Virchows Arch (2000) 436:617–621

ORIGINAL ARTICLE

S. Philippou · P. Otto · P. Reinhold · M. Elschner
H.-J. Streckert

Respiratory syncytial virus-induced chronic bronchiolitis in experimentally infected calves

Received: 14 October 1999 / Accepted: 15 November 1999

Abstract Human (RSV) and bovine (BRSV) respiratory syncytial virus cause similar infections of the lower respiratory tract. Therefore, experimentally infected calves are suited to the study of RSV-induced chronic bronchiolitis. Colostrum-fed calves aged 17–24 days were successfully infected with BRSV. BRSV strain 375 was applied as an aerosol on 4 consecutive days. Clinical symptoms were already evident on the 1st day after infection. The calves were necropsied 12 weeks after the first infection. Focal severe chronic bronchiolitis with atelectasis and focal bronchiolitis obliterans were demonstrated. The bronchiolar lumina were filled with secretion. Transmission electron microscopy revealed an alteration of the ciliogenesis and partial loss of cilia. Immunhistochemically virus P protein could still be detected, mainly in the epithelial cells of the inflamed bronchioli.

Key words Respiratory syncytial virus · RSV · Chronic bronchiolitis · RSV persistence

Introduction

Respiratory syncytial virus (RSV)-induced infections of the lower respiratory tract in infancy and early childhood are the most frequent cause of a hyperreactive bronchial system and obstructive lung disease [14, 24, 26, 28].

Glezen et al. [8] and Henderson et al. [10] showed that 34% of the children with acute bronchiolitis of known origin are caused by RSV. At present, approximately 1% of the children with acute bronchiolitis die. The possibility of developing a hyperreactive bronchial system is over 50%. Recent investigations [13, 16, 19] show that about 10–20% of adult cases of hyperreactive bronchial system may be caused by virus infections.

Our knowledge of this virus infection is based on clinical and serological studies. Morphological studies of the bronchial wall are rarely available for technical reasons.

Study of the morphological alterations of the respiratory tract in vivo subsequent to virus infection is dependent on the availability of an experimental animal model. Application of the findings of animal experiments to humans can involve problems, however. In particular, the variability in the reactivity to certain pathogenic agents in various species must be taken into consideration.

In the past we have shown[] the morphological alterations of the lower respiratory tract in the initial stage of the infection subsequent to aerogenic RSV application in an animal model [20–22].

In this study the same animal model was used, and light and electron microscopic investigations of the chronic stage of the RSV infection were performed.

Materials and methods

Five conventionally kept and colostrum-fed Friesian crossbred calves aged 17–24 days were infected with BRSV strain 375 (ATCC no.: VR-1339). The animals were exposed to aerogenic infection (jet nebulizer, Pari Provokationstest I, Medanz, Starnberg, Germany) with the BRSV as described by Otto et al. [20].

Twelve weeks after the first infection the animals were killed painlessly under general anaesthesia (1 g thiamylal sodium/50 kg body weight) by bleeding. The left lung of each calf was fixed while still in situ, whereas the right lung remained unfixed for virological and immunological studies. The lung fixation was performed as described elsewhere by Philippou et al. [22].

The lung tissue was prepared as usual for light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Approximately 80 Epon 812* blocks and 20 histological blocks taken from all sections of the fixed lung of each animal were processed.

The murine monoclonal antibody 3 C 4 (directed towards RSV P protein, dilution 1:25) and antibodies CD 4 (Medac, dilution 1:50) and CD 8 (Dako, dilution 1:100) were used for the immunohistochemical studies.

S. Philippou (✉)
Institute of Pathology, Augusta Hospital, Zeppelinstrasse 18, 44791 Bochum, Germany
Fax: +49-234-9585604

H.-J. Streckert
Dr. Streckert-Diagnostica, Witten, Germany

P. Otto · P. Reinhold · M. Elschner
Federal Institute for Health Protection of Costumers and Veterinary Medicine, Jena Branch, Germany

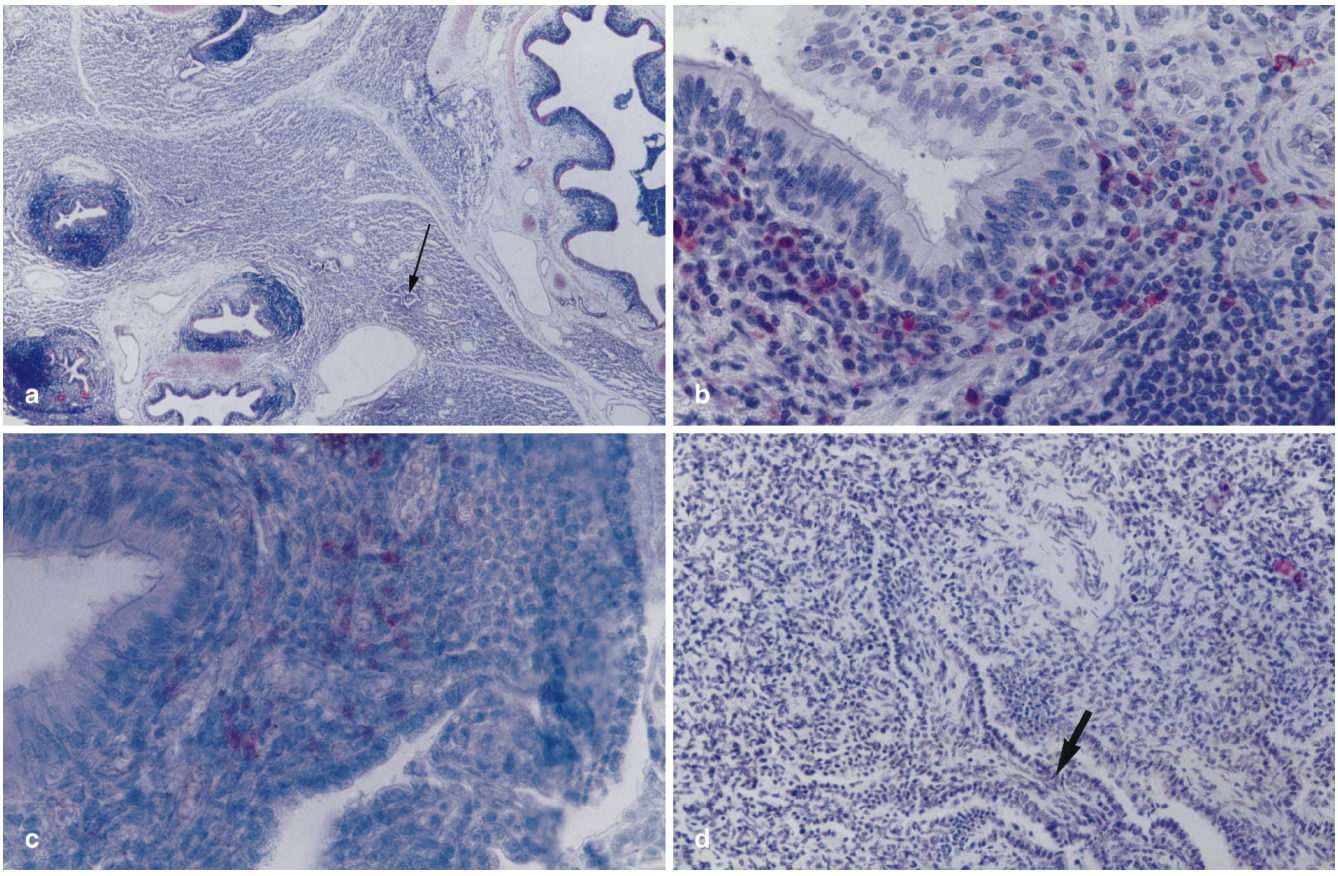


Fig. 1 a Atelectatic lung parenchyma with dense lymphocytic infiltrate in the bronchiolar wall. In the bronchioli respiratorii granulation tissue was evident (*arrow*). PAS, original magnification ×20 **b** Most of the lymphocytes were against CD 4 positive. ABC method, anti-CD 4, original magnification ×200 **c** In between few CD 8 positive lymphocytes could be depicted. ABC method, antiCD 8, original magnification ×200 d The lumen of a bronchiolus respiratorious was filled with granulation tissue (*arrow*). H&E, original magnification ×100

Results

Clinical data

Each calf developed coughing, runny eyes and nasal discharge within a few days after the first inhalation of virus. No significant increase in rectal temperature was observed. Owing to the infection, the respiratory rate measured in resting animals increased significantly.

The bronchial hyperresponsiveness was monitored [20] in the 1st week after the infection and during the next 11 weeks.

Replication of virus

Infectious BRSV was reisolated from lung tissue and cells from BAL fluid 1 week after the experimental infection.

Macroscopic findings

Irregularly distributed atelectasis developed in both central and peripheral regions, mainly in the basal parts of the upper, middle and lower lobes. The remaining lung parenchyma was ventilated evenly, and the visceral pleura was smooth and shiny.

Light microscopic findings

The lumen of the corresponding bronchioli to the atelectasis was narrow and focally filled with secretion. The superficial bronchial epithelial cells were swollen. In the bronchiolar wall a variably dense lymphocytic infiltrate was evident. Lymphocytic aggregates and follicles were visible (Fig. 1a). Immunohistochemically most lymphocytes were positive against CD 4 (Fig. 1b). A small number of CD 8-positive lymphocytes were depicted among the others (Fig. 1c). Further bronchioli were filled with granulation tissue (Fig. 1d). On the surface were columnar, swollen epithelial cells. The lung parenchyma corresponding to the bronchioli exhibited atelectasis. Immunohistochemically a pronounced reaction to the antibody 3 C 4 (against virus P protein) was obvious (Fig. 2). The reaction was positive in the epithelial cells of the inflamed bronchioli. A positive reaction was also visible in the epithelial cells of the peribronchial glands and in a few pneumocytes.

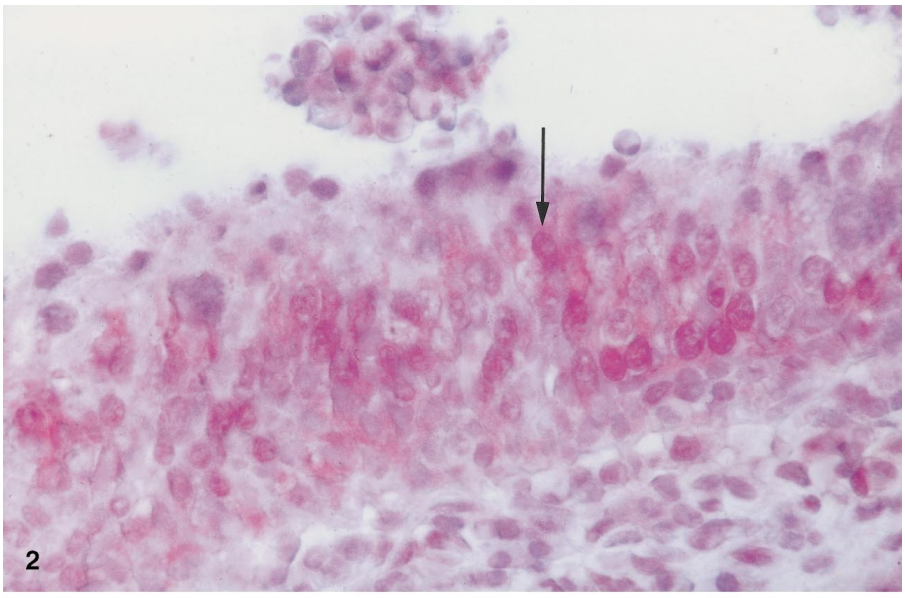
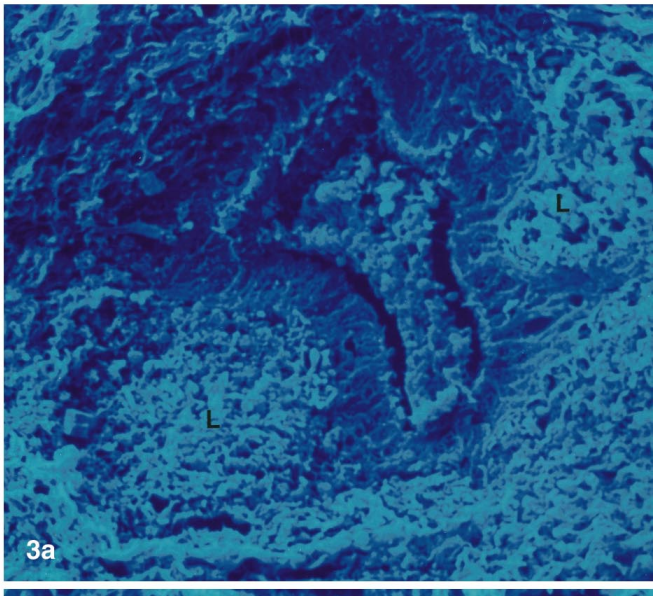


Fig. 2 A positive reaction to the antibody 3C 4 (against virus-P protein) in the epithelial cells of the inflamated bronchioli was obvious (*arrow*). ABC method, anti virus-P protein, original magnification ×400



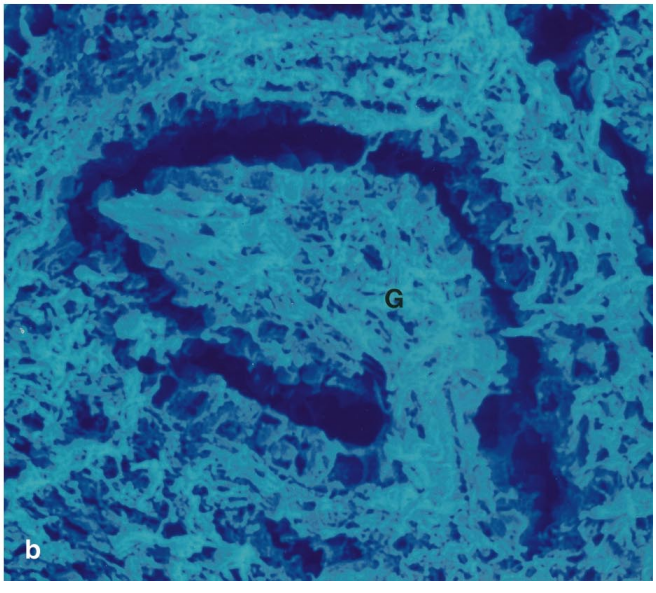


Fig. 3 **a** In bronchiolar lumen secretion and cell debris were shown. In the bronchiolar wall lymphocytic aggregates (*L*) were visible. SEM. Original magnification ×400 **b** Granulation (*G*) tissue in a bronchiolar lumen. SEM. Original magnification ×602

Electron microscopic findings

Scanning electron microscopy showed that the bronchiolar lumina were partly filled with secretion and granulation tissue (Fig. 3a, b). A small residual lumen was visualized. On the surface of the granulation tissue a columnar epithelium without cilia was obvious. In the bronchiolar wall the lymphocytic aggregates were visible.

On transmission electron microscopy the number of cilia at the cell surface was seen to be significantly reduced (Fig. 4). Besides this, there were cilia with partly elevated, double-contoured elementary membranes. Also, compound cilia were demonstrable. The structure of the microvilli was severely altered. There were plump microvilli varying in length.

In the apical luminal cytoplasm, nondirectional tubulus systems similar to those formations in axonemes were found. Some of them were arranged parallel to the surface in the cytoplasm. Beside them in the perinuclear area basal bodies without axonemes were found.

The intercellular spaces were focally distended. The contact zones between the epithelial cells were intact.

No virus inclusions in the epithelial cells or fully developed RSV particles could be found.

Discussion

In recent years several experimental studies with RSV infections in sheep, guinea-pigs and calves have been performed. In contrast to our studies, only minimal lesions of the respiratory tract and only the early stages of the infection have been reported from others [1–6, 12, 17, 18, 23, 25].

The discrepancy is probably due to the differing mode of virus application and to the various species. In these studies and in contrast to ours the virus isolate was ap-

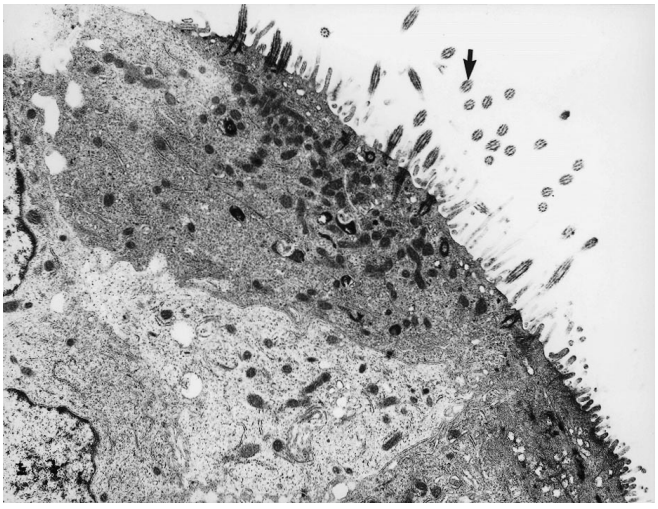


Fig. 4 The number of cilia (*arrow*) at the bronchiolar epithelial cell surface was significantly reduced. TEM. Original magnification ×7000

plied intranasally and/or intratracheally. This route of infection does not represent the natural pathway of infection and explains the discrepancy between the localization of the development of bronchiolitis in our studies and in those of Ciszewski et al. [6].

The age of the animals also plays an important part in the experimentally induced RSV infection. Bryson [3] and McNulty [17] induced infections of the respiratory tract with BRSV in calves younger than 7 days who had not received any colostrum after birth.

In both humans and calves, owing to the protective function of the maternal antibodies no RSV infection occurs in the lower respiratory tract during the 1st month of life [11]. The peak of infections requiring treatment occurs during the 2nd month of life. Based on this observation and the similarities between the BRSV infection and the human RSV infection of the respiratory tract, we decided to perform our experiments with 17- to 24-day-old calves.

In contrast to previous studies in a guinea-pig model [6, 7, 9, 27], the morphological changes of the lower respiratory tract in the calf model were significant and severe. In the previous studies only a mild inflammation of the bronchioli and few dystelectases were observed. In our study severe chronic bronchiolitis with atelectasis was demonstrable. Bronchiolitis obliterans was focally obvious. The electron microscopic examination revealed a partial loss of the cilia, and also compound cilia. The lumen of the bronchioli was focally filled with secretion. These findings show that the mucociliary clearance is damaged not only in the early stage of the infection but also in the next 12 weeks.

Iinflammation of the wall of the bronchioli reflects a predominance of CD 4 lymphocytes.

In the epithelial cells of the bronchioli viral P protein was still present, as proved by immunohistochemical marking. Persistence of virus proteins for 6 weeks after the experimental infection were demonstrated in the guinea-pig model by Streckert et al. [27]. In their study the inflammation of the bronchioli was only very mild. Similar investigations are not known in the calf model.

In conclusion, using a calf model, 12 weeks after an experimental BRSV infection chronic, partly follicular, bronchiolitis and also bronchiolitis obliterans can be demonstrated. Viral P protein persisted for at least 12 weeks after infection, and over the same period airway hyperresponsiveness was unchanged in infected animals. The morphological changes to the lower respiratory tract and the persistence of viral proteins may be responsible for persistent airway hyperresponsiveness. The persistence of the viral proteins is obviously responsible for increased IL-8 release [15], which may in turn be responsible for the airway hyperresponsiveness. Further investigations are necessary to examine this idea.

References

1. Al-Darraji AM, Cutlip RC, Lehmkuhl HD, Graham DL (1982) Experimental infection of lambs with bovine respiratory syncytial virus and *Pasteurella haemolytica*: pathology studies. Am J Vet Res 43:224–229
2. Baker JC, Werdin RE, Ames TR, Markham RJF, Larson VL (1986) Study on etiology role of bovine respiratory syncytial virus in pneumonia fo dairy calves. J Am Vet Med Assoc 189: 66–70
3. Bryson DG, McNulty MS, Logan EF, Cush PF (1983) Respiratory syncytial virus pneumonia in young calves: clinical and pathologic findings. Am J Vet Res 44:1648–1655
4. Castleman WL, Chandler SK, Slauson DO (1985) Experimental bovine respiratory syncytial virus infection in conventional calves: ultrastructural respiratory lesions. Am J Vet Res 46: 554–560
5. Castleman WL, Lay JC, Dubovi EJ, Slauson DO (1985) Experimental bovine respiratory syncytial virus infection in conventional calves: light microscopic lesions, microbiology, and studies of lavaged lung cells. Am J Vet Res 46:547–553
6. Ciszewski D, Baker J, Slocombe R, Reindel R, Haines D, Clark E (1991) Experimental reproduction of respiratory tract disease with bovine respiratory syncytial virus. Vet Microbiol 28:39–60
7. Folkerts G, Verheyen A, Nijkamp F (1992) Viral infection in guinea pigs induces a sustained nonspecific airway hyperresponsiveness and morphological changes of the respiratory tract. Eur J Pharmacol 228:121–130
8. Glezen WP, Loda FA, Clyde WA, et al (1971) Epidemiologic patterns of acute lower respiratory disease of children in pediatric practice. J Pediatr 78:397
9. Hegele RG, Hayashi S, Bramley AM, Hogg JC (1994) Persistence of respiratory syncytial virus genome and protein after acute bronchiolitis in guinea-pigs. Chest 105:1848–1854
10. Henderson FW, Clyde WA, Collier AM, et al (1979) The etiologic and epidemiologic spectrum of bronchiolitis in pediatrci practice. J Pediatr 95:183
11. Holberg CJ, Wright AL, Martinez FD, Ray CG, Taussig LM, Lebowitz MD (1991) Risk factors for respiratory syncytial virus-associated lower respiratory illness in the first year of life. Am J Epidemiol 133:1135–1151
12. Jacobs JW, Edington N (1975) Experimental infection of calves with respiratory syncytial virus. Res Vet Sci 18: 299–306
13. Johnston SL, Bardin PG, Pattemore PK (1993) Viruses as precipitants of asthma symptoms. III. Rhinoviruses: molecular biology and prospects for future intervention. Clin Exp Allergy 23:237–246

14. Kimman TG, Westenbrink F (1990) Immunity to human and bovine respiratory syncytial virus. Arch Virol 112:1–25
15. König B, Krusat T, Streckert HJ, König W (1996) IL-8 release from human neutrophils by the respiratory syncytial virus is independent of viral replication. J Leucoc Biol 60:253–260
16. McIntosh K, Chanock RM (1990) Respiratory syncytial virus. In: Fields BN, et al (eds) Virology, 2nd edn. Raven Press, New York, pp 1045–1072
17. McNulty MS, Bryson DG, Allan GM (1983) Experimental respiratory syncytial virus pneumonia in young calves: microbiologic and immunofluorescent findings. Am J Vet Res 44: 1656–1659
18. Mohanty SB, Ingling AL, Lillie MG (1975) Experimentally induced respiratory syncytial viirus infections in calves. Am J Vet Res 36:417–419
19. Ogra P (1989) Allergy, respiratory tract infections and bronchial hyperreactivity Pediatr Infect Dis J 8:347–352
20. Otto P, Elschner M, Reinhold P, Köhler H, Streckert H-J, Philippou S, Werchau H (1996) A model for respiratory syncytial virus (RSV) infection based on experimental aerosol exposure with bovine RSV in calves. Comp Immun Microbiol Infect Dis 19:85–97
21. Philippou S, Streckert H-J, Humbert B, Otto P, Reinhold P (1993) Respiratory syncytial virus induced bronchiolitis. Eur Respir J 6 [Suppl 17]:539
22. Philippou S, Streckert H-J, Otto P, Reinhold P, Elschner M, Werchau H, Morgenroth K (1996) Morphological studies of the respiratory syncytial virus induced bronchiolitis in experimentally infected calves. Pathol Res Pract 192: 1045–1056
23. Pirie HM, Petrie L, Pringle CR, Allan EM, Kenedy GJ (1981) Acute fatal pneumonia in calves due to respiratory syncytial virus. Vet Res 108:411–416
24. Reinhardt D, Griese M, Morgenroth K (1987) Beziehungen zwischen Infekten und Allergien bei obstruktiven Atemwegserkrankungen im Kindesalter. Monatsschr Kinderheilk 135:615–621
25. Riedel F, Oberdiek B, Streckert H-J, Philippou S, Krusat T, Marek W (1997) Persistence of airway hyperresponsiveness and viral antigen following respiratory syncytial virus bronchiolitis in young guinea pigs. Eur Respir J 10:639–645
26. Stott EJ, Taylor G (1985) Respiratory syncytial virus. Arch Virol 84:1–52
27. Streckert HJ, Philippou S, Riedel F (1996) Detection of respiratory syncytial virus (RSV) antigen in the lungs of guinea-pigs 6 weeks after experimental infection and despite the production of neutralizing antibodies. Arch Virol 141: 401–410
28. Welliver RC (1983) Viral infections and obstructive airway disease in early life. Pediatr Clin North Am 30: 819–828