronchoalveolar lavage (4–6, 14, 45); however, as yet there have been no detailed studies of the nature of the cellular infiltrate which occurs within the tracheal epithelium during Sendai virus infection. As most viral infections of the respiratory tract begin in the nasopharynx and descend the tracheobronchial tree into the lung, infection in the trachea represents a critical stage at which effective antiviral immunity may prevent further spread of the virus and hence should represent the focus of efforts to develop efficient mucosal vaccination strategies.

Dendritic cells (DC) are a critical element in the generation of immune responses. Our laboratory has recently described a network of DC which are resident within the epithelial lining of the trachea both in rodents (19) and humans (18), where they function to sample antigenic material, process this material into a form in which it is associated with surface major histocompatibility complex (MHC) class II molecules, and present it to naive T cells within regional lymph nodes, hence facilitating the generation of protective local immune responses. In the present study, we examined the nature of the cellular changes which take place within the epithelial lining of the rat trachea during Sendai virus infection with particular reference to changes in the resident DC population.

## MATERIALS AND METHODS

**Animals.** Brown Norway rats (mean age, 5 weeks; approximately 130 g [body weight]) were used throughout this study. The animals were specific-pathogen-free derived and barrier maintained. Animals were housed on low-dust bedding to minimize background airway inflammation, as detailed previously (43), and were serologically free of Sendai virus infection and other known pathogens.

**Virus and inoculations.** The Enders strain of Sendai virus was grown in the allantoic cavity of 10-day-old embryonated chicken eggs, and allantoic fluid was stored at −70°C. Virus-free allantoic fluid was also stored at −70°C. Animals were anesthetized intraperitoneally with chloral hydrate (250 μl of 5.7% [wt/vol]

in phosphate-buffered saline [PBS] per 50 g of body weight) and 50 μl of allantoic fluid containing $10^3$ hemagglutinating units administered intranasally.

**Antibodies.** The following monoclonal antibodies (MAbs) were used: Ox6 (IgG1 anti-Ia antigen [29]), V65 (IgG1 anti-rat T-cell receptor [TCR] γδ [26]), ED2 (IgG2a anti-rat tissue macrophage [10]), 10/78 (anti-rat NKR-P1 produced by selecting for competition with 3.2.3 antibody [20a]), R73 (anti-rat TCR αβ [21]), RLN-9D3 (IgG2a anti-rat B cell [Seikagaku Corporation] [46]), Ox19 (IgG1 anti-rat T lymphocyte [49]), RP3 (IgM anti-rat neutrophil [44]), and W3/25 (IgG1 anti-rat CD4) and OX8 (anti-rat CD8) (2). WS16 (anti-Sendai virus nucleoprotein) was the generous gift of A. Portner, Virology Department, St. Jude's Children's Research Hospital, Memphis, Tenn. Anti-rat ICAM-1 (CD54; clone 1A29) was purchased from Seikagaku Corporation.

**Immunohistochemical examination of the trachea.** At appropriate times after Sendai virus infection, animals were sacrificed by intraperitoneal injection of lethobarb. After the aorta was severed, animals were perfused with 20 ml of PBS containing 0.2% (wt/vol) bovine serum albumin and 1% (wt/vol) heparin by injection into the right ventricle. Tracheas were removed and immediately fixed in cold ethanol for 30 min. Then the tissue was rehydrated in PBS, embedded in 100% OCT (Tissue Tek; Miles, Elkhart, Ind.), and snap frozen in liquid nitrogen-cooled isopentane. Tangential sections (18), 8- to 10-μm thick, were cut at −23°C and allowed to air dry at room temperature. Then sections were immunostained with primary antibody for 1 h at room temperature, washed three times (10 min for each wash) with PBS, and incubated with secondary antibody (biotinylated sheep anti-mouse Ig [Amersham] with 10% [vol/vol] normal rat serum) and streptavidin conjugated to horseradish peroxidase (Amersham), which was visualized by reaction with 3,3′-diaminobenzidine and 0.015% (vol/vol) hydrogen peroxide in PBS.

Sections were counterstained with hematoxylin, dehydrated, and mounted. Enumeration of stained cells was carried out as previously described (43).

All animal experimentation was done with the prior approval of the Institute for Child Health Animal Ethics Committee, which complies with conditions set down by the Australian National Health and Medical Research Council.

**In vitro culture of tracheal tissue.** Tracheas were removed from uninfected rats and cut into small segments of approximately 3 $mm^2$. These were cultured in RPMI medium (Gibco, Melbourne, Australia) containing 10% (vol/vol) fetal bovine serum (Trace; Biosearch Australia) at 37°C and 5% $CO_2$. Some segments were incubated with either Sendai virus-containing allantoic fluid or sterile allantoic fluid for up to 3 days. Other segments were incubated with 100 U of human recombinant gamma interferon (IFN-γ) per ml. At daily intervals, the tracheal segments were processed and immunohistochemically stained for Sendai virus nucleoprotein and class II expression as described above.

**Bronchoalveolar lavage (BAL).** Rat tracheas were exposed and catheterized. Lungs were then lavaged with two lots of 10-ml volumes of PBS containing 0.2% (wt/vol) bovine serum albumin (Sigma Chemical Co., Sydney, Australia) and 0.2% (wt/vol) lignocaine. Lavage fluids were centrifuged, and cytospin preparations were made with pelleted cells. These were air dried and stained with Leishmans stain (BDH), and differential cell counts were performed.

## RESULTS

**Viral infection of the tracheal epithelium.** To determine the exact time course of viral infection of the rat tracheal epithelium, we inoculated young adult brown Norway rats intranasally (i.n.) with Sendai virus in allantoic fluid. Although infected rats did not appear to exhibit any indication of respiratory distress throughout the course of the infection, immunohistochemical staining of tracheal tissue using a MAb directed against the nucleoprotein of Sendai virus demonstrated that infection of the epithelium took place. Figure 1 illustrates the time course of epithelial infection. One day after i.n. inoculation, we could not detect any significant viral infection of the epithelium (Fig. 1A) (in this case, we have presented a tangential section of epithelium which provides an en face view of the epithelial surface in order to show a greater surface area); however, between days 2 and 4 after infection, almost the entire tracheal epithelial surface became heavily infected with Sendai virus (Fig. 1B). As can be seen from this photomicrograph, virus infection was limited to those epithelial cells exposed to the lumen of the airway and the infection did not extend further than one or perhaps two cells into the epithelial layer. At no time during the course of infection did we detect any staining of subsurface or basal layers of the epithelium for nucleoprotein antigen. By day 5 after infection (Fig. 1C), viral antigen could be detected only in isolated epithelial cells, and by day 7, virtually no viral antigen was present (not shown).

**Induction of class II antigen expression.** We have previously demonstrated (18) that under steady-state conditions, the only class II (Ia) staining present within the epithelial lining of the rat trachea is associated with constitutive expression by a population of resident DC which form a contiguous network of antigen-presenting cells throughout the tracheal lining. The DC network in uninfected control animals from these experiments is illustrated in Fig. 2A. By using the MAb Ox6 to detect Ia expression, these same cells do not express antigens recognized by the MAbs OX12 (pan-B cell), ED2 (panmacrophage), and G4.18 (CD3; pan-T cell) but do stain with the OX62 MAb, which marks TCR γδ cells and DC (34).

We characterized the changes in Ia expression which occur within the tracheal epithelium during a primary Sendai virus infection. Until 2 days after i.n. introduction of Sendai virus, intraepithelial Ia expression remained restricted to DC-like cells, as in uninfected animals. However, during the period from days 3 to 5 after infection, Ia expression was increasingly associated with epithelial cells until by day 5 large areas of the epithelial lining layer stained strongly for Ia (Fig. 2B). Enumeration of tracheal intraepithelial $Ia^+$ DC during this period could be performed only in areas where Ia expression on epithelial cells was low to moderate. The numbers obtained (Fig. 3A) suggest large increases in DC density at that time; however, these may considerably underestimate the true number of DC. By day 7, the intensity of class II staining on epithelial cells was considerably reduced and was totally absent in many areas. At that time, accurate counts of DC could be made; from Fig. 2C and 3A, it is apparent that while epithelial class II expression was absent, the number of $Ia^+$ DC remained considerably higher than normal. By day 14, all epithelial class II cells had disappeared, while the number of DC remained high (Fig. 3A). Control animals treated with sterile allantoic fluid showed no change in the number of DC over a 14-day period (Fig. 3A) nor did they show any expression of class II antigen on their epithelial cells.

**Inflammatory infiltration of the tracheal epithelium.** By using MAbs to identify specific inflammatory cell types, we examined the nature and size of the inflammatory infiltrate occurring within the tracheal epithelium during the course of Sendai virus infection (Fig. 3 and 4A, B, and E). The first evidence of inflammatory cellular infiltrate was detectable at day 3 postinfection, when in conjunction with the increase in the number of resident $Ia^+$ DC (Fig. 3A), there was an influx of NKR-$P1^+$ NK cells (Fig. 3D) and $RP3^+$ neutrophils (Fig. 3B). No influxing $ED2^+$ macrophages were present until day 4 postinfection (Fig. 3C). In all cases, the maximum number of infiltrating cells was seen at day 5 (Fig. 3 and 4A, B, and E). Thereafter, the numbers of $ED2^+$ macrophages and $RP3^+$ neutrophils declined rapidly until by day 7 the numbers had essentially returned to steady-state levels. Between days 5 and 7, there was a reduction in the number of NKR-$P1^+$ NK cells; however, the numbers continued to remain significantly elevated until after day 14. The changes in the number of resident $Ia^+$ DC are particularly interesting in that there was a decrease between days 5 and 7 which appeared to plateau thereafter and the numbers remained significantly elevated after day 14. We have not continued the time course past day 14 to determine how long the DC numbers remained elevated.

Animals treated with sterile allantoic fluid showed little or no influx of any of the cell types examined (Fig. 3). No B cells were detected in the trachea at any stage of infection.

**Induction of ICAM-1.** Epithelial sections taken at various times during the course of Sendai virus infection were stained for ICAM-1 expression. Until day 3 after infection, little or no ICAM-1 could be detected; however, between days 3 and 5,

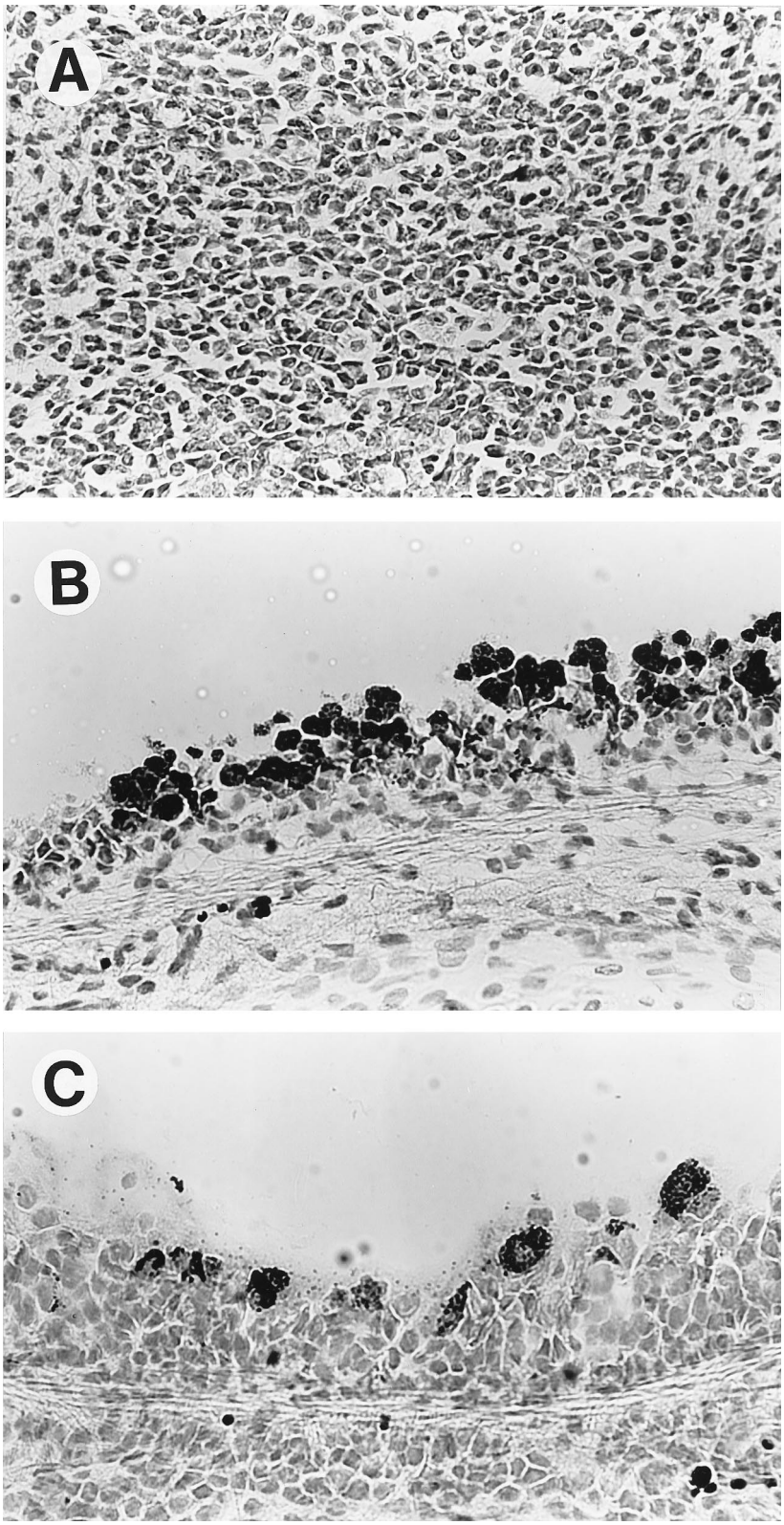


FIG. 1. Tracheal sections from Sendai virus-infected rats. Photomicrographs of frozen sections stained with MAb WS16 directed against the nucleoprotein of Sendai virus are shown. (A) A tangential section of tracheal epithelium 1 day after i.n. infection with Sendai virus that shows the total absence of any virus-infected epithelial cells. (B) Tracheal section that shows the pattern of viral infection seen from days 2 to 4 after infection. Only those epithelial cells exposed to the tracheal lumen were infected; basal cells remained free of virus. (C) Tracheal section 7 days after infection that shows that only relatively few epithelial cells remained infected with Sendai virus.

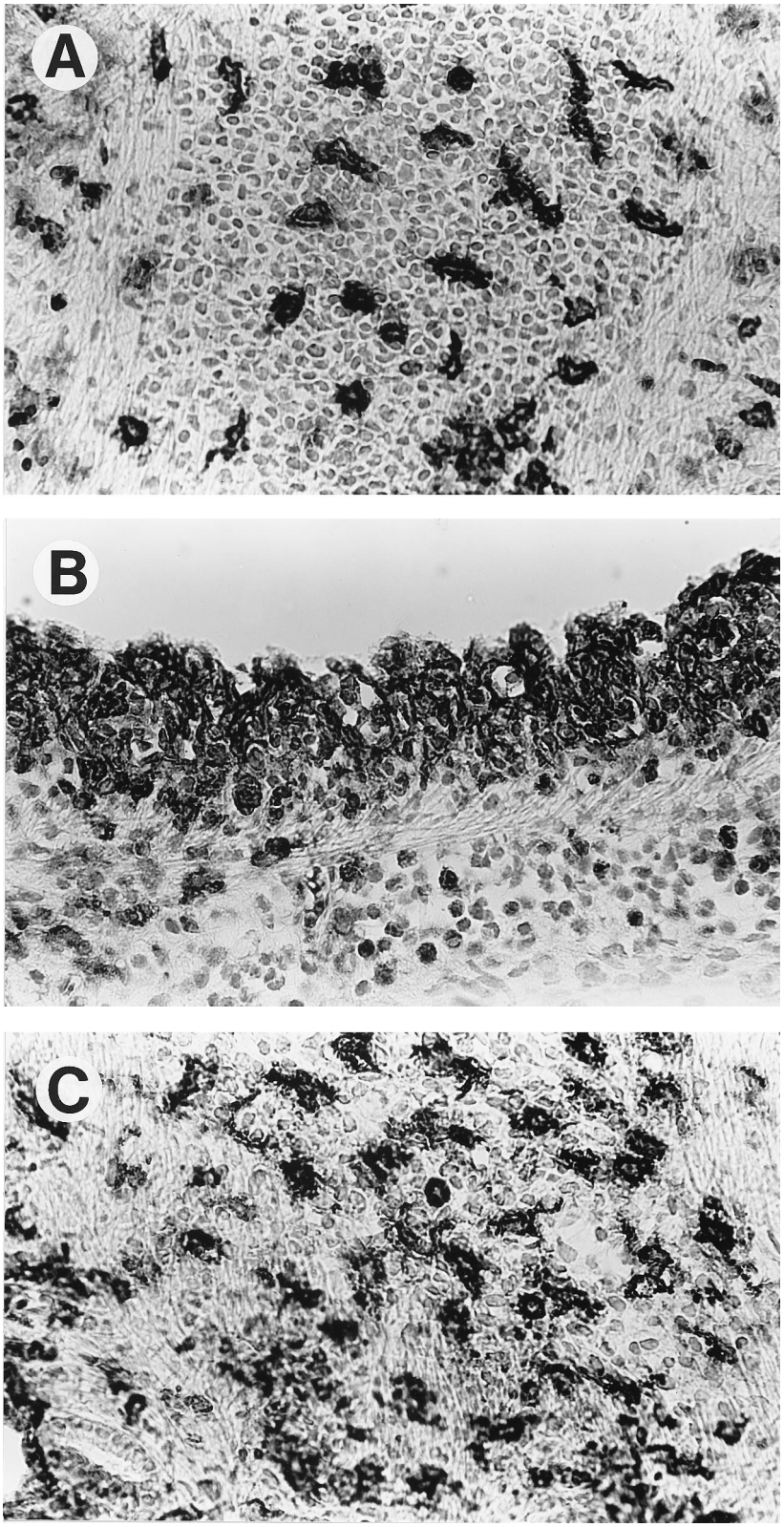


FIG. 2. Representative photomicrographs of rat tracheal epithelium immunohistochemically stained with MAb Ox6 (anti-Ia). (A) Constitutive $Ia^+$ staining pattern associated with resident DC under steady-state uninfected conditions and in epithelial tissue during the first 2 days after Sendai virus infection; (B) $Ia^+$ staining pattern 5 days after infection, showing massive induction of class II antigen expression on tracheal epithelial cells; (C) $Ia^+$ staining pattern 7 days after infection, showing downregulation of epithelial class II expression with a persistence in the increased number of class II-expressing DC.

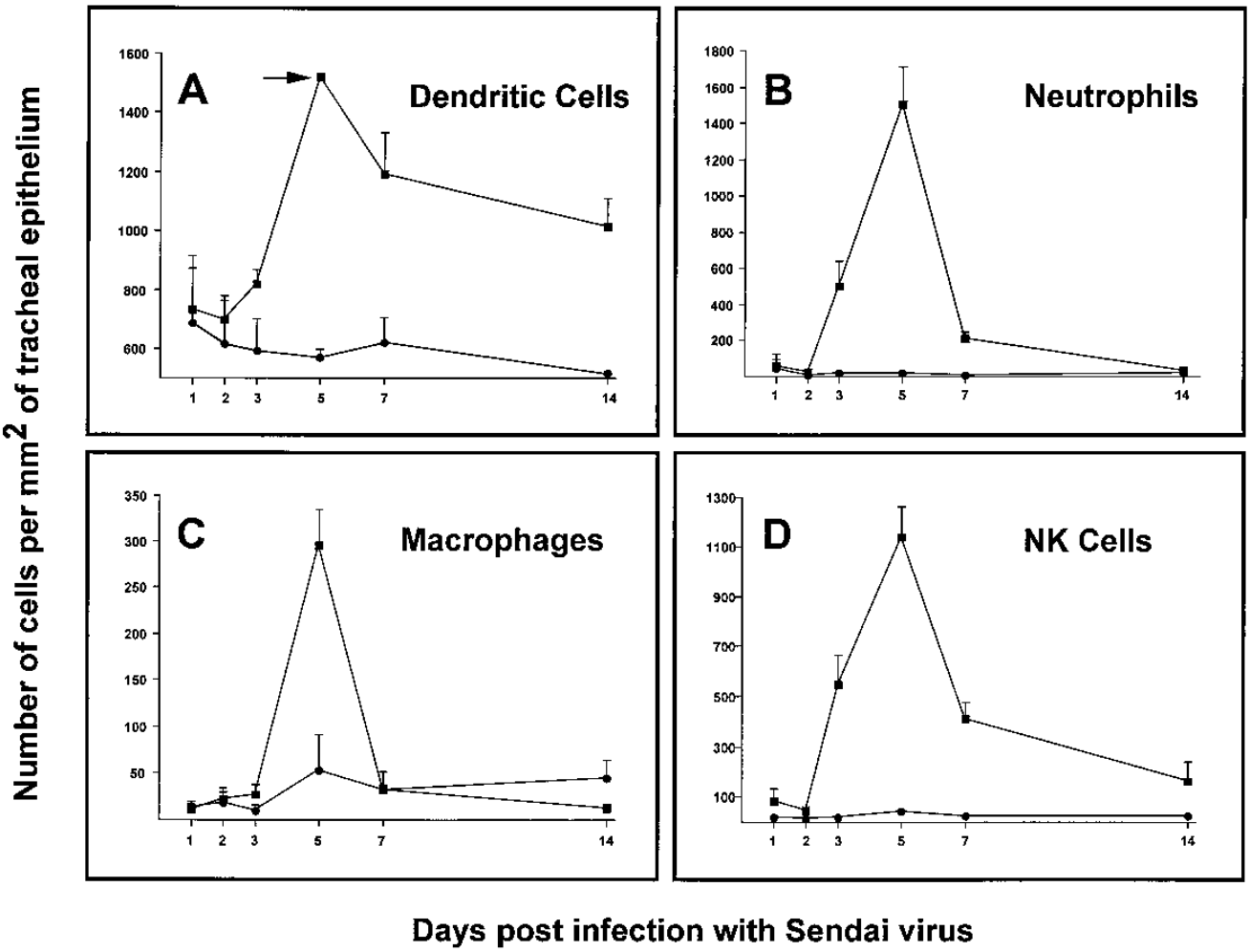


FIG. 3. Cellular inflammatory response in the tracheal epithelium after infection with Sendai virus. At designated times after i.n. infection, frozen tracheal tissue was sectioned and immunohistochemically stained with MAbs specific for each of the cell types examined. Data are the mean number of cells per square millimeter of tracheal epithelium derived from four to five animals per time point plus the positive standard deviation. Symbols: ■, Sendai virus-infected animals; ●, sterile allantoic fluid-inoculated control animals.

there was a steady increase in epithelial ICAM-1 expression, which first occurred as a band of staining associated with those epithelial cells closest to the basement membrane (Fig. 4F), while those cells closer to the lumen remained negative. This was the pattern of expression in most of the epithelium; however, in some areas, ICAM-1 expression extended throughout the epithelium to the lumenal surface. ICAM-1 expression appeared to parallel the influx of inflammatory cells described above in that expression was maximal at day 5 and then proceeded to disappear until day 7 when it was almost undetectable.

**Infiltration of T cells into the tracheal epithelium.** Using a mixture of MAbs Ox19 and R73, we determined the total number of T lymphocytes entering the tracheal epithelium during Sendai virus infection (Fig. 5A). The numbers rose rapidly after day 3 and peaked at day 5. Between days 5 and 7, the numbers rapidly declined, but between days 7 and 14, there was a much slower rate of decline. The profile representing changes in $R73^+$ αβ TCR lymphocytes (Fig. 5B) reflects almost exactly that of the total T-cell influx. However, the changes in $V65^+$ γδ TCR lymphocytes were somewhat different. Figure 4C and D are photomicrographs depicting the maximum influx of αβ TCR T cells and γδ TCR T cells, respectively. Uninfected animals had practically no $\gamma\delta^+$ cells resident within the tracheal epithelium; however, a significant influx of these cells was apparent at day 3 in infected animals, with numbers peaking at day 5. Compared to the magnitude of the αβ T-cell response (approximately 1,800 cells per $mm^2$), the γδ response (130 cells per $mm^2$) was quite small, and the numbers of γδ cells fell rapidly until by day 7 steady-state numbers were restored. This rapid decline also contrasted with the relatively slow decline in αβ cell numbers.

Tracheal sections were stained with MAbs W3/25 and OX8 to detect CD4- and CD8-expressing cells, respectively. The results (Fig. 6) show a large influx of both CD4- and CD8-positive cells with maximum numbers at day 5 postinfection.

**Induction of class II expression by IFN-γ.** To determine whether IFN-γ was responsible for induction of class II expression on the tracheal epithelium, we stained tracheal tissue taken at day 3 postinfection with MAb DB1 (25), which is specific for rat IFN-γ. The results of these experiments were inconsistent in that some sections stained positive for IFN-γ while others were negative. We believe that this may be a

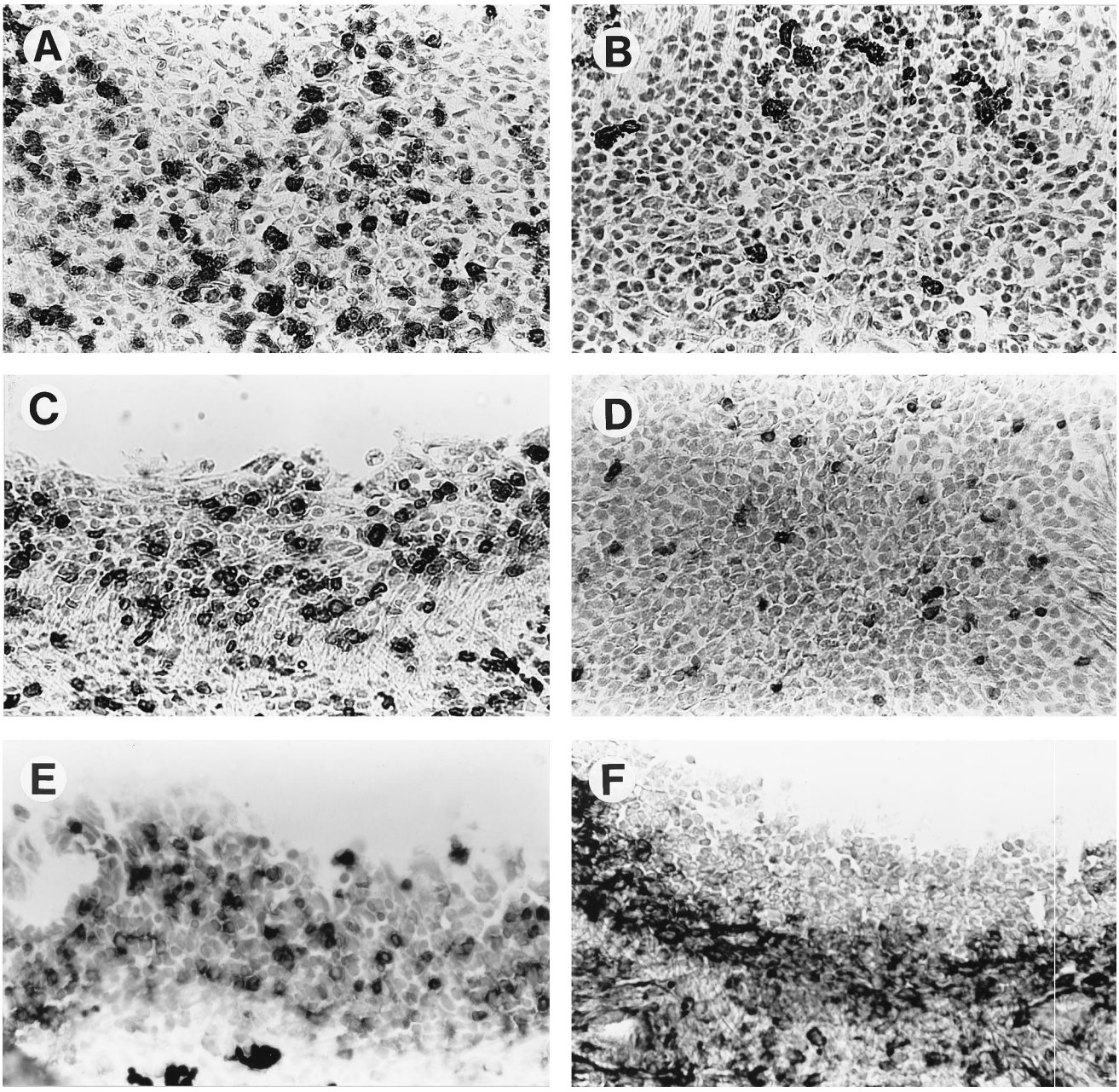


FIG. 4. Representative photomicrographs of rat tracheal epithelium obtained 5 days after Sendai virus infection and immunohistochemically stained with MAbs RP3 for neutrophils (A), ED2 for macrophages (B), R73 for αβ T cells (C), V65 for γδ T cells (D), 10/78 for NKR-P1-positive NK cells (E), and anti-ICAM (F).

reflection of technical problems associated with the fixation of tissue for IFN-γ staining.

We further sought to establish whether class II induction was a consequence of de novo synthesis resulting from viral infection of epithelial cells and whether exogenous IFN-γ could induce epithelial class II expression. To accomplish this, we cultured small segments of tracheal tissue from normal animals and infected some of these with Sendai virus while others were incubated with human recombinant IFN-γ. Immunohistochemical staining of virus-infected tracheal segments showed that, as expected, class II expression was present on resident DC shortly after removal (Fig. 7A); however, after 2 or 3 days in culture, this staining had virtually disappeared (Fig. 7B), which is consistent with known patterns of migration of DC out of isolated tissue segments (1). By day 3 after viral infection, nucleoprotein staining was still intense on cultured epithelial segments (Fig. 7C), indicating that no clearance of the virus by immune mechanisms was occurring. During the 4 days in which virus-infected tracheal segments were examined, no epithelial class II induction was detected, indicating clearly that Sendai virus is not able to directly induce MHC class II expression on epithelial cells. In a parallel experiment in which recombinant human IFN-γ was added to noninfected cultures, class II induction was observed (Fig. 7D); however, this occurred in patches and was not as widespread as that seen in in vivo Sendai virus infections. These results strongly suggest that

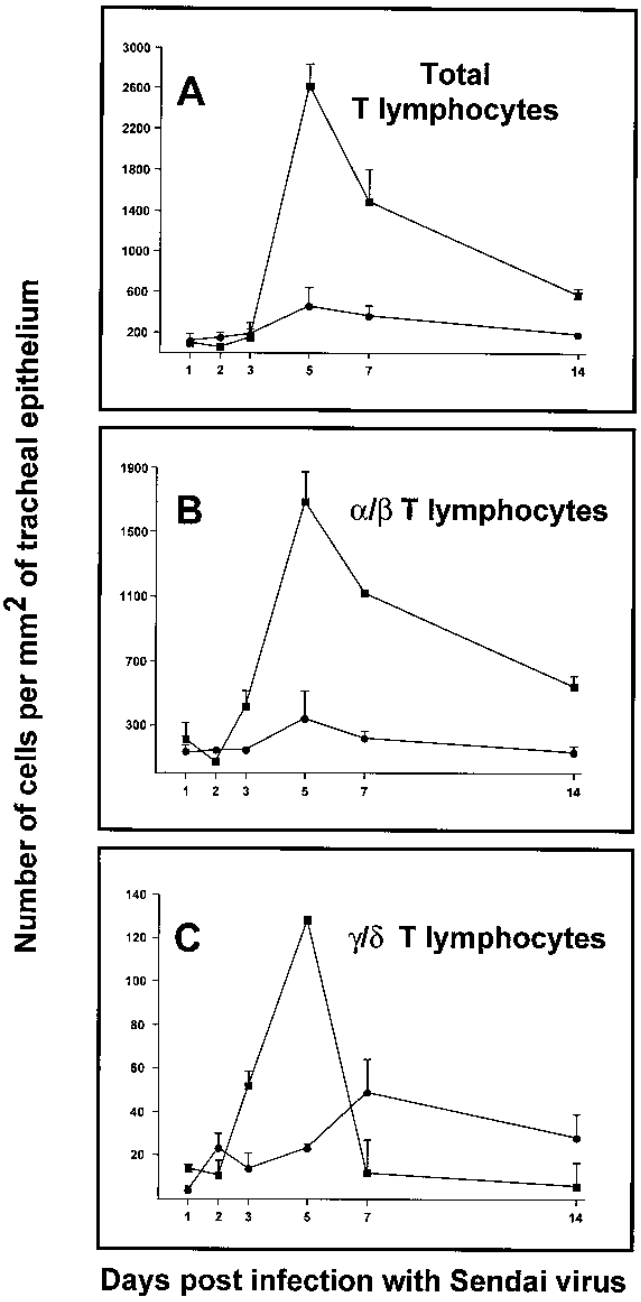


FIG. 5. T-cell influx into the tracheal epithelium during the course of infection with Sendai virus. At day 5 after i.n. infection with Sendai virus, frozen tracheal tissue was sectioned and immunohistochemically stained with MAbs Ox19 and R73 for total T-cell numbers (A), R73 for αβ T cells (B), and V65 for γδ T cells (C). Data are the mean number of cells per square millimeter of tracheal epithelium derived from four to five animals per time point plus the positive standard deviation. Symbols: ■, Sendai virus-infected animals; ●, sterile allantoic fluid-inoculated control animals.

IFN-γ produced as part of the host response to the virus is responsible for the induction of MHC class II expression on the tracheal epithelium during Sendai virus infection.

**BAL cellular response.** Leukocytes migrating into the lumen of the airways or lung air spaces were recovered by BAL from animals infected with Sendai virus. Cytospin preparations were differentially stained with Leishmans stain or immunohistochemically stained with Ox6. BAL fluids from infected animals contained almost 100% alveolar macrophages until day 3 after infection when BAL fluids contained 25% neutrophils (Fig. 8). The percentage of neutrophils increased until by day 5 approximately 80% of BAL cells were neutrophils. By day 7, the percentage had been reduced to 50%. The profile of neutrophils recovered by BAL corroborates the cellular influx of neutrophils into the tracheal epithelium and demonstrates that once neutrophils have migrated into the epithelium, they continue through the epithelium into the lumen of the airway. Staining of BAL cells with Ox6 MAb (not shown) demonstrated that some cells became class II positive between days 3 and 7 postinfection; however, only weak class II staining was present and the number of these cells was very low. These cells appeared regular in shape, with a foamy appearance to the cytoplasm normally seen in rat alveolar macrophages, and did not have the characteristic comet morphology associated with DC in BAL fluid. The fact that none of the BAL cells appeared morphologically like DC suggests that DC influxing into the epithelium do not enter the lumen but remain within the epithelium until migrating to regional lymph nodes.

## DISCUSSION

It is becoming increasingly clear that the nature of the immune response mounted within the trachea is a critical element in the development of protective antiviral immunity within the airways (36). It is also clear that the presence of antigen-presenting cells at the site of antigenic exposure is crucial for the development of effective local and systemic immune responses. In the case of herpes simplex virus type 1 (HSV-1) infection of the cornea, it has been shown that the resident Langerhans cells control the development of immunologically mediated inflammatory responses to the virus (16).

Previous work from our laboratory has demonstrated the presence of an extensive network of resident DC situated within the epithelial lining of the rat trachea (18). Furthermore, we have shown that this is a dynamic population of cells with a much shorter turnover time compared with those of other DC populations, such as Langerhans cells present within the epidermis of the skin (17), and that the tracheal DC population is also able to expand rapidly in response to local inflammatory stimuli, such as whole bacteria (32). We undertook the present study to determine the response of these epithelial DC to a virus-induced inflammation and to correlate this response with the influx of other bone marrow-derived cells into the epithelium. In this way, we hoped to elucidate the role of the tracheal DC population in inflammatory and immune responses in the upper airways. Prior to this study, there have been no published reports examining the nature of the tracheal epithelial inflammatory response during Sendai virus infection. To enable us to correlate cellular changes within the epithelium with viral infection of epithelial cells, we immunohistochemically stained tracheal sections taken throughout the course of a Sendai virus infection with a MAb specific for the nucleoprotein antigen of Sendai virus. This enabled us to confirm previous observations that virus infection is limited to those epithelial cells exposed to the lumen of the airway and that more basally located cells are not infected (30). This limited cellular specificity is particularly intriguing, considering the enormous influx of inflammatory cells which was found in the epithelium during infection, and highlights the importance of these epithelial cells in initiating inflammatory and immune responses in this tissue. Since the Sendai virus was administered i.n., this should allow deposition of infectious virus directly onto the epithelial surface. However, since viral antigen became detectable in most animals only 2 or 3 days after i.n.

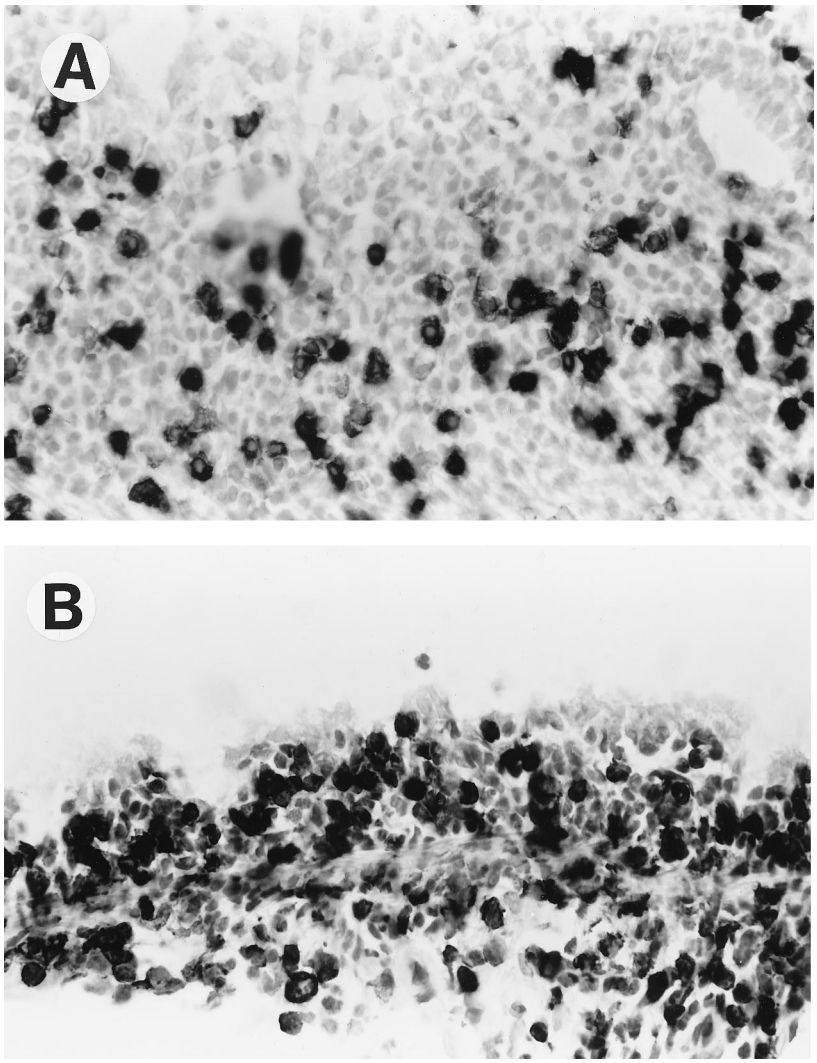


FIG. 6. At day 5 after i.n. infection with Sendai virus, frozen tracheal tissue was sectioned and immunohistochemically stained with MAbs W3/25 to detect CD4-expressing cells (A) and OX8 to detect CD8-expressing cells (B).

infection, this lag period presumably represents the time necessary for infection to occur and for a sufficient amount of viral antigen to be produced to enable detection by immunohistochemical methods. The maximum amount of epithelial viral antigen was present 4 or 5 days after infection and thereafter rapidly declined until only isolated staining was present at day 7.

A detailed immunohistochemical analysis was performed on epithelial sections with a series of MAbs previously validated and commonly used as markers of specific inflammatory and immune cell types. These studies revealed that as early as day 3 postinfection, noticeable changes within the epithelium were commencing. These manifestations were increases in the number of resident epithelial DC and an influx of NK cells and neutrophils into the epithelial layer. This was reflected in the large increase in neutrophils recovered from the airways by BAL on day 3. Changes in macrophage numbers followed approximately 1 day behind this initial influx. The maximum numbers of influxing cells were found on day 5 for all cell types and declined thereafter; however, the rate of decline differed, depending on the cell type. Whereas macrophage and neutrophil numbers returned almost to steady-state levels by day 7, DC and NK cell numbers remained high for at least another 7 days. This slow decline may suggest that it is important for the epithelium to maintain high numbers of these particular cells for some time after the initial infection, either as a protection against viral superinfection or infection by other opportunistic organisms.

Similar experiments examined the nature of the T-cell influx. In this case, a large increase in the number of TCR $\alpha\beta$ T cells was observed with kinetics similar to those seen with DC and NK cells. While there was also an increase in TCR $\gamma\delta$ T cells, the numbers reached only approximately 10% of those of $\alpha\beta$ T cells. While these two populations of T cells displayed similar influx kinetics, they differed significantly in terms of their rates of disappearance from the epithelium. Whereas $\gamma\delta$ T cells were essentially gone by day 7, the numbers of $\alpha\beta$ T cells were still significantly elevated after day 14, resembling the slow decline seen in DC and NK numbers. Previous studies investigating the involvement of $\gamma\delta$ T cells in viral airway infections have used BAL fluids as their source of cells.(11) In the murine influenza virus infection model (3), there seems to be a staged response

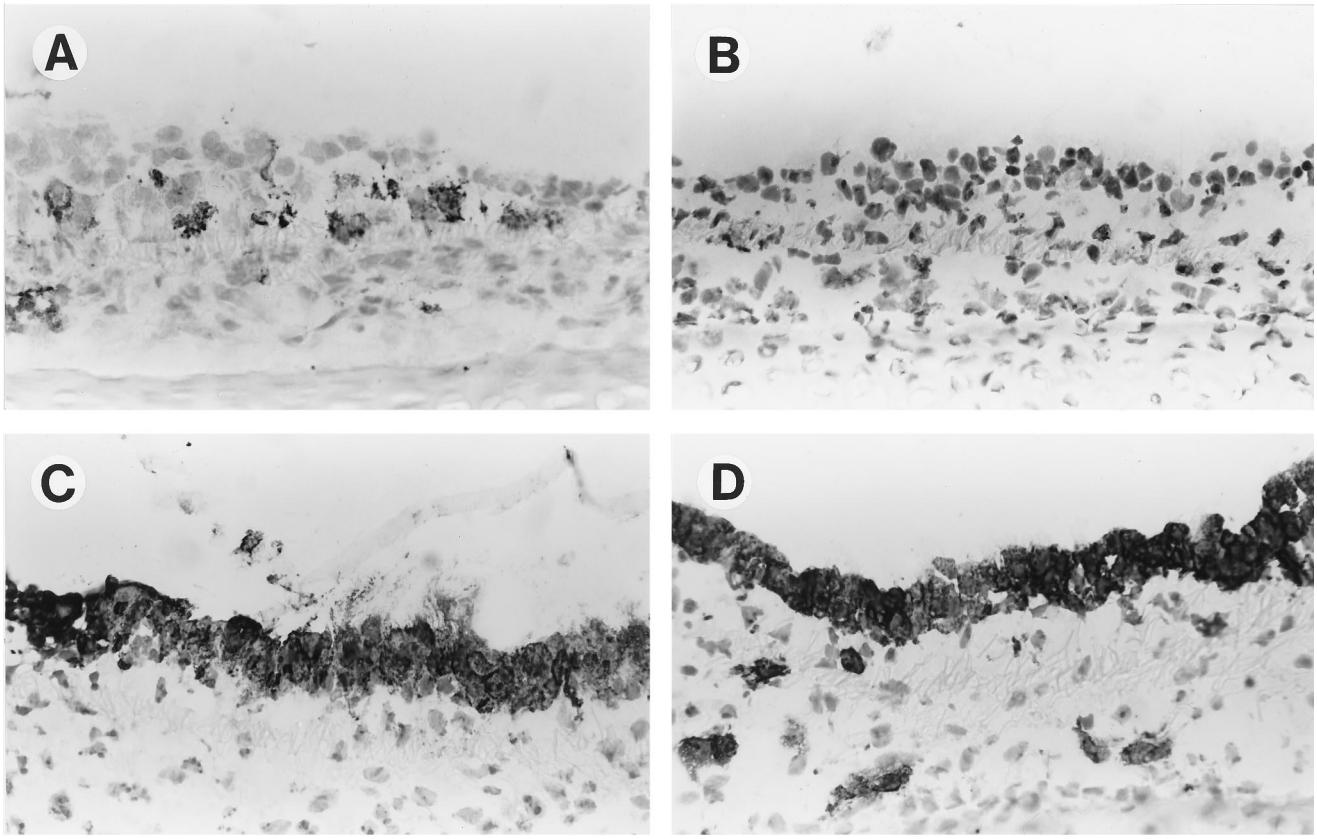


FIG. 7. In vitro culture of tracheal segments. Segments of normal rat trachea were cultured in vitro, infected with Sendai virus, and stained with MAb, WS16, against Sendai virus nucleoprotein. A representative photomicrograph of the staining pattern seen from days 1 to 3 is shown (C). Similarly infected segments were stained for Ia expression with the MAb Ox6 after days 1 (A) and 3 (B). Other cultured segments were not infected with virus but were incubated with 100 U of IFN-γ per ml and stained with Ox6 after day 3 (D).

in which αβ T cells appear first during viral infection and are followed some days later, after clearance of the virus, by the appearance of γδ T cells. Interestingly, in this latter study (3), more γδ T cells were found by in situ hybridization than were detected by flow cytometry, suggesting that the method used to detect these cells is important. The situation with Sendai virus infection in mice is somewhat different in that murine infections have an early and relatively minor TCR γδ response (20, 37) which is similar to that found in the rat trachea. The murine TCR αβ response also appears to be significantly prolonged(37). Using a murine model of respiratory syncytial virus, Openshaw (38) also found that the BAL γδ T-cell response was relatively minor compared to the αβ T-cell response.

The observation that the entire tracheal epithelium became class II positive during the peak phase of Sendai virus infection is intriguing; however, the function of class II expression by epithelial cells during infection is unclear. In other situations where viral infection has been found to upregulate MHC class II antigen expression on epithelial surfaces, such as HSV-2 infection of the mouse vaginal epithelium (33), it is not clear whether it is the virus which directly induces class II expression or whether IFN-γ is responsible. In the case of cytomegalovirus induction of class II expression in vivo in rat tissues (47) or in isolated endothelial cells (48), the virus appears to be able to directly induce class II expression. On epithelial surfaces, induction of class II expression may serve any of several functions. Thus, human bronchial epithelial cells expressing class II antigen are known to be capable of acting as accessory cells in stimulating immune responses (24), and there is evidence that HSV infection of the corneal epithelium may be important in presentation of viral antigen to T cells (12). Recently, Jiang et al. (23) demonstrated that target expression of class II molecules may regulate NK cell function and target recognition via a mechanism involving endogenous peptide presentation on class II molecules. If, as suggested by Ljunggren and Kärre (27), NK cells choose their targets cells on the basis of their expression of MHC antigens, then class II expression by airway epithelial cells may provide a mechanism whereby influxing NK cells are able to recognize and kill only those cells expressing class II complexed with viral antigen, while those expressing class II molecules with self peptides are spared. Since virtually all of the lumenal tracheal epithelial cells appeared to express class II, this process would result in killing only those outer epithelial cells infected with Sendai virus and hence expressing viral peptide within MHC class II while those basal cells not infected would remain untouched. Although we have no direct evidence to support this hypothesis, a mechanism such as this may be vitally important in maintaining the integrity of the epithelial covering of the respiratory tract and ensuring rapid recovery of a sensitive tissue after viral infection.

Induction of epithelial class II expression in our model could result from exposure to either granulocyte-macrophage colony-stimulating factor or IFN-γ. We have had variable results in staining the epithelium for IFN-γ; however, it is clear that isolated segments of tracheal tissue are not able to upregulate

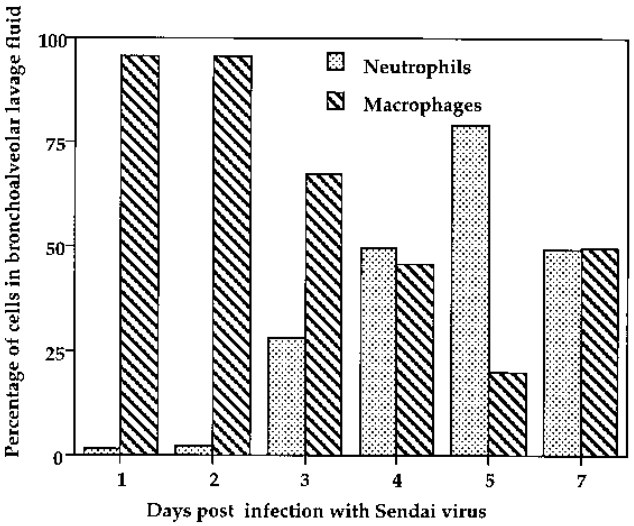


FIG. 8. Cellular composition of BAL fluid during Sendai virus infection. At designated times after Sendai virus infection, BAL was performed with PBS containing 0.35% (wt/vol) lignocaine. Cytospin preparations of the cells obtained were prepared and stained with Leishmans stain, and differential counts were performed. Data are percentages of macrophages and neutrophils and are the means of three to five animals per time point.

class II expression in direct response to Sendai virus infection or in the absence of influxing inflammatory cells. Since these segments are able to express class II when cultured with recombinant IFN-γ and since Sendai virus is known to be a potent inducer of IFN-γ from peripheral blood mononuclear cells (8), this strongly suggests that IFN-γ produced by either macrophages, T cells, or NK cells is responsible for the induction of class II expression during viral infection. Production of IFN-γ by inflammatory cells may also be responsible for the induction of epithelial ICAM-1 (28, 40) expression observed during Sendai virus infection. We are currently using PCR to confirm this and to determine the source and time course of IFN-γ production. In the murine Sendai virus model, BAL fluid IFN-γ is maximal 7 to 10 days after infection (33); however, in our rat model, epithelial class II expression has resolved by this time.

In a previous work (32), we showed that acute inflammatory stimuli, such as whole bacteria, are able to rapidly recruit large numbers of DC into the tracheal epithelium and that this influx occurs at the same time as the neutrophil response. Here we used a rat model of Sendai virus infection to examine changes in the epithelial DC population. Again, it is clear that there is a rapid influx of DC into the tracheal epithelium which occurs at the same time as the neutrophil and NK cell influx, i.e., day 3, approximately 1 day after virus can first be detected. It is not known whether these cells come from bone marrow reserves or from circulating precursor populations in the blood; however, it is clear that DC numbers remain significantly elevated for some time after the virus has been cleared and that epithelial class II expression has disappeared. After leaving the trachea, these DC migrate to the draining lymph nodes, where they are able to present processed viral antigen to naive T cells. Thus, DC able to present viral antigen have been isolated from the regional lymph nodes of mice infected with influenza virus (15).

In summary, we have presented data which describe important cellular changes that occur within the tracheal epithelium during the course of a viral infection and have highlighted the potentially important role played by epithelial DC in the development of antiviral immune responses. These data support previous work by Nonacs et al. (35) describing the potency of DC in presenting viral antigens and that by Frankel et al. (13) describing DC involvement in the pathology of viral infections at epithelial surfaces as well as the suggestions by Janeway (22), Matzinger (31), and Ridge et al. (42) that the DC may exist primarily to alert the immune system to the impending danger of a viral infection.

## ACKNOWLEDGMENTS

This work was supported by GlaxoWellcome Australia. P.G.H. was supported by the National Health and Medical Research Council of Australia.

## REFERENCES

1. **Austyn, J. M.** 1993. Dendritic cells in transplantation. Adv. Exp. Med. Biol. **329:**489–494.
2. **Barclay, A. N.** 1981. The localization of populations of lymphocytes defined by monoclonal antibodies in rat lymphoid tissues. Immunology **42:**593–600.
3. **Carding, S. R., W. Allan, S. Keyes, A. Hayday, K. Bottomly, and P. Doherty.** 1990. Late dominance of the inflammatory process in murine influenza by $\gamma/\delta^+$ T cells. J. Exp. Med. **172:**1225–1231.
4. **Castleman, W. L.** 1983. Respiratory tract lesions in weanling outbred rats infected with Sendai virus. Am. J. Vet. Res. **44:**1024–1031.
5. **Castleman, W. L.** 1984. Alterations in pulmonary ultrastructure and morphometric parameters induced by parainfluenza (Sendai) virus in rats during postnatal growth. Am. J. Pathol. **114:**322–335.
6. **Castleman, W. L., L. J. Brundage-Anguish, L. Kteitzer, and S. B. Neuenschwander.** 1987. Pathogenesis of bronchiolitis and pneumonia induced in neonatal and weanling rats by parainfluenza (Sendai) virus. Am. J. Pathol. **129:**227–286.
7. **Castleman, W. L., P. J. Northrop, and P. K. McAllister.** 1989. Replication of parainfluenza (Sendai) virus in isolated rat pulmonary type II alveolar epithelial cells. Am. J. Pathol. **134:**1135–1142.
8. **Costas, M. A., D. Mella, M. Criscuolo, A. Diaz, S. Finkielman, V. E. Nahmod, and E. Arzt.** 1993. Superinduction of mitogen-stimulated interferon-γ production and other lymphokines by Sendai virus. J. Interferon Res. **13:** 407–412.
9. **Denny, F. W.** 1995. The clinical impact of human respiratory virus infection. Am. J. Respir. Crit. Care Med. **152:**S4–S12.
10. **Dijkstra, C. D., E. A. Dopp, P. Joling, and G. Kraal.** 1985. The heterogeneity of mononuclear phagocytes in lymphoid organs: distinct macrophage subpopulations in rat recognized by monoclonal antibodies ED1, ED2 and ED3. Adv. Exp. Med. Biol. **186:**409–419.
11. **Doherty, P. C., W. Allan, and M. Eichelberger.** 1992. Roles of αβ and γδ T cell subsets in viral immunity. Annu. Rev. Immunol. **10:**123–151.
12. **Fahy, G. T., D. C. Cooper, and D. L. Easty.** 1993. Antigen presentation of herpes simplex virus by corneal epithelium, an in vitro and in vivo study. Br. J. Ophthalmol. **77:**440–444.
13. **Frankel, S. S., B. M. Wenig, A. P. Burke, P. Mannan, L. D. R. Thompson, S. L. Abbondanzo, A. M. Nelson, P. Melissa, and R. M. Steinman.** 1996. Replication of HIV-1 in dendritic cell-derived syncytia at the mucosal surface of the adenoid. Science **272:**115–117.
14. **Giddens, W. E., G. L. Van Hoosier, and L. E. Garlinghouse.** 1987. Experimental Sendai virus infection in laboratory rats. II. Pathology and immunohistochemistry. Lab. Anim. Sci. **37:**442–448.
15. **Hamilton-Easton, A., and M. Eichelberger.** 1995. Virus-specific antigen presentation by different subsets of cells from lung and mediastinal lymph node tissues of influenza-infected mice. J. Virol. **69:**6359–6366.
16. **Hendricks, R. L., M. Janowicz, and T. M. Tumpey.** 1992. Critical role of corneal Langerhans cells in the CD4- but not CD8-mediated immunopathology in herpes simplex virus-1-infected mouse corneas. J. Immunol. **148:**2522–2529.
17. **Holt, P. G., S. Haining, D. Nelson, and J. D. Sedgwick.** 1994. Origin and steady-state turnover of class II MHC-bearing dendritic cells in the epithelium of the conducting airways. J. Immunol. **153:**256–261.
18. **Holt, P. G., M. A. Schon-Hegrad, J. Oliver, B. J. Holt, and P. G. McMenamin.** 1990. A contiguous network of dendritic antigen-presenting cells within the respiratory epithelium. Int. Arch. Allergy Appl. Immunol. **91:**155–159.
19. **Holt, P. G., M. A. Schon-Hegrad, M. J. Phillips, and P. G. McMenamin.** 1989. Ia-positive dendritic cells form a tightly meshed network within the human airway epithelium. Clin. Exp. Allergy **19:**597–601.
20. **Hou, S., J. M. Katz, P. C. Doherty, and S. R. Carding.** 1992. Extent of gamma delta T cell involvement in the pneumonia caused by Sendai virus. Cell. Immunol. **143:**183–193.

20a. **Hünig, T.** Personal communication.

21. **Hünig, T., H.-J. Wallny, J. K. Hartley, A. Lawetzky, and G. Tiefenthaler.** 1989. A monoclonal antibody to a constant determinant of the rat T cell antigen receptor that induces T cell activation. J. Exp. Med. **169:**73–86.
22. **Janeway, C. A.** 1992. The immune system evolved to discriminate infectious nonself from noninfectious self. Immunol. Today **13:**11–16.
23. **Jiang, Y. Z., D. Couriel, D. A. Mavroudis, P. Lewalle, V. Malkovska, N. F. Hensel, S. Dermime, J. Molldrem, and A. J. Barrett.** 1996. Interaction of natural killer cells with MHC class II: reversal of HLA-DR1-mediated protection of K562 transfectant from natural killer cell-mediated cytolysis by brefeldin-A. Immunology **87:**481–486.
24. **Kalb, T. H., M. T. Chuang, Z. Marom, and L. Mayer.** 1991. Evidence for accessory cell function by class II MHC antigen-expressing airway epithelial cells. Am. J. Respir. Cell. Mol. Biol. **4:**320–329.
25. **Kingston, A. E., K. Bergsteinsdottir, K. R. Jessen, P. H. Van der Meide, M. J. Colston, and R. Mirsky.** 1989. Schwann cells co-cultured with stimulated T cells and antigen express major histocompatibility complex (MHC) class II determinants without interferon-gamma pretreatment: synergistic effects of interferon-gamma and tumor necrosis factor on MHC class II induction. Eur. J. Immunol. **19:**177–183.
26. **Kuhnlein, P., J.-H. Park, T. Herrman, A. Elbe, and T. Hunig.** 1994. Identification and characterization of rat γ/δ T lymphocytes in peripheral lymphoid organs, small intestine, and skin with a monoclonal antibody to a constant determinant of the γ/δ T cell receptor. J. Immunol. **153:**979–986.
27. **Ljunggren, H.-G., and K. Kärre.** 1990. In search of the 'missing self': MHC molecules and NK cell recognition. Immunol. Today **11:**237–244.
28. **Look, D. C., S. R. Rapp, B. T. Keller, and M. J. Holtzman.** 1992. Selective induction of intercellular adhesion molecule-1 ny interferon-gamma in human airway epithelial cells. Am. J. Physiol. **263(1):**L79–L87.
29. **Mason, D. W., R. P. Arthur, M. J. Dallman, J. R. Green, G. P. Spickett, and M. L. Thomas.** 1983. Functions of rat T-lymphocyte subsets isolated by means of monoclonal antibodies. Immunol. Rev. **74:**57–82.
30. **Massion, P., C. C. P. Funari, I. Ueki, S. Ikeda, D. M. McDonald, and J. A. Nadel.** 1993. Parainfluenza (Sendai) virus infects ciliated cells and secretory cells but not basal cells of rat tracheal epithelium. Am. J. Respir. Cell. Mol. Biol. **9:**361–370.
31. **Matzinger, P.** 1994. Tolerance, danger, and the extended family. Annu. Rev. Immunol. **12:**991–1045.
32. **McWilliam, A. S., D. Nelson, J. A. Thomas, and P. G. Holt.** 1994. Rapid dendritic cell recruitment is a hallmark of the acute inflammatory response at mucosal surfaces. J. Exp. Med. **179:**1331–1336.
33. **Mo, X. Y., S. R. Sarawar, and P. C. Doherty.** 1995. Induction of cytokines in mice with parainfluenza pneumonia. J. Virol. **69:**1288–1291.
34. **Nelson, D. J., C. McMenamin, A. S. McWilliam, M. Brenan, and P. G. Holt.** 1994. Development of the airway intraepithelial dendritic cell network in the rat from class II MHC (Ia) negative precursors: differential regulation of Ia expression at different levels of the respiratory tract. J. Exp. Med. **179:**203–212.
35. **Nonacs, R., C. Humborg, J. P. Tam, and R. M. Steinman.** 1992. Mechanisms of mouse spleen dendritic cell function in the generation of influenza-specific, cytolytic T lymphocytes. J. Exp. Med. **176:**519–529.
36. **Novak, M., Z. Moldoveanu, D. P. Schafer, J. Mestecky, and R. W. Compans.** 1993. Murine model for evaluation of protective immunity to influenza virus. Vaccine **11:**55–60.
37. **Ogasawara, T., M. Emoto, K. Kiyotani, K. Shimokata, T. Yoshida, Y. Nagai, and Y. Yoshikai.** 1994. Sendai virus pneumonia: evidence for the early recruitment of γδ T cells during the disease course. J. Virol. **68:**4022–4027.
38. **Openshaw, P. J.** 1991. Pulmonary epithelial T cells induced by viral infection express T cell receptors αβ. Eur. J. Immunol. **21:**803–806.
39. **Parr, M. B., L. Kepple, M. R. McDermott, M. D. Drew, J. J. Bozzola, and E. L. Parr.** 1994. A mouse model for studies of mucosal immunity to vaginal infection by herpes simplex virus type 2. Lab. Invest. **70:**369–380.
40. **Pilewski, J. M., D. J. Scott, J. M. Wilson, and S. M. Albelda.** 1995. ICAM-1 expression on bronchial epithelium after recombinant adenovirus infection. Am. J. Respir. Cell. Mol. Biol. **12:**142–148.
41. **Ramphal, R., R. C. Cogliano, J. W. Shands, and P. A. Small.** 1979. Serum antibody prevents lethal murine influenza pneumonitis but not tracheitis. Infect. Immun. **25:**992–997.
42. **Ridge, J. P., E. J. Fuchs, and P. Matzinger.** 1996. Neonatal tolerance revisited: turning on newborn T cells with dendritic cells. Science **271:**1723–1726.
43. **Schon-Hegrad, M. A., J. Oliver, P. G. McMenamin, and P. G. Holt.** 1991. Studies on the density, distribution, and surface phenotype of intraepithelial class II major histocompatibility complex antigen (Ia)-bearing dendritic cells (DC) in the conducting airways. J. Exp. Med. **173:**1345–1356.
44. **Sekiya, S., S. Gotoh, T. Yamashita, T. Watanabe, S. Saitoh, and F. Sendo.** 1989. Selective depletion of rat neutrophils by in vivo administration of a monoclonal antibody. J. Leukocyte Biol. **46:**96–102.
45. **Sorden, S. D., and W. L. Castleman.** 1991. Brown Norway rats are high responders to bronchiolitis, pneumonia, and bronchiolar mastocytosis induced by parainfluenza virus. Exp. Lung Res. **17:**1025–1045.
46. **Takami, T., C.-F. Qi, H. Kimura, K. Kawata, T. Yamada, S. Ojima, S. Koizumi, T. Uede, and K. Kikuchi.** 1988. Analysis of rat B lymphocytes by using monoclonal antibodies. Trans. Soc. Pathol. Jpn. **77:**178.
47. **Ustinov, J., R. Loginov, C. Bruggeman, J. Suni, P. Hayry, and I. Lautenschlager.** 1994. CMV-induced class II antigen expression in various rat organs. Transplant. Int. **7:**302–308.
48. **Ustinov, J. A., T. T. Lathtinen, C. A. Bruggeman, P. J. Hayry, and I. T. Lauenschlager.** 1994. Direct induction of class II molecules by cytomegalovirus in rat heart microvascular endothelial cells is inhibited by ganciclovir (DHPG). Transplantation **58:**1027–1031.
49. **Vermeer, L. A., N. K. de Boer, C. Bucci, N. A. Bos, F. G. M. Kroese, and S. Alberti.** 1994. MRC OX19 recognizes the rat CD5 surface glycoprotein, but does not provide evidence for a population of $CD5^{bright}$ B cells. Eur. J. Immunol. **24:**585–592.