

# Immunohistochemical Detection of Respiratory Syncytial Virus Infection in the Lung of Child Autopsy Cases

Naomi YAMASAKI<sup>1)</sup>, Masataka INADA<sup>1)</sup>, Toshio KAWAHARA<sup>1)</sup>, Yoshikazu HIGAMI<sup>1)</sup>, Keisuke IWASAKI<sup>2)</sup>, Mikihiro AOKI<sup>3)</sup>, Isao SHIMOKAWA<sup>1)</sup>

1) First Department of Pathology, Nagasaki University School of Medicine

2) Division of Pathology, Sasebo City General Hospital

3) Division of Pediatrics, Sasebo City General Hospital

Viral infection in the respiratory tract is suspected in some cases of infant death; however, in most of those cases, routine postmortem examination has been unable to determine a definitive etiology. Using immunohistochemistry with a specific antibody to respiratory syncytial virus (RSV) in paraffin sections, we investigated a possible association of RSV infection with interstitial pneumonia or bronchitis in four child autopsy cases while two adult cases with cytomegalic inclusion disease, pneumocystis carinii pneumonia, or acute interstitial pneumonia were also included as negative control. Immunoreactivity for RSV was detected in one of the 4 child cases; the bronchial and bronchiolar epithelium were immunostained. No immunoreactivity was observed in the two adult cases. Retrospective microscopic examination in routinely stained slides could find no distinctive findings indicating RSV infection in this case as well as the other three cases. Although further evidence, e.g., detection of the viral nucleic acid in specimens, may be needed, the present results suggest that this antibody can be utilized for detection of RSV infection in autopsy samples.

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**Key Words:** immunohistochemistry, respiratory syncytial virus, postmortem examination, sudden infant death syndrome

## Introduction

Viral infection in the lower respiratory tracts occasionally causes sudden death in infants and children.

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**Address Correspondence:** Isao Shimokawa, M.D.  
First Department of Pathology, Nagasaki University School of Medicine 1-12-4 Sakamoto, Nagasaki City 852-8523  
TEL: +81-95-849-7050, FAX: +81-95-849-7052  
E-mail: shimo@net.nagasaki-u.ac.jp

However, pathogens have been obscure in most cases during routine postmortem examination. Recent developments in immunologic and molecular techniques enable us to detect viral antigens and nucleic acids in paraffin sections obtained from autopsy. Although histo-in situ hybridization and polymerase chain reaction methods for detecting viral nucleic acids have been popular in laboratory settings, immunohistochemical detection of viral antigens is advantageous in terms of its cost effectiveness and accurate histopathologic analyses.

In infants less than 2 years old, respiratory syncytial virus (RSV), parainfluenzavirus 3, and less commonly, adenovirus may produce severe bronchiolitis and pneumonia. In older children, influenzaviruses cause severe respiratory infection<sup>1)</sup>. RSV is most commonly isolated in a series of sudden infant deaths<sup>2)</sup>. At present, a mouse monoclonal antibody to RSV, which can be used in paraffin sections, is commercially available. In this study, using this antibody, we attempted to detect RSV immunohistochemically in archival postmortem tissue sections of cases suspected of lower respiratory tract viral infection.

## Materials and Methods

### Cases

After reviewing approximately 4000 autopsy records in the Division of Pathology of Sasebo City General Hospital and the Department of Pathology, Nagasaki University School of Medicine, four infant or child cases, in which postmortem examination suspected non-bacterial interstitial pneumonia, bronchitis, or bronchiolitis, were chosen. Acute bronchopneumonia, mostly caused by bacterial infection, was excluded in the present study. A 39-year-old case with concomitant infection of

cytomegalic inclusion disease and pneumocystis carinii pneumonia, and an 80-year-old case with acute interstitial pneumonia were also included as a negative control.

### Immunohistochemistry

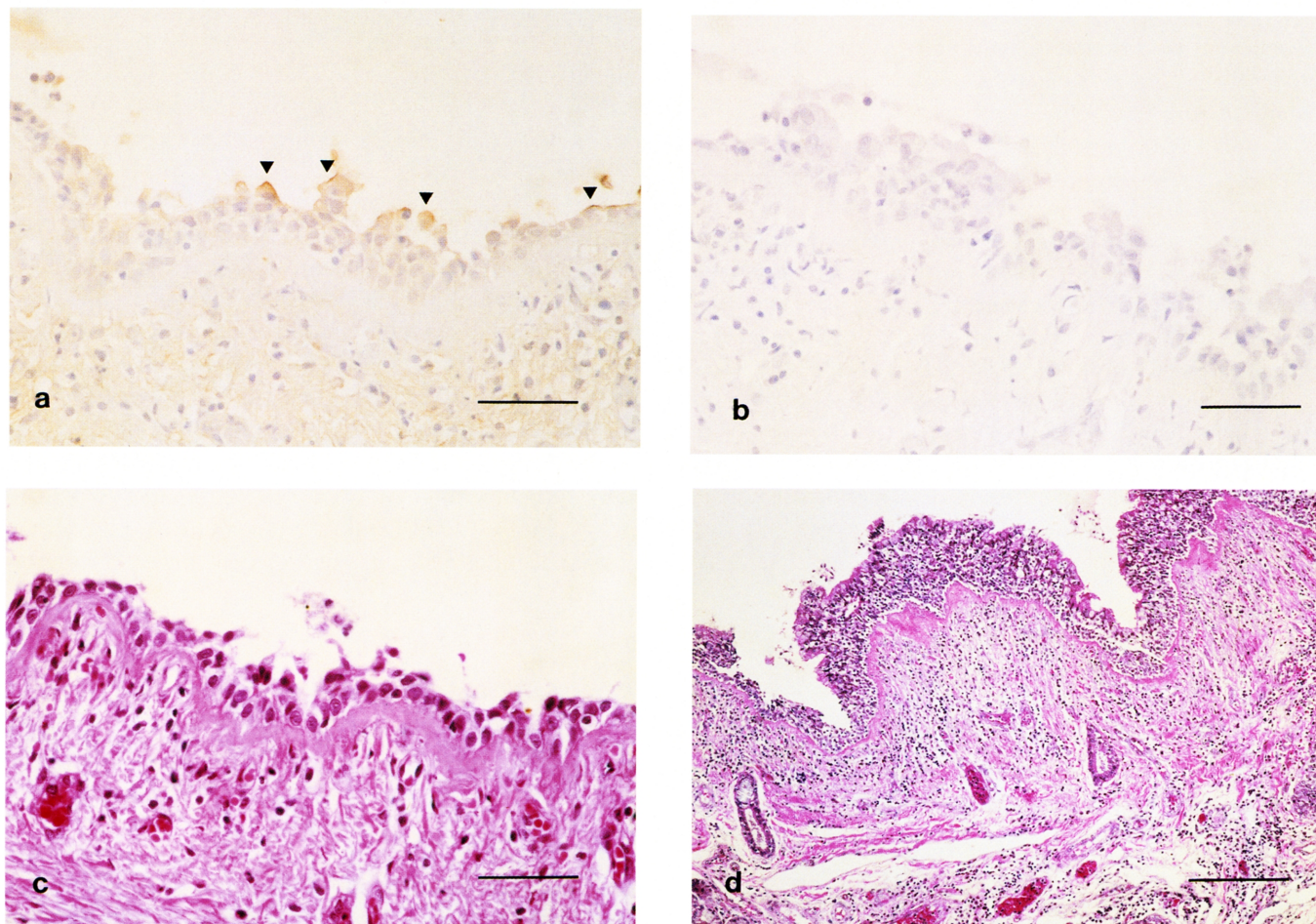
Paraffin sections of these 6 cases were subjected to immunohistochemistry. Following the manufacturer's protocol, deparaffinized sections were autoclaved in 0.01 M-sodium citrate buffer (pH 6.0) at 121°C for one minute for antigen unmasking. The primary antibody, mouse IgG (NCL-RSV3, NOVO CASTRA, UK), was used at a dilution 1:200. The standard ABC technique was performed to visualize the localization using an ABC kit (Nichirei Co. Ltd., Tokyo). Negative control was achieved by replacement of the primary antibody with non-immunized mouse IgG (DAKO A/S, Glostrup, Denmark) at the same dilution.

## Results

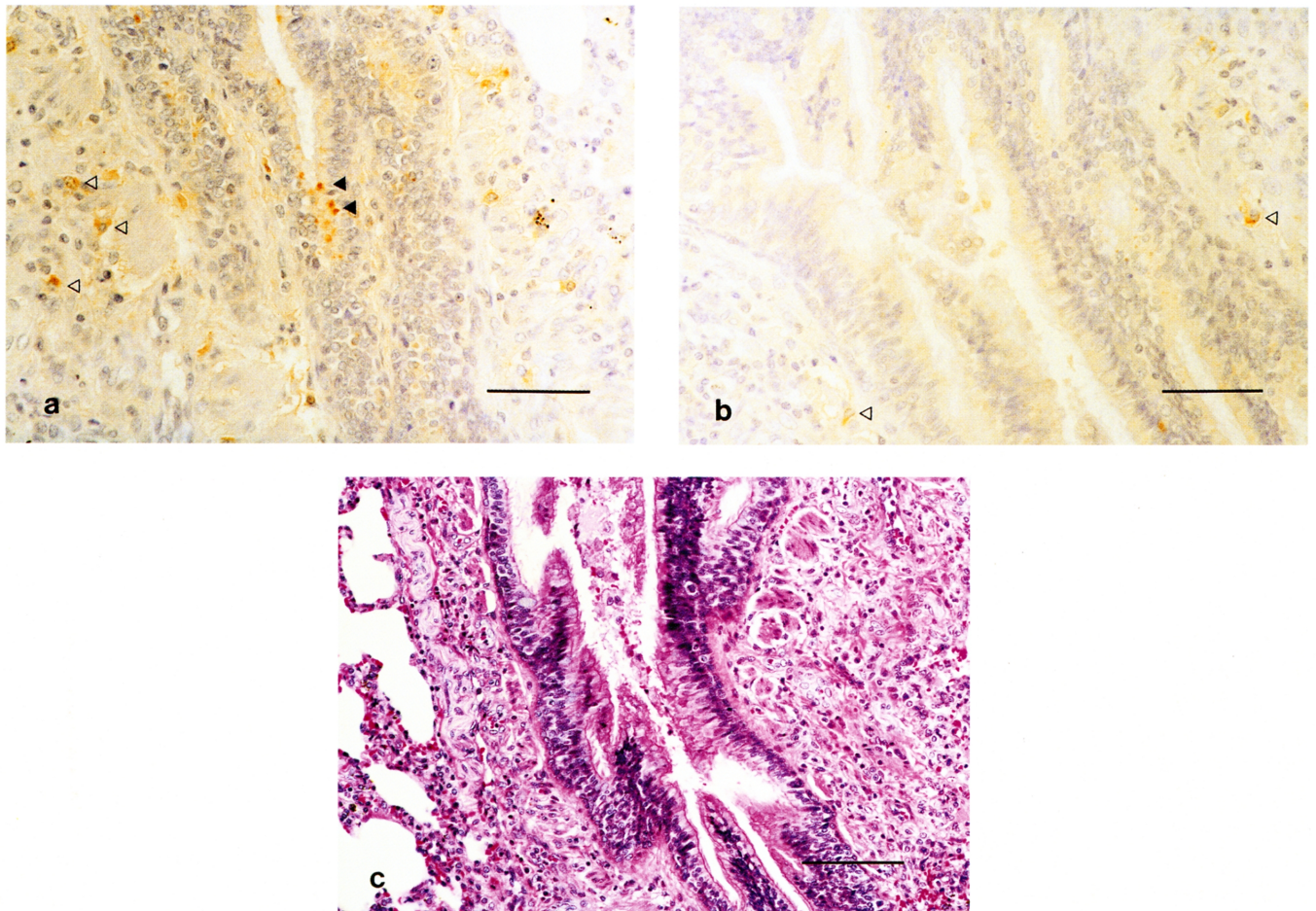
### Summary of the Cases

Case 1: This 2-year and 5-month-old boy, without a prior history of overt illness, had a fever a day before death. The postmortem examination revealed necrotizing bronchitis (Figure 1), focal lymphocytic infiltrates in the interstitium, and patchy areas of atelectasis. The bronchiole appeared to be constricted. Lymphocytic infiltrates in the bronchiole were scant, while some bronchioles contained foamy macrophages (Figure 2).

Case 2: This 4-month-old infant girl, with no specific signs or symptoms, was found cyanotic 1 hour and 20 minutes after she had been fed milk, and underwent cardiac arrest on reaching hospital. Resuscitation efforts were continued in vain. The postmortem examination showed mild lymphocytic bronchitis and necrotizing lymphocytic peri-bronchiolitis. Most parts of the lung



**Figure 1.** a) Immunoreactivity of RSV is observed (arrow head) in the surface and the cytoplasm of bronchiolar epithelium in case 1 (bar; 100  $\mu$  m). b) Negative control (non-immunized IgG) of an adjacent section (bar; 100  $\mu$  m). Note that the immunoreactivity in the bronchial epithelium is considerably decreased. c, d) The bronchus is desquamated (c, bar; 100  $\mu$  m) or infiltrated by lymphocytes (d, bar; 200  $\mu$  m) in case 1. No cytoplasmic inclusion was seen (Hematoxylin-Eosin stain).



**Figure 2.** a) Immunoreactivity of RSV is also observed in a few cells of the bronchiolar epithelium of case 1 (arrow head, bar; 100  $\mu$ m). A few inflammatory cells are also positive (open arrow head). These are, however, also present in negative control sections (see Fig. 2-b); thereby, they were considered false-positive. b) Negative control (non-immunized IgG) of an adjacent section (bar; 100  $\mu$ m). Note that the immunoreactivity disappears in the bronchiolar epithelium, while it remains only in a few inflammatory cells. c) The bronchiole appears to be constricted, while only a few lymphocytes are infiltrated (Hematoxylin-Eosin stain, bar; 200  $\mu$ m).

underwent atelectasis. Pulmonary hemorrhages were also noted.

**Case 3:** This 5-month-old infant boy with congenital biliary atresia was admitted to hospital at 4 months of age with suspected bronchiolitis. In spite of antibiotics and gamma globulin treatment, he died one month after the admission. On autopsy, thickened alveolar septum with mild lymphocytic infiltrates, activated type 2 pneumocytes, patchy areas of atelectasis, and intra-alveolar hemorrhages were observed.

**Case 4:** This 4 year-old boy had been admitted to hospital repeatedly after birth for pneumonia and cerebral palsy. Growth retardation and malnutrition had also been noted. Clinical examination also suspected muscular dystrophy. He was found dead after one day of stridor. The postmortem examination revealed lymphocytic bronchitis and multifoci of lymphocytic cell infiltrates in the alveolar septum, features similar to lymphoid interstitial pneumonia.

We could find no intranuclear or intracytoplasmic inclusions in all the cases.

#### *Immunohistochemistry*

As demonstrated in Figure 1, immunoreactivity was observed in the bronchial epithelium of case 1. A few cells in the bronchiolar epithelium were also immunostained (Fig. 2); no reaction product was observed in these cells in the negative control sections of this case. A few inflammatory cells were weakly stained by the anti-RSV antibody; however, similar stainings remained in the negative control sections. Therefore, we concluded that the bronchial and bronchiolar cells were positive for RSV, while the inflammatory cells were false-positive. We also found no immunostained cells or structures in the other 5 cases, except a few inflammatory cells considered false-positive.

## Discussion

In the autopsy records reviewed in this study, we could find no case given a diagnosis of RSV infection before death; therefore, definitely positive case was not included in this immunohistochemical study. However, no immunoreactivity was seen in the other 5 cases as well as in negative control sections of case 1. It has been reported that RSV antigens can be immunohistochemically detected in bronchial, bronchiolar, and alveolar epithelial cells, and in alveolar syncytial giant cells<sup>3)</sup>. In the present case 1, immunoreactivity was observed mostly in bronchial epithelium, in which desquamation and lymphocytic infiltrates are noted, and in the bronchioles, although only a few cells were positive; these immunohistochemical findings were similar to those reported by Neilson et al.<sup>3)</sup>. It is, therefore, unlikely that the immunoreactivity in case 1 is false-positive.

Histopathologic features suspecting of viral infection in the lower respiratory tract are the followings; necrotizing bronchitis or bronchiolitis, interstitial pneumonia with diffuse alveolar damage, and intranuclear or intracytoplasmic inclusions<sup>1)</sup>. Although polymorphonuclear cells participate in the inflammatory response, the inflammatory reaction consists primarily of mononuclear cells. Several patterns of the inflammatory response and viral inclusions may specify a type of viruses. However, these patterns are not mutually exclusive<sup>1)</sup>. In the present study, even retrospective microscopic examination could find no distinctive findings indicating RSV infection in case 1 as well as case 2-4. In this sense, immunohistochemistry using the specific antibody is a useful diagnostic tool for the detection of RSV infection in tissues routinely processed for histopathologic examination.

Using the histo-in situ hybridization technique in paraffin sections, An et al.<sup>4)</sup> have reported that 11 of 45 cases of sudden infant death syndrome (SIDS) and one of 30 cases of non-SIDS are positive for RSV. A recent study using the histo-in situ hybridization technique and the reverse transcriptase-polymerase chain reaction method has also demonstrated the presence of RSV nucleic acid in archival postmortem tissues in one

fourth of 99 cases of SIDS<sup>5)</sup>. However, a careful case-control study in that report has also revealed that RSV is detected in a considerable number of a control non-SIDS group, although the percentage is slightly larger in the SIDS group than the non-SIDS group. The detection of RSV does not necessarily imply a cause of death in SIDS cases. In the present case 1, significant histopathologic findings were found only in the lung. Although a possible association of the other viruses in this case should be examined, at present, we have concluded that RSV induced the lung lesions, leading to respiratory failure in case 1.

A combination of immunohistochemistry with these molecular techniques could increase the accuracy of diagnosis for RSV infection. In addition, efforts to detect viral antigens and nucleic acids should be extended to other viruses including parainfluenza virus, adenovirus, and influenza virus to elucidate the possible presence of a pathogen in infant or child death by respiratory infection.

## Acknowledgements

The authors wish to thank Ms. Yuko Moriyama for her technical assistance. This study was conducted for the research seminar of the 4th year medical students (N. Y. & M. I.) of Nagasaki University school of Medicine.

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