Histol Histopathol (1997) 12: 931-935

Histology and Histopathology

Bronchial epithelium associated to lymphoid tissue does not selectively express vimentin

L.M. Alonso, A. Cortés, M.G. Barrutia, T. Romo, A. Varas and A.G. Zapata
Department of Cell Biology, Faculty of Biology, Complutense University, Madrid, Spain

Summary. The existence of a lymphoepithelium containing M cells in the bronchus-associated lymphoid tissue (BALT) of several species has repeatedly been questioned. In previous electron microscopical studies we failed to ultrastructurally identify these cells in the epithelium covering bronchial lymphoid tissue of adult rats. In the present study, we analyze immunohistochemically the expression of vimentin, an intermediate filament, reported to be a sensitive marker for rabbit M cells, in both BALT and Peyer's patches. Our results demonstrate, however, the absence of vimentin expression in the epithelium covering the bronchial lymphoid aggregates as well as in the lymphoepithelium of the Peyer's patches. On the contrary, both epithelia are strongly cytokeratin positive. Furthermore, numerous vimentin-positive lymphocytes appear in both lymphoid organs. Results are discussed from a view of the possible relationship between BALT and the so-called mucosae-associated lymphoid tissue (MALT).

Key words: M cells, Vimentin, Cytokeratins, BALT, Peyer's patches

Introduction

The relationship between bronchial-associated lymphoid tissue (BALT) and the so-called mucosae-associated lymphoid tissue (MALT) is a matter of discussion. Apart from other histological differences and the extensive variations observed between species, follicle-associated cells (M cells) containing short microvilli and a few small vesicles presumably involved in antigen processing have not been ultrastructurally identified in the rat BALT (Fernández-Cabezudo, 1990) and it has been emphasized that these only appear after repeated antigenic stimulation (Racz et al., 1977; Plesch, 1982; van der Brugge-Gamelkoorn et al., 1986).

On the other hand, there are no cell markers available capable of identifying these cells in other epithelia. Owen and Bhalla (1983) reported reduced expression of brush-border alkaline phosphatase in M cells, but a high variability in enzymatic activity and intermediate cell types, with unknown functional characteristics, have also been reported (Bye et al., 1984; Smith et al., 1987, 1988). In addition, cathepsin E, an aspartic proteinase involved in antigen processing (Bennet et al., 1992), has also been used to immunohistochemically identify M cells in the follicle-associated epithelium of intestine and tonsils (Finzi et al., 1993). In the rat BALT, Tatrai et al. (1994) found differences in the composition of the surface carbohydrate between ciliated bronchial and non-ciliated lymphoepithelium. Recently, the protein vimentin has been proposed as a sensitive marker for M cells of the rabbit ileum and caecum (Gebert et al., 1992; Jepson et al., 1992; Gebert, 1995) and these same authors described a few vimentin-positive epithelial cells in the lymphoepithelium of rabbit BALT (Gebert and Hach, 1992). However, M cells from rats, mice and humans do not appear to contain vimentin (Jepson et al., 1992).

In order to further analyze the existence or absence of M cells in rat BALT and to confirm vimentin as a possible universal cell marker for M cells we studied immunohistochemically the expression of either vimentin or keratin in the lymphoepithelium of both Peyer's patches and BALT. Our results, in agreement with those by Jepson et al. (1992), demonstrate that the rat lymphoepithelium covering the bronchial lymphoid aggregates, as well as the other cells of the airway epithelium, are vimentin negative and cytokeratin positive.

Materials and methods

Lungs as well as Peyer's patches, aseptically removed from adult Wistar Furth rats, were embedded in Tissue-Tek and snap-frozen in liquid nitrogen. For immunohistochemistry, 7 μm-thick cryostat sections of the tissues were cut, air-dried and fixed in acetone at room temperature for 10 min. After, they were rehydrated in PBS for 5 minutes and incubated for 1 h at room temperature with 100 μl of a 1:100 dilution in PBS of the following monoclonal antibodies (mAb): Vim 13.2 (vimentin-specific; Bio-Genex Laboratories,

Offprint requests to: Dr. A. G. Zapata, Department of Cell Biology, Faculty of Biology, Complutense University, 28040 Madrid, Spain

Dublin, CA) and LEG-1 (cytokeratin specific, gift from Dr. Jorcano, CIEMAT, Madrid). Endogenous peroxidase activity was blocked by 15 min incubation in an H_2O_2 : methanol (1:50) solution. After washing in PBS for 15 min, rabbit anti-mouse immunoglobulins conjugated to horseradish peroxidase (DAKO) were applied to the sections at a dilution of 1:40 in PBS for 30 min at room temperature. Peroxidase activity was detected as a brown colour following 5 min incubation with diaminobenzidine (DAB) in PBS containing H_2O_2. Sections were counterstained with methylene blue, dehydrated and mounted in DPX.

Results

The BALT of adult rats is formed by distinct morphological units, named BALUs (Sminia et al., 1989), consisting of large aggregates of lymphoid tissue under the bronchial epithelium. The lymphoid masses constitute true cell infiltrates in the connective tissue and the smooth muscle of the walls of the bronchial ducts, rather than isolated organs. Moreover, neither a histological regionalization nor germinal centers were observed in the rat BALT (Fig. 1). Immunodetection of vimentin in the BALT of adult rats demonstrated the lack of staining of the bronchial epithelium after using the mAb 13.2 specifically raised against this molecule (Fig. 2). Nevertheless, numerous small cells, morphologically similar to lymphocytes, appeared strongly stained in the bronchial lymphoid tissue (Fig. 2) as well as in the bronchial epithelium (Figs. 2, 3). On the contrary, the epithelium covering the bronchial lymphoid tissue, but

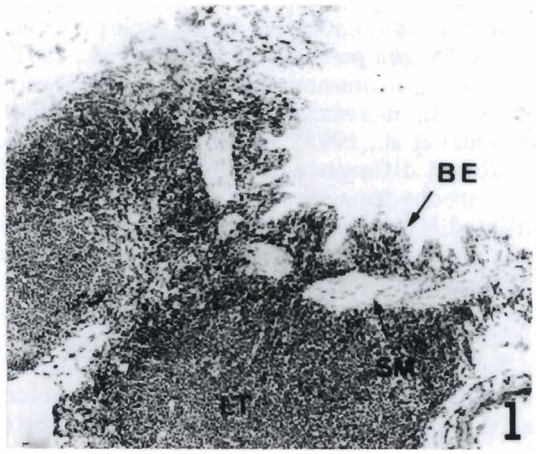


Fig. 1. Lymphoid aggregates in the bronchus of an adult rat. The lymphoid tissue (LT) accumulates in the connective tissue and the smooth muscle (SM) of the bronchial walls under the bronchial epithelium (BE). Note the lack of histological regionalization and germinal centers in the bronchial tissue. x 65

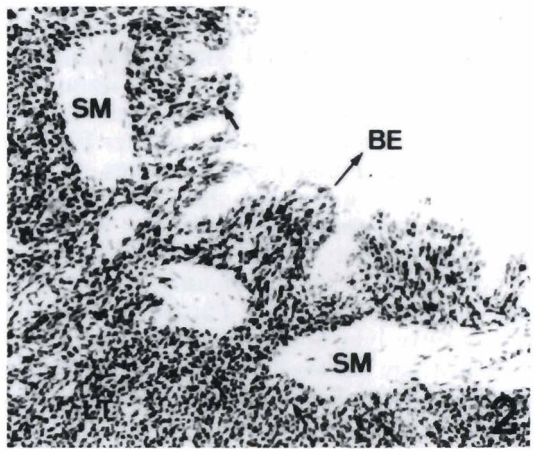


Fig. 2. Numerous vimentin-positive lymphoid cells (→) occur in the bronchial lymphoid tissue (LT) and infiltrate the epithelium (BE), whereas it remains unstained. SM: smooth muscle. x 130

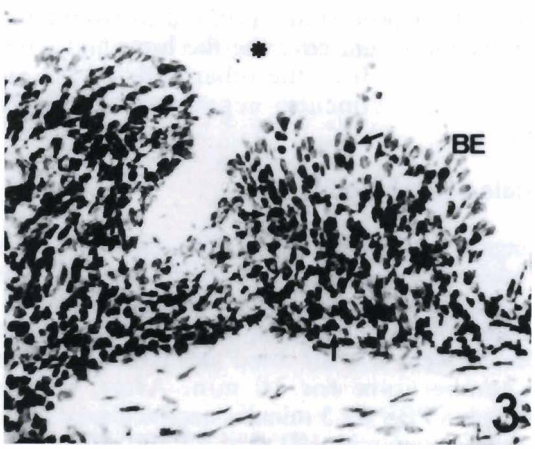


Fig. 3. Vimentin-positive lymphocytes (→) infiltrate the epithelium which covers the bronchial lymphoid tissue. Black circle: bronchial lumen. x 260

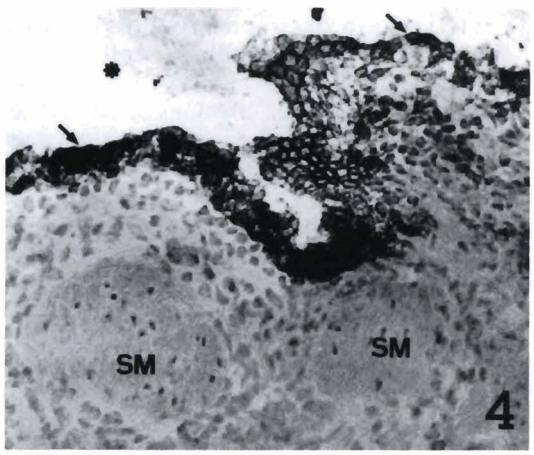


Fig. 4. Homogeneous staining for cytokeratins of the bronchial epithelium (→), but not of the underlying lymphoid tissue (LT) of rat BALT. SM: smooth muscle; Black circle: bronchial lumen. x 260

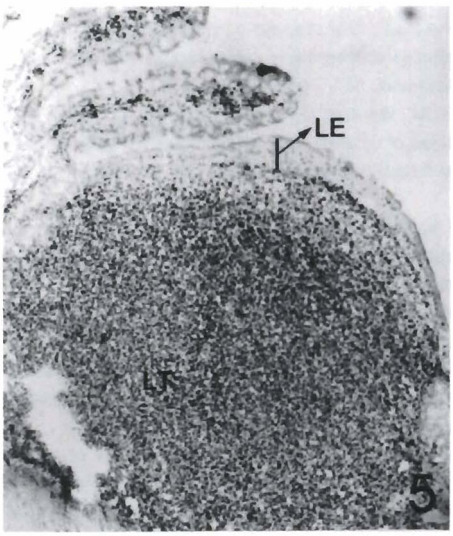
not the lymphoid tissue itself, was homogeneously stained with a mAb (LEG-1) specific to cytokeratins (Fig. 4). Likewise, whereas the lymphoepithelium which covered the dome region in rat Peyer's Patches remained unstained for vimentin (Fig. 5), numerous vimentin-positive small lymphocytes appeared in the intestinal lymphoid tissue and infiltrating the lymphoepithelium (Fig. 6).

Discussion

M cells were described in the lymphoepithelium covering the gut-associated lymphoid tissue (GALT) and were considered to be involved in the transport of antigens from gut lumen to the underlying lymphoid tissue. They ultrastructurally exhibit short microvilli and contain vesicles in the apical cytoplasm presumably involved in antigen uptake (Ermak et al., 1989). The existence of M cells in other MALT locations, and more specifically in the BALT, is a matter of discussion. In fact, the relationship between BALT itself and the common mucosal immune system is controversial. To date, there are important differences in the histological organisation of both tissues (van der Brugge-Gamelkoorn, 1986; Sminia et al., 1989); a lympho-epithelium covering the bronchial aggregates has only been observed after extensive immunization, especially in rodents (Sminia et al., 1989) and attempts to induce immune responses in the lung have led to contradictory results (for review see Alonso et al., 1994). Our own results suggest that the immune response in the pulmonary lymphoid tissue is a local phenomenon with activation of the systemic immune responses rather than of the MALT (Alonso, 1993; Alonso et al., 1994).

In a series of previous studies, vimentin and cytokeratins were demonstrated to appear in the M cells but not in the enterocytes of rabbit GALT (Gebert et al., 1992; Jepson et al., 1992). Later, these authors extended their studies to the epithelial cells of rabbit BALT and suggested that vimentin might be a cell marker for M cells (Gebert and Hach, 1992). However, Jepson et al (1992) were unable to observe expression of vimentin in rat, mouse and human M cells of either Peyer's patches or appendix. Our present results confirm that neither the lymphoepithelium of Peyer's patches nor the bronchial epithelium associated to lymphoid tissue of adult rats express immunohistochemically-identifiable vimentin. In agreement, Gebert et al. (1994) have reported the absence of vimentin expression by M cells of porcine Peyer's patches. On the contrary, they suggested that cytokeratin 18 (not tested in the present study) would be a useful marker for detecting porcine M cells. It is important to note that the BALT is very well developed in rabbits but is much less abundant in rodents and almost irrelevant in pigs (Jericho, 1970; Pabst and Gehrke, 1990), dogs (Brownstein et al, 1980) and humans (Pabst and Gehrke, 1990). In fact, a clear bronchial lymphoepithelium, containing infiltrating lymphocytes and non-ciliated cells (Racz et al., 1977; Tenner-Racz et al., 1979) with ability to transport antigens from the airway lumen to the bronchial lymphoid tissue (van der Brugge-Gamelkoorn et al., 1986) has only been reported in rabbits, whereas in rats it only occurs after extensive immunization (Sminia et al, 1989). Furthermore, many authors consider that antigens gain entrance to lung via the parenchyma macrophages (Holt et al., 1985; Rochester et al., 1988; Reynolds, 1989) (our own unpublished observations) or through the lung draining lymph nodes (van der Brugge-Gamelkoorn et al., 1985; Sminia et al., 1987; Bice and Shopp, 1988).

On the other hand, numerous vimentin-expressing lymphocytes appear in the dome area and infiltrate the epithelium of both bronchus and Peyer's patches. Gebert and Hach (1992) reported that only using a high concentration of a mAb anti-vimentin did lymphocytes



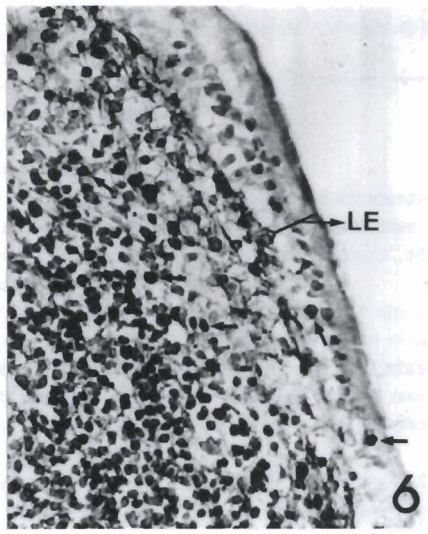


Fig. 5. Peyer's patches of adult rat. The lymphoepithelium (LE) covering the lymphoid tissue (LT) does not immunohistochemically express vimentin. x 65

Fig. 6. Vimentin-positive lymphocytes (→) in both the dome and the lymphoepithelium (LE) of the Peyer's patches of an adult rat.

of both bronchial lymphoid tissue and lymphoepithelium express this intermediate filament. In fact, vimentin is presumably the only intermediate filament of lymphocytes and other haematopoietic cells (Tsuru et al., 1990). It is a growth-responsive gene in T cells (Hornbeck et al., 1993) which is induced in normal resting murine T lymphocytes by diverse factors, including IL-2 (Podolin and Prystowsky, 1991).

Vimentin expression on rabbit M cells has been correlated with the origin of M cells, an aspect which is also a subject of debate. Some authors suggested these are formed from mature enterocytes possibly under the influence of lymphocytes (Smith and Peacock, 1982). Ermark et al. (1989) demonstrated, however, that M cell differentiation may occur independently of lymphocyte inductive signals and other authors have claimed their direct origin from crypt cells (Bye et al., 1984; Jepson et al., 1993). With regard to this, Gebert et al. (1992) described an increase in vimentin expression but a decrease in keratin expression by these cells in their migration from the mouth of the crypts towards the apex of the dome area in rabbit Peyer's patches. Nevertheless, analysis of the follicle-associated epithelium (FAE) differentiation of chicken bursal during ontogeny demonstrates that the FAE co-expresses vimentin and cytokeratins during the entire period of embryogenesis, but that later vimentin expression disappears (Olah and Glick, 1992).

In summary, vimentin could be useful to characterize rabbit M cells but it is not, obviously, a universal marker for this cell type. On the other hand, although both the epithelium covering bronchial lymphoid aggregates and the lymphoepithelium of Peyer's patches do not express vimentin, we cannot conclude from the current results that they exhibit similar morpho-functional features, as members of the common mucosal immune system.

Acknowledgements. We wish to thank Dr. Jorcano (CIEMAT, Madrid, Spain) for providing mAb LEG-1. This work was supported in part by Spanish Ministry of Education and Science grants CICYT PB 91-0374 and CICYT PB 94-0332. L.M. Alonso is fellow of the Spanish Ministry of Education and Science.

References

Alonso L.M. (1993). Respuestas primarias y secundarias en pulmón a antígenos T-dependientes (TNP-KLH) y T-independientes (TNP-LPS) administrados por distintas rutas. MSc, Complutense Univ. Madrid. Spain.

Alonso L.M., van Appeldorf A., Bañuelos M., Barrutia M.G. and Zapata A.G. (1994). Antibody forming cells (AFCs) in the lung lymphoid tissue after primary and secondary immunization through different routes. A semiquantitative analysis in adult rats using T-dependent (TNP-KLH) and T-independent (TNP-LPS) antigens. Adv. Exp. Med. Biol. 355, 277-281.

Bennett K., Levine T., Ellis J.S., Peanosky R.S., Salmoff I.M., Kay J. and Chain B.M. (1992). Antigen processing for presentation by class II major histocompatibility complex requires cleavage by cathepsin E. Eur. J. Immunol. 22, 1519-1524.

Bice D.E. and Shopp G.M. (1988). Antibody responses after lung immunization. Exp. Lung Res. 14, 133-155.

Brownstein D.G., Reber A.H., Bice D.E., Muggenburg B.A. and Hill J.O. (1980). Immunology of the lower respiratory tract. Serial morphologic changes in the lungs and tracheobronchial lymph nodes of dogs after intrapulmonary immunization with sheep erythrocytes. Am. J. Pathol. 98, 499-514.

Bye W.A., Allen C.H. and Trier J.S. (1984). Structure, distribution and origin of M cells on Peyer's patches of mouse ileum. Gastroenterology 86, 789-801.

Ermak T.H., Steger H.J., Strober S. and Owen R.L. (1989). M cells and granular mononuclear cells in Peyer's patch domes of mice depleted of their lymphocytes by total lymphoid irradiation. Am. J. Pathol. 134, 529-533.

Fernández-Cabezudo M.J. (1990). Desarrollo postnatal del tejido linfoide en el pulmón de rata. Doctoral Thesis. Complutense Univ. Madrid. Spain.

Finzi G., Cormaggia M., Capella C., Fiocca R., Bosi F., Solcia E. and Samloff I.M. (1993). Cathepsin E in follicle associated epithelium of intestine and tonsils: localisation to M cells and possible role in antigen processing. Histochemistry 99, 201-211.

Gebert A. (1995). Identification of M cells in the rabbit tonsil by vimentin immunohistochemistry and in vivo protein transport. Histochem. Cell. Biol. 104, 211-220.

Gebert A. and Hach G. (1992). Vimentin antibodies stain membranous epithelial cells in the rabbit bronchus associated lymphoid tissue (BALT). Histochemistry 98, 271-273.

Gebert A., Hach G. and Bantels H. (1992). Co-localisation of vimentin and cytokeratins in M cells of rabbit GALT. Cell Tissue Res. 269, 331-340.

Gebert A., Rothkotter H.J. and Pabst R. (1994). Cytokeratin 18 is an M-cell marker in porcine Peyer's patches. Cell. Tissue Res. 276, 213-221.

Holt P.G., Degebrodt A., Venaille J., O'Leary C., Krska K., Flexman J., Shellman G.R., Young P., Penhale J., Robertson T. and Papadimitriou J.M. (1985). Preparation of interstitial lung cells by enzymatic digestion of tissue slides. Preliminary characterisation by morphology and performance in functional arrays. Immunology 54, 139-147.

Hornbeck P.V., Garrels J.I., Capetanaki Y. and Heimer S. (1993). Vimentin expression is differentially regulated by Il-2 and Il-4 in mouse T cells. J. Immunol. 151, 4013-4021.

Jepson M.A., Mason C.M., Bennett M.K., Simmons N.L. and Hirst B.H. (1992). Co-expression of vimentin and cytokeratin in M cells of rabbit intestinal lymphoid follicle-associated epithelium. Histochem. J. 24, 33-39.

Jepson M.A., Clark M.A., Simmons N.L. and Hirst B.H. (1993). Epithelial M cells in the rabbit caecal lymphoid patch display distinctive surface characteristics. Histochemistry 100, 441-447.

Jericho K.W.F. (1970). Intrapulmonary lymphoid tissue of healthy pigs. Res. Vet. Sci. 11, 548-552.

Olah Y. and Glick B. (1992). Dynamic changes in the intermediate filaments of the epithelial cells during development of the chicken's bursa of Fabricius. Poult. Sci. 71, 1857-1872.

Owen R.L. and Bhalla D.K. (1983) Cytochemical analysis of alkalin phosphatase and esterase activities and of lectin-binding and anionic sites in rat and mouse Peyer's patch M cells. Am. J. Anat. 168, 199-212.

Pabst R. and Gerhke Y. (1990). Is the bronchus-associated lymphoid tissue (BALT) an integral structure of the lung in normal mammals including man? Am. J. Respir. Cell. Mol. Biol. 3, 131-135.

Plesch B.E.C. (1982) Histology and immunohistochemistry of bronchus-associated lymphoid tissue (BALT) in the rat. In: in vivo Immunology. Nieuwenhuis P., van de Broek A.A., and Hanna M.G. (eds). Plenum Publ. Corp. New York. pp 491-497.

Podolin P.L. and Prystowsky M.B. (1991). The kinetics of vimentin RNA and protein expression in Il-2 stimulated T lymphocytes. J. Biol. Chem. 266, 5870-5875.

Racz P., Tenner-Racz K., Myrvik Q.N. and Fainter L.K. (1977) Functional architecture of BALT and lymphoepithelium in pulmonary cell-mediated reactions in the rabbit. J. Reticuloendoth. Soc. 22, 59-83.

Reynolds H.Y. (1989). Pulmonary host defenses. State of the art. Chest. 95 (Suppl.), 223S-230s.

Rochester C.L., Goodel E.M., Stoltenberg J.K. and Bowers W.E. (1988). Dendritic cells from rat lungs are potent accesory cells. Am. Rev. Respir. Dis. 138, 121-128.

Sminia T., van de Ende M.B. and Jeurissen S.H.M. (1987). Specific antibody formation in mucosa associated lymphoid tissue after intratracheal and intraintestinal administration of TNP-KLH. Differences between BALT and GALT. Adv. Exp. Med. Biol. 216B, 981-987.

Sminia T., van der Brugge-Gamelkoorn G.J. and Jeurissen S.H.M. (1989). Structure and function of bronchus-associated lymphoid tissue (BALT). Crit. Rev. Immunol. 9, 119-150.

Smith M.W., Jones P.S. and Tivey D.R. (1987). M cell numbers increase after transfer of SPF mice to a normal animal house environment. Am. J. Pathol. 128, 385-389.

Smith M.W., Jones P.S., Tivey D.R. and Brown D. (1988). Automated histochemical analysis of cell populations in the intact follicle-associated epithelium of the mouse Peyer's patch. Histochem. J. 20, 443-448.

Smith M.W. and Peacock M.A. (1982). Lymphocyte induced formation of antigen transporting M cells from fully differentiated mouse enterocytes. In : Mechanisms of intestinal adaption. Robinson J.W.L., Dowling R.H. and Reicken E.D. (eds.). M.T.P. Lancaster. pp 573-583.

Tatrai E., Ungvary G. and Adamis Z. (1994). On the structural differences in the membranes between ciliated bronchial and non-ciliated lymphoepithelium in rats. Eur. J. Histochem. 38, 59-64.

Tenner-Racz K., Racz P., Myrvik Q.N., Ockens J.R. and Geister R. (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, 106-110.

Tsuru A., Nakamura N., Takayama E., Suzuki Y., Hirayoshi K. and Nagata K. (1990). Regulation of the expression of vimentin gene during the differentiation of mouse myeloid leukemia cells. J. Cell. Biol. 110, 1655-1664.

van der Brugge-Gamelkoorn G.J. (1986) Structure and function of bronchus-associated lymphoid tissue (BALT) in the rat. Ph.D. Thesis. Free University. Amsterdam. Holland.

van der Brugge-Gamelkoorn G.J., Plesch B.E.C., Sminia T. and Langevoort H.L. (1985). Histological changes in rat bronchus-associated lymphoid tissue (BALT) after administration of five different antigens. Respiration 48, 29-36.

van der Brugge-Gamelkoorn G.J., Claasen E. and Sminia T. (1986). Anti TNP-forming cells in bronchus-associated lymphoid tissue (BALT) and paratracheal lymph node (PTLN) of the rat after intratracheal priming and boosting with TNP-KLH. Immunology 57, 405-409.

Accepted March 4, 1997