INFECTION AND IMMUNITY, Apr. 1991, p. 1514–1520
0019-9567/91/041514-07$02.00/0

Vol. 59, No. 4

Morphological and Immunohistochemical Studies of the Lungs and Bronchus-Associated Lymphoid Tissue in a Rat Model of Chronic Pulmonary Infection with *Pseudomonas aeruginosa*

MASATOSHI IWATA* AND ATSUHIKO SATO

The Second Department of Internal Medicine, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu-shi, Japan 431-31

Received 12 July 1990/Accepted 19 December 1990

***Pseudomonas aeruginosa* is one of the most frequently encountered bacterial pathogens in patients with chronic pulmonary infections, including cystic fibrosis and diffuse panbronchiolitis. Bronchus-associated lymphoid tissue (BALT), noted frequently in patients with cystic fibrosis and diffuse panbronchiolitis, is considered to play an important role in the local immunologic defense mechanisms in the respiratory tract. To investigate the role of BALT in chronic pulmonary infections, we developed an animal model for chronic pulmonary infection and studied the morphological and immunohistochemical characteristics of BALT. Experimental pneumonia was produced in rats by intratracheal inoculation of *P. aeruginosa* enmeshed in agar beads. The histological changes corresponded to those occurring in chronic bronchiolitis. Immunohistochemically, surface immunoglobulin M-positive ($sIgM^+$) cells and $sIgA^+$ cells were recognized in the inflamed bronchial walls from day 4, and $sIgG^+$ cells were recognized from day 14. $W3/25^+$ cells exceeded $OX8^+$ cells in number until day 14. In the BALT, there was a massive accumulation of lymphocytes in the lymphatics and high endothelial venules. The development of germinal centers was accompanied by increased numbers of $sIgM^+$ and $sIgA^+$ cells. $W3/25^+$ cells exceeded $OX8^+$ cells in number in the BALT until day 14. On the other hand, $OX8^+$ cells were predominant in comparison with $W3/25^+$ cells at day 21, and then both $sIgM^+$ and $sIgA^+$ cells and inflammatory changes in the lung decreased at day 28. These findings suggest that BALT regulates the local immune responses against chronic pulmonary infection due to *P. aeruginosa*.**

Pseudomonas aeruginosa is one of the most frequently encountered bacterial pathogens in patients with chronic pulmonary infections, including cystic fibrosis (CF) and diffuse panbronchiolitis (DPB) (8, 9). When grown on agar media, *P. aeruginosa* strains of chronically infected patients generally have a mucoid appearance (7, 8, 18). Mucoid *P. aeruginosa* is intractable and virtually impossible to eradicate. Despite all the advances that have been made in the diagnosis and assessment of these infectious diseases, the mechanisms of transmission and colonization of *P. aeruginosa* remain unclear. In order to investigate the interactions between microbial virulence factors and host defense mechanisms, a model of chronic pulmonary infection had to be achieved. Cash et al. (5) developed a rat model of chronic *P. aeruginosa* pulmonary infection by using infective agar beads instilled intratracheally. The inflammatory histologic changes were similar to those of chronic bronchiolitis.

Hyperplastic lymphoid tissues along the airways (bronchus-associated lymphoid tissue [BALT]) are frequently noted in the lungs of patients with CF and DPB (13, 24). Although BALT is considered to play an important role in the local immunologic defense mechanism against foreign antigens in the respiratory tract (1, 2, 20), exact details are poorly understood.

To investigate the role of BALT in chronic pulmonary infections, we developed an animal model of chronic pulmonary infection with *P. aeruginosa* and studied the morphological and immunohistochemical characteristics of BALT.

* Corresponding author.

MATERIALS AND METHODS

Organism. The *P. aeruginosa* strain was isolated from sputum cultures of patients with chronic pulmonary infections (gift from E. Tanaka, Chest Disease Research Institute, Kyoto University, Kyoto, Japan). This strain was serum sensitive and produced a mucoid phenotype when grown on blood agar. Strains were stored frozen in skim milk at −80°C.

Experimental animals. Male Sprague-Dawley rats weighing 150 to 200 g (specific-pathogen-free rats, approximately 6 to 8 weeks old) were purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals. These animals were separated from other animals and were housed in the laboratory with standard care and feeding conditions.

Experimental model of infection. A suspension of bacteria enmeshed in agar beads was made by using slight modifications of previously described methods (5). Briefly, 5 ml of *P. aeruginosa* in melted 2.5% tryptic soy agar was added to 50 ml of heavy mineral oil at 50°C as the oil was stirred rapidly with a magnetic stirring bar. The cold oil-agar mixture was then washed with 0.5% sodium deoxycholate in phosphate-buffered saline (PBS), washed again with 0.25% sodium deoxycholate in PBS, and then washed three more times with PBS. *P. aeruginosa* enmeshed in agar beads was suspended in PBS to 4×10^7 to 6×10^7 CFU/ml. Rats were anesthetized with ether. The trachea was exposed by midline cervical incision, and 0.1 ml of bead suspension was inoculated into the distal bronchus via a curve needle. For a control, sterile agar beads were instilled in the same way, and the control rats were housed in the same laboratory (different cage) with the infected rats.

Bacteriology and histopathology. Each group of animals was exsanguinated by cardiac puncture under anesthesia.

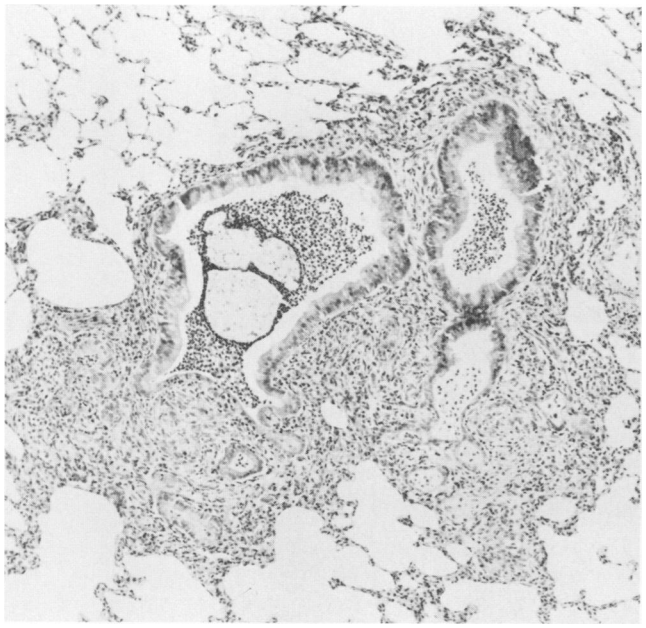
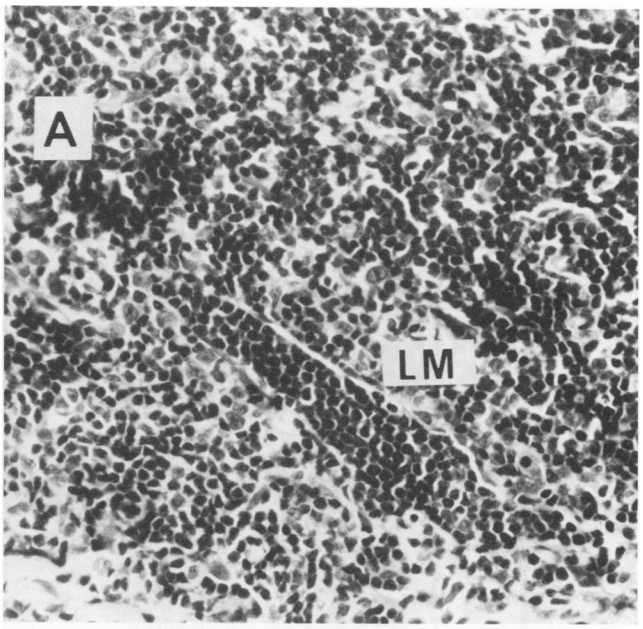


FIG. 1. Light micrograph of lung from a rat infected for 10 days with agar beads containing *P. aeruginosa*. *P. aeruginosa*-impregnated agar beads are seen within the bronchioli. The lumens of the bronchioli are narrowed by a massive cell infiltration, consisting of neutrophils, lymphocytes, and granulation tissues. Magnification, ×25. Cells were stained with hematoxylin and eosin.

The lungs were removed aseptically. The right lungs, used for bacteriological analysis, were homogenized and were cultured quantitatively by serial dilution on blood agar plates. A part of the left lung, used for histologic study, was fixed in 10% buffered formalin by using an inflation fixation apparatus. The lungs were sectioned and stained with hematoxylin and eosin. Elastica van Gieson or Gram staining was carried out when it seemed of particular value.

Immunohistochemistry. The remaining part of the left lungs, used for immunohistochemical study, was fixed in periodate-lysine-paraformaldehyde. Immunohistochemical staining was accomplished by using the avidin-biotin-peroxidase complex technique (10). Isobe's method (11) was applied to quench endogenous peroxidase activity. After being washed in PBS, sections were pretreated with 10% normal rabbit or goat serum to inhibit nonspecific background staining. The slides were incubated with goat anti-rat immunoglobulin G (IgG) (polyclonal antibody; Cappel Laboratories), mouse anti-rat IgA and mouse anti-rat IgM (monoclonal antibodies; Zymed Laboratories), OX6 (monoclonal antibody against rat Ia antigen), W3/25 (against rat T helper cells), and OX8 (against rat T nonhelper cells; Sera Laboratories) for 8 h at 4°C. After being washed in PBS, sections were incubated in turn with a second antibody such as biotinylated rabbit anti-goat IgG or goat anti-mouse IgG for 60 min and then stained by the avidin-biotin-peroxidase complex technique for 30 min (both in Vectastain ABC Kits). The second antibody alone was used for controls. After the final washing, samples were stained with 3.3-diaminobenzidine and counterstained with 1% methyl green. Slides were graded by two independent observers as follows: strongly positive (++), more than 20 positive cells; positive (+), less than 20 cells; negative (−); or equivocal (±), a few questionably stained cells.



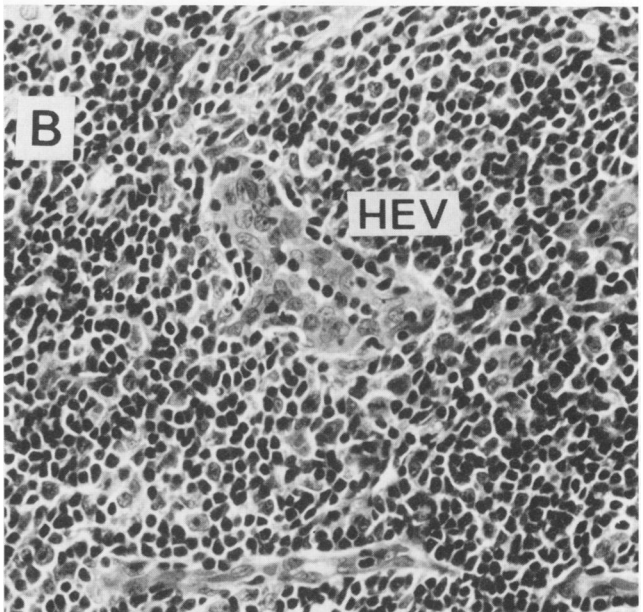


FIG. 2. The PFA of BALT from a rat infected for 7 days. In the PFA, there is a massive accumulation of lymphocytes in both lymphatics (A) and HEV (B). Magnification, ×100. Cells were stained with hematoxylin and eosin. LM, Lymphatics.

RESULTS

Bacteriology. Each group of rats was inoculated intratracheally with 10^6 CFU of *P. aeruginosa* enmeshed in agar beads. Six rats per group were killed at days 1, 4, 7, 10, 14, 21, and 28 after inoculation. The mean CFU recoverable from the homogenized lungs increased to 10^8 CFU at day 1 and decreased to 10^5 CFU at day 4. The number of bacteria remained fairly constant, however, at the level of 10^4 CFU until day 28. In contrast, no bacteria were grown in control groups.

Pathology. Microscopic examination demonstrated both

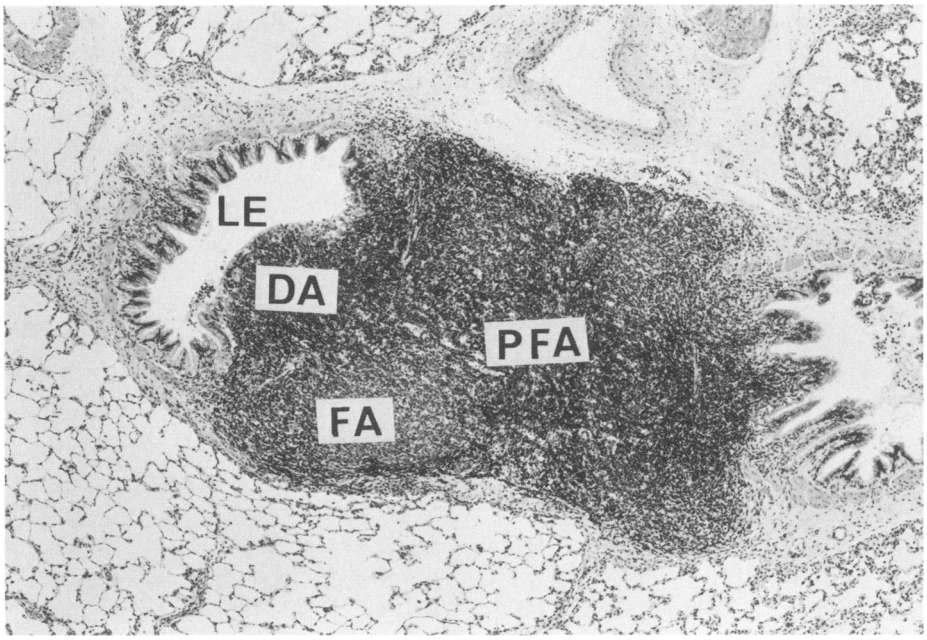


FIG. 3. BALT from a rat infected for 21 days. Enlarged FA with germinal centers can be seen within the BALT. The airway is narrowed by BALT hyperplasia protruding into the bronchial lumen. Magnification, ×16. Cells were stained with hematoxylin and eosin.

bronchial and parenchymal changes. Hyperplasia of goblet cells was frequently seen in bronchial mucosa. Agar beads with *P. aeruginosa*, which were surrounded by a great number of neutrophils, could be seen within large and small airways. Acute bronchopneumonia was observed along the airways from days 1 to 7. The lumens of the bronchioli were narrowed by infiltration of neutrophils, lymphocytes, and granulation tissues from day 7 to day 10 (Fig. 1). Accumulations of foamy macrophages accompanied by lymphocytes were seen around the bronchioli. In later stages, bronchiectasis was a common finding. These chronic inflammatory histologic changes corresponded closely to findings of chronic bronchiolitis observed in patients with CF and DPB. These findings weakened slightly but were recognized until day 28.

Hyperplasia of BALT occurred frequently in the peribronchial area adjacent to airways containing beads. BALT was divisible into four different areas: the lymphoepithelium (LE), dome area (DA), follicular area (FA), and parafollicular area (PFA). The LE was devoid of cilia and was infiltrated with lymphocytes. Proliferation of the high endothelial venules (HEV) and dilatation of the lymphatics were seen in the BALT, and there was a massive accumulation of lymphocytes in both the lymphatics and the HEV at day 7 (Fig. 2). The development of germinal centers in the FA was characteristic from day 7 to day 28. The airways were narrowed as a result of BALT hyperplasia protruding into the bronchial lumen (Fig. 3). The histologic changes of the lungs and BALT observed in rats infected with agar beads containing serum-sensitive mucoid *P. aeruginosa* are summarized in Table 1.

Neither inflammatory reactions in the lung nor germinal center development in BALT was recognized in control groups.

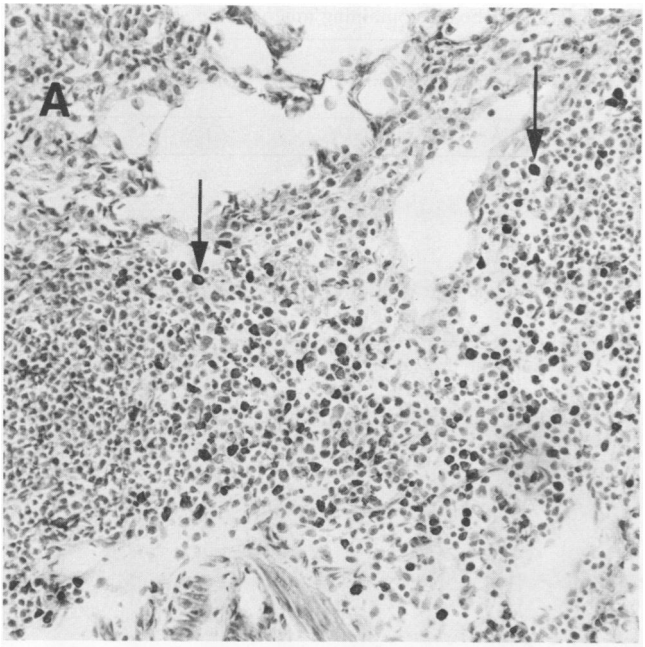
Immunohistochemistry. Surface IgA-positive ($sIgA^+$) cells and $sIgM^+$ cells were first recognized around the inflamed bronchioli at day 4, while $sIgG^+$ cells appeared from day 14 (Fig. 4). $W3/25^+$ cells and $OX8^+$ cells were observed from day 4. $W3/25^+$ cells exceeded $OX8^+$ cells in number at day 14, while the ratio was reversed at day 21. $OX6^+$ (Ia^+) cells first appeared on day 4 and remained in great numbers until day 28 (Table 2).

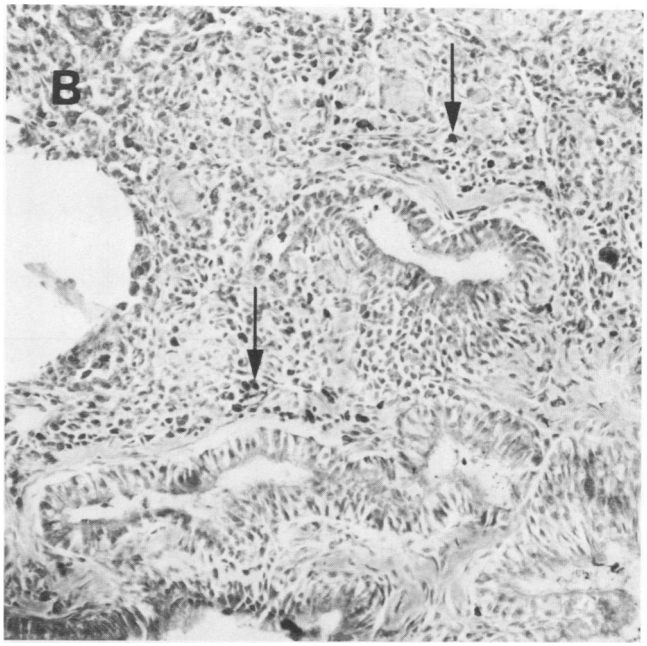
In the BALT (Table 3), the development of germinal centers was accompanied by increased numbers of $sIgM^+$ cells, $sIgA^+$ cells, and T cells. On closer examination, $sIgM^+$ cells were identified mainly in the FA, whereas $sIgA^+$ cells were distributed primarily in the PFA and DA. The majority of $W3/25^+$ cells and $OX8^+$ cells were confined to the PFA, although a few were situated in the FA. In the analysis of the immunocytes in the PFA, the characteristic predominance of $W3/25^+$ cells until day 14 reversed at day 21 (Fig. 5). $OX6^+$ cells were diffusely scattered in BALT from day 4 until day 28 (Fig. 5), whereas $OX6^+$ cells were recognizable only in the FA in the control group. The

TABLE 1. Histologic changes of the lung and BALT from rats infected with agar beads containing mucoid *P. aeruginosa*[a]

No. of days after infection	Degree of cell infiltration[b]							Development of follicular area in BALT
	Bronchioli				Alveoli			
	N	L	F	GT	N	L	F	
1	+	−	−	−	+	−	−	−
4	+	±	−	−	++	±	−	−
7	+	± to +	−	+	+	±	±	+
10	±	± to +	±	+	+	+	±	+
14	− to ±	+	+	−	±	+	+	+
21	− to ±	+	+	−	− to ±	+	+	+
28	−	+	±	−	−	+	±	+

[a] Symbols are as defined in Materials and Methods.
[b] N, Neutrophil; L, lymphocyte; F, foamy cell; GT, granulation tissue.





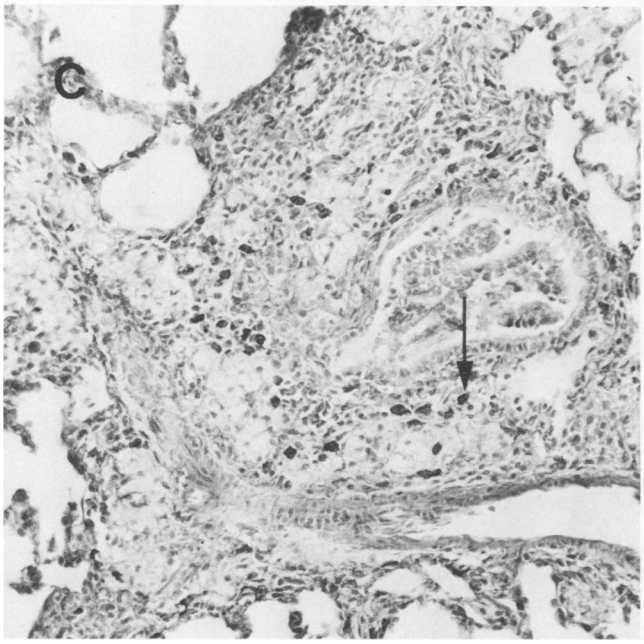


FIG. 4. Distribution of $sIgM^+$ cells (infection for 10 days) (A), $sIgA^+$ cells (infection for 14 days) (B), and $sIgG^+$ cells (infection for 21 days) (C) from infected rats. $sIgM^+$ cells (A), $sIgA^+$ cells (B), and $sIgG^+$ cells (C) are observed around the inflamed bronchioli. The arrows indicate positive cells. Cells were stained with methyl green. Magnification, ×50.

inflamed bronchial walls and BALT exhibited almost identical immunocyte changes. On the other hand, control slides without primary antibody were not stained (Fig. 6).

DISCUSSION

Although chronic pulmonary infections with mucoid strains of *P. aeruginosa* are common in patients with CF and DPB, the mechanisms of transmission and colonization of *P. aeruginosa* remain unknown. Once *P. aeruginosa* is present in patients with CF or DPB, it is rarely eradicated from their lungs, despite the use of various antibiotics. Ultimately, patients die of respiratory failure or consequences of respiratory infection.

To investigate the pathogenesis in chronic pulmonary infection with *P. aeruginosa*, we established the animal model of chronic pulmonary infections caused by *P. aeruginosa* by using the method described above. The results were that the lumens of the bronchioli were narrowed by infiltration of lymphocytes, granulation tissues, and foamy macrophages, which led to bronchiectasis in later stages. These histological changes corresponded to those observed in patients with chronic bronchiolitis which accompanied CF or DPB. Previously, chronic pulmonary infections were not achieved in the absence of immunosuppressive agents, so the discussion should focus on the factors of the host-parasite relationship which might influence the achievement of a chronic pulmonary infection model.

One of the most important factors seemed to be the virulence that instilled pathogens possess. Differences of clinical aspects could be explained by the fact that *P.*

TABLE 2. Distribution of cells infiltrated in the airways of rats infected with agar beads containing mucoid *P. aeruginosa*

No. of days after infection	Degree of cell infiltration in inflamed bronchial wall[a]					
	IgG^+	IgA^+	IgM^+	$OX6^+$ (Ia^+)	$W3/25^+$	$OX8^+$
1	−	−	−	±	−	−
4	−	±	±	+	±	− to ±
7	−	±	±	++	±	±
10	−	+	++	++	+	±
14	±	+	+	++	++	+
21	+	+	+	++	+	++
28	±	±	±	+	±	+

[a] Specificities of $W3/25^+$ were helper T cells, macrophages, and dendritic cells, and specificities of $OX8^+$ were cytotoxic/suppressor T cells and NK cells. Symbols are as defined in Materials and Methods.

TABLE 3. Distribution of cells infiltrated in BALT of rats infected with agar beads containing mucoid *P. aeruginosa*

No. of days after infection	Degree of cell infiltration in BALT[a]								
	IgG$^+$	IgA$^+$	IgM$^+$	OX6$^+$ (Ia$^+$)		W3/25$^+$		OX8$^+$	
				FA	PFA	FA	PFA	FA	PFA
1	±	±	+	+	±	±	+	− to ±	+
4	±	±	+	+	+	±	+	±	+
7	±	+	++	++	+	+	+ to ++	±	+
10	±	+	++	++	+ to ++	+	++	±	+ to ++
14	±	+	++	++	++	+	++	± to +	++
21	±	+	++	++	++	± to +	+ to ++	± to +	++
28	±	±	+	++	+ to ++	±	+ to ++	±	+ to ++

[a] Symbols are as defined in Materials and Methods.

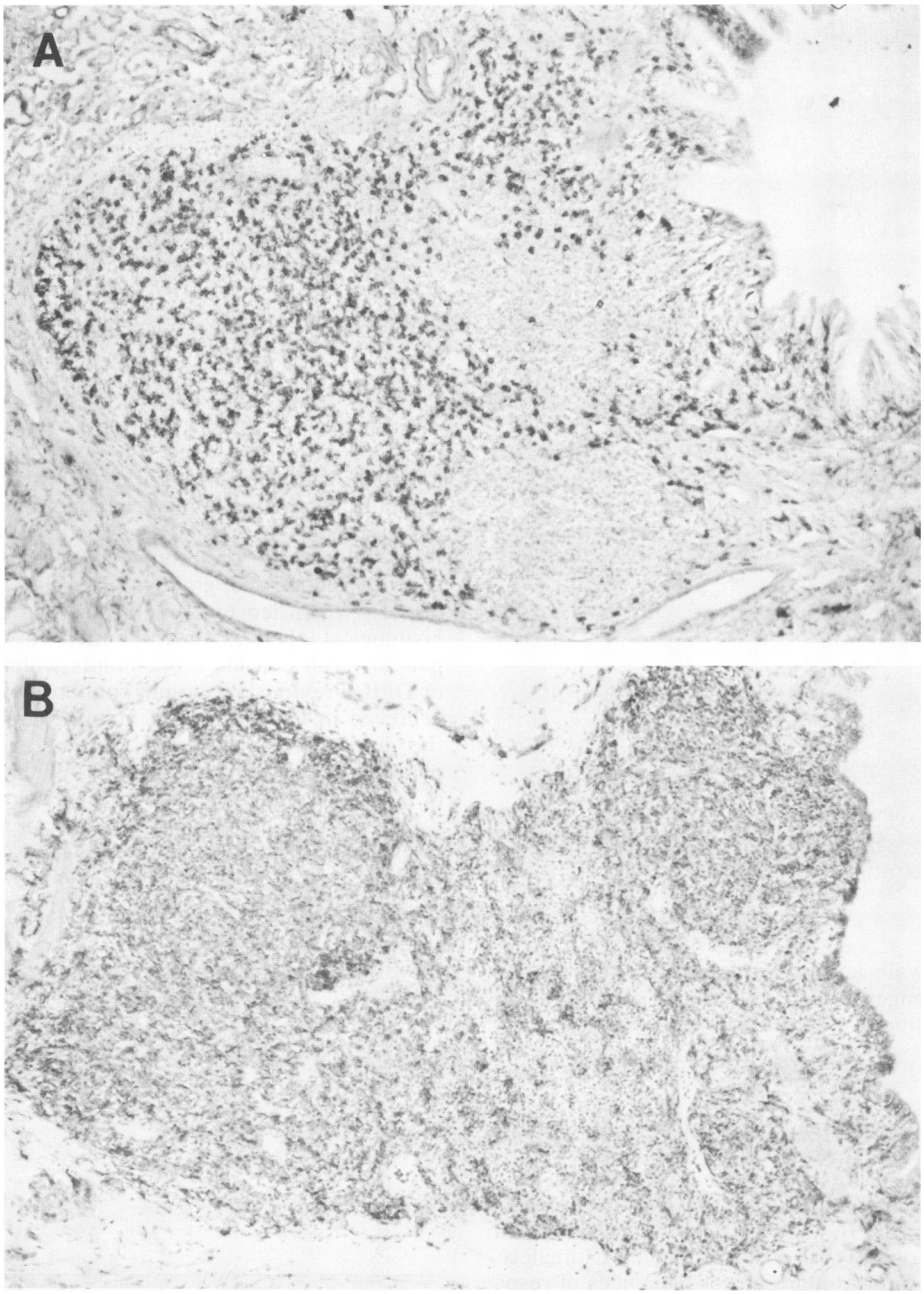


FIG. 5. Distribution of OX8$^+$ cells and OX6$^+$ cells in BALT from infected rats. OX8$^+$ cells (A) increase mostly in PFA at day 21. OX6$^+$ cells (B) are observed diffused throughout BALT at day 28. Magnification, ×33. Cells were stained with methyl green.

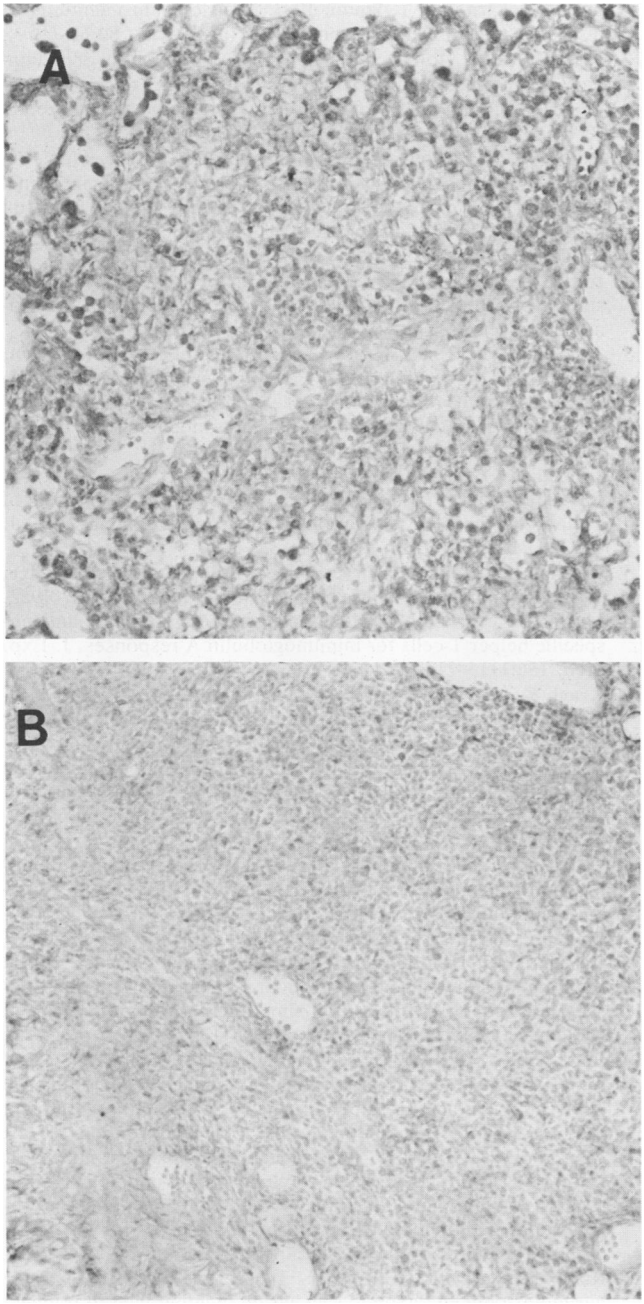


FIG. 6. Control slides without primary antibody. The lymphocytes in the chronic inflammation (A) (second antibody, IgM) or BALT hyperplasia (B) (second antibody, OX8) were not stained. Magnification, ×50. Cells were stained with methyl green.

aeruginosa strains isolated from patients with CF or DPB are sensitive to the bactericidal activity of human serum, whereas *P. aeruginosa* strains isolated from patients with other diseases, such as from an immunocompromised host, are sometimes resistant (8). Furthermore, bacterial adherence to the airway endothelium is a very important process in pulmonary infections. Ramphal and Pier (22) suggest that the mucoid exopolysaccharide produced by mucoid strains of *P. aeruginosa* may play an important role in the adhesion process. It has also been reported that most of the mucoid exopolysaccharide isolated from patients with CF and DPB is resistant to phagocytosis because of the inhibition of opsonophagocytosis mediated by the antibody against the cell wall lipopolysaccharide (4, 22). These observations suggest that the virulence and mucoid production of *P. aeruginosa* should be primary considerations when establishing a chronic lung infection model with *P. aeruginosa*.

Another factor which may affect the achievement of a chronic infection model is the use of agar beads. In 1979, Cash et al. (5) succeeded in developing a rat model of chronic respiratory infection with *P. aeruginosa* by using bacteria enmeshed in agar beads. The histological analysis of this model disclosed that agar beads caused the bronchial narrowing or obstruction, which facilitated the local persistent infections. In clinical cases, bronchial mucin or secretion, inflamed bronchial walls, or BALT hyperplasia usually induces bronchial narrowing. It thus appears that the mechanical narrowing of bronchioli is partly attributable to BALT hyperplasia. In fact, BALT is well expressed in animals that have undergone various antigenic stimulations including those by bacteria, viruses, and mycoplasmas (13, 14).

Immunologic factors in the host defense mechanism are also very important, and BALT is considered to play important roles in the local and systemic immune responses of the lung. BALT has been defined by Bienenstock et al. (1, 2) as lymphoid tissue along the airways consisting of both lymphoid aggregates and lymphocytes that are well organized into follicles and covered with a specialized LE, which in many respects resembles gut-associated lymphoid tissue. In humans, BALT is often found in the lungs of patients with chronic recurrent pneumonia, CF, and DPB (12, 17, 24). As a direct result of BALT hyperplasia, bronchiolar stenosis is frequently recognized in DPB (12). However, studies of the immunologic responses of BALT in chronic pulmonary infections are lacking. We studied BALT both morphologically and immunohistochemically.

Morphologically, BALT was found mostly at the bifurcations from the bronchi to bronchioli and was divisible into four different areas (the LE, DA, FA, and PFA), as seen in previous reports. This localization and structure are thought to allow antigen uptake to BALT through the LE. The LE covering the BALT has the same structural appearance as in Peyer's patches (3). Specialized M (microfold) cells within the LE have the capacity to sample various antigens (20). By using horseradish peroxidase and inhaled bacillus Calmette-Guérin, Racz et al. (19) reported that the LE covering BALT represented a major portal of entry of antigens to the lung. Fournier et al. (6) have shown that the BALT LE could selectively sample antigens in the airways at a higher rate than the surrounding epithelium.

In our model, $OX6^+$ cells increasingly diffused throughout BALT from day 4 until day 28, and germinal centers developed from day 7 after inoculation. Antigen-presenting cells including dendritic and interdigitating macrophages in DA and FA help antigens which penetrate through the LE interact with lymphocytes (20). Then immune responses are performed in concert with B cells in the FA (B-cell-dependent area) and T cells in the PFA (T-cell-dependent area). At the same time, we observed a massive accumulation of small lymphocytes in dilated lymphatics, and HEV proliferated in PFA. Since it has been reported that antigens may be transported via macrophages from BALT to the draining lymph node and that HEV may play a main part in recirculation of lymphocytes (21, 23), it can be surmised from a cytokinetic viewpoint that our findings represent the efflux and influx of lymphocytes through lymphatics and HEV,

respectively. These responses may amplify the immune responses in BALT (16). In Peyer's patches, it was reported that initial appearance of helper T cells may induce the differentiation of the IgA cells or may cause isotype switching from IgM cells to $sIgA^+$ cells (15). In our study, both $sIgM^+$ and $sIgA^+$ cells in BALT increased from day 7. At the same time, $W3/25^+$ cells and $OX6^+$ cells in BALT also increased. So these immunocyte changes in BALT must be regulated by the same mechanism. In later stages (after day 21), the number of $OX8^+$ cells increased and exceeded the number of $W3/25^+$ cells, which might be associated with the termination of immune responses in BALT. These immunocyte changes were almost identical between the BALT and the inflamed bronchial walls. In fact, both $sIgM^+$ and $sIgA^+$ cells slightly decreased, and morphologic inflammatory changes of the lung decreased slightly at day 28.

Although specific antibody-producing cells to *P. aeruginosa* should be identified and quantified, these results suggest that BALT may have a specific role in developing the local immune responses against chronic lung infections due to *P. aeruginosa*.

ACKNOWLEDGMENTS

We wish to thank T. Yoshimi, Department of Medicine, and A. Yamashita, Department of Anatomy, Hamamatsu University School of Medicine, for their encouragement and K. Chida, Department of Medicine, Hamamatsu University School of Medicine, for his advice.

REFERENCES

1. **Bienenstock, J., N. Johnston, and D. Y. E. Perry.** 1973. Bronchial lymphoid tissue. I. Morphological characteristics. Lab. Invest. **28**:686–692.
2. **Bienenstock, J., N. Johnston, and D. Y. E. Perry.** 1973. Bronchial lymphoid tissue. II. Functional characteristics. Lab. Invest. **28**:693–698.
3. **Bockman, D. E., and W. Stevens.** 1977. Gut-associated lymphoepithelial tissue: bidirectional transport of tracer by specialized epithelial cells associated with lymphoid follicles. RES J. Reticuloendothel. Soc. **21**:245–254.
4. **Cabral, D. A., B. A. Loh, and D. P. Speert.** 1987. Mucoid Pseudomonas aeruginosa resists nonopsonic phagocytosis by human neutrophils and macrophages. Pediatr. Res. **22**:429–431.
5. **Cash, H. A., D. E. Wood, B. McCullough, W. G. Johanson, and J. A. Bass.** 1979. A rat model of chronic respiratory infection with Pseudomonas aeruginosa. Am. Rev. Respir. Dis. **119**:453–459.
6. **Fournier, N., F. Vai, J. P. Derenne, and R. Pariente.** 1977. Bronchial lymphoepithelial nodules in the rat. Morphologic features and uptake and transport of exogenous proteins. Am. Rev. Respir. Dis. **116**:685–694.
7. **Høiby, N., G. Doring, and P. O. Schiotz.** 1987. Pathogenic mechanisms of chronic Pseudomonas aeruginosa infections in cystic fibrosis patients. Antibiot. Chemother. (Basel) **39**:60–76.
8. **Høiby, N., and S. Olling.** 1977. Pseudomonas aeruginosa infection in cystic fibrosis. Bactericidal effect of serum from normal individuals and patients with cystic fibrosis on P. aeruginosa strains from patients with cystic fibrosis or other diseases. Acta Pathol. Microbiol. Scand. Sect. C **85**:107–114.
9. **Homma, H., A. Yamanaka, S. Tanimoto, M. Tamura, Y. Chijimatsu, S. Kira, and T. Izumi.** 1983. Diffuse panbronchiolitis. A disease of the transitional zone of the lung. Chest **83**:63–69.
10. **Hsu, S. M., L. Raine, and H. Franger.** 1981. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J. Histochem. Cytochem. **29**:577–580.
11. **Isobe, Y., S. T. Chen, P. K. Nakane, and W. R. Brown.** 1977. Studies on translocation of immunoglobulins across intestinal epithelium. I. Improvements in the peroxidase-labeled antibody method for application to study of human intestinal mucosa. Acta Histochem. Cytochem. **10**:161–171.
12. **Iwata, M., A. Sato, and H. Hayakawa.** 1987. Histopathological studies of bronchus-associated lymphoid tissue (BALT) in pulmonary diseases. Jpn. J. Lymphol. **10**:81–85.
13. **Jericho, K. W. F., P. K. C. Austwick, R. T. Hodge, and J. B. Dixon.** 1971. Intrapulmonary lymphoid tissue of pigs exposed to aerosols of carbon particles, of Salmonella oranienburg, of Mycoplasma granularum, and to an oral inoculum of larvae of Metastrongylus apri. J. Comp. Pathol. **81**:13–21.
14. **Jericho, K. W. F., J. B. Derbyshire, and J. E. T. Jones.** 1971. Intrapulmonary lymphoid tissue of pigs exposed to aerosols of haemolytic Streptococcus group L and porcine adenovirus. J. Comp. Pathol. **81**:1–11.
15. **Kiyono, H., J. R. McGhee, L. M. Mosteller, J. H. Eldridge, W. J. Koopman, J. F. Kearney, and S. M. Michalek.** 1982. Murine peyer's patch T cell clones. Characterization of antigen-specific helper T cells for immunoglobulin A responses. J. Exp. Med. **156**:1115–1130.
16. **Kraal, G., and A. Twisk.** 1984. The interaction of high endothelial venules with T and B cells in peripheral lymph nodes after antigenic stimulation. Eur. J. Immunol. **14**:586–588.
17. **Meuwissen, H. J., and H. Hussain.** 1982. Bronchus-associated lymphoid tissue in human lung: correlation of hyperplasia with chronic pulmonary disease. Clin. Immunol. Immunopathol. **23**:548–561.
18. **Pier, G. B.** 1985. Pulmonary disease associated with Pseudomonas aeruginosa in cystic fibrosis: current status of the host-bacterium interaction. J. Infect. Dis. **151**:575–580.
19. **Racz, K. T., P. Racz, Q. N. Myrvik, J. R. Ockers, and R. Geister.** 1979. Uptake and transport of horseradish peroxidase by lymphoepithelium of the bronchus-associated lymphoid tissue in normal and bacillus Calmette-Guerin-immunized and challenged rabbits. Lab. Invest. **41**:106–115.
20. **Racz, P., K. T. Racz, Q. N. Myrvik, and L. K. Fainter.** 1977. Functional architecture of bronchial associated lymphoid tissue and lymphoepithelium in pulmonary cell-mediated reactions in the rabbit. RES J. Reticuloendothel. Soc. **22**:59–83.
21. **Racz, P., K. T. Racz, Q. N. Myrvik, B. T. Shannon, and S. H. Love.** 1978. Sinus reactions in the hilar lymph node complex of rabbits undergoing a pulmonary cell-mediated immune response: sinus lymphocytosis and immature sinus histiocytosis. RES J. Reticuloendothel. Soc. **24**:499–525.
22. **Ramphal, R., and G. B. Pier.** 1985. Role of *Pseudomonas aeruginosa* mucoid exopolysaccharide in adherence to tracheal cells. Infect. Immun. **47**:1–4.
23. **Stamper, H. B., and J. J. Woodruff.** 1977. An in vitro model of lymphocyte homing. I. Characterization of the interaction between thoracic duct lymphocytes and specialized high endothelial venules of lymph nodes. J. Immunol. **119**:772–780.
24. **Starke, J. R., M. S. Edwards, C. Langston, and C. J. Baker.** 1987. A mouse model of chronic pulmonary infection with Pseudomonas aeruginosa and Pseudomonas cepacia. Pediatr. Res. **22**:698–702.