Histol Histopathol (2023) 38: 889-892

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORIGINAL ARTICLE

Open Access

Ex vivo detection of lipopolysaccharide immunopositivity in Rushton bodies

Sirke Virkkunen[1], Jaana Willberg[2], Caj Haglund[1,3,6], Malin Sund[1,3], Timo Sorsa[4,5] and Jaana Hagström[1,2,6]

[1]Department of Pathology, University of Helsinki and Helsinki University Hospital, Helsinki, [2]Department of Oral Pathology and Radiology, University of Turku, Turku, [3]Department of Surgery, [4]Department of Oral and Maxillofacial Diseases, University of Helsinki and Helsinki University Hospital, Helsinki, Finland, [5]Section of Periodontology and Dental Prevention, Department of Dental Medicine, Karolinska Institutet, Stockholm, Sweden and [6]Translational Cancer Medicine Research Program, Faculty of Medicine, University of Helsinki, Finland

Summary. Aim. Our aim was to investigate how bacterial lipopolysaccharide (LPS) is immunoexpressed in periapical lesions. By surprise we detected Rushton bodies (RBs) whose origin has been debatable to be positive for LPS.

Methodology. Samples of radicular cysts (N=70) were stained in order to identify variations in LPS immunoexpression indicating bacterial background. For immunostaining, we used an anti-LPS antibody from Escherichia coli, and for visualization Horse Radish Peroxidase labeled polymer as the secondary antibody.

Results. RBs showed positivity for LPS in radicular cysts. After collection of radicular cyst samples (70 in total), we noted that all RBs (N=25) histologically detected in tissue samples were positive for LPS. Furthermore, calcification in the cyst capsule showed immunopositivity.

Conclusion. We demonstrate for the first time that LPS is present in RBs, indicating that host response to bacteria might be the initial cause of the formation of these hyaline bodies in the cyst epithelium and cyst capsule calcifications.

Key words: Lipopolysaccharide, Periapical lesions, Radicular cyst, Rushton bodies

Introduction

Odontogenic cysts are slow growing, benign osteolytic lesions of the jaw bones originating from the epithelial components of the odontogenic apparatus or its remnants. They are classified as developmental and inflammatory based on their pathogenesis. Dentigerous and odontogenic keratocysts are developmental, whereas radicular, residual, and lateral periodontal cysts are inflammatory by origin (Mosqueda-Taylor et al., 2002; Srinath et al., 2014). In this study, we focus on inflammatory radicular cysts.

Radicular cysts are the most common cysts affecting the jaws and are usually found at the apices of involved teeth as a result of root canal inflammation caused by bacteria. Most radicular cysts are lined by nonkeratinized stratified squamous epithelium with an associated inflammatory process that consists predominantly of polymorphonuclear leukocytes, whereas the adjacent fibrous capsule is infiltrated mainly by chronic inflammatory cells (Browne, 1975; Latoo et al., 2009).

In 1955, peculiar, eosinophilic, linear, curved, or hairpin-shaped hyaline structures occurring with variable frequency in the epithelial lining of odontogenic cysts were described in detail by Martin Rushton (Rushton, 1955). Rushton bodies (RBs) are most often seen in the epithelial linings of radicular cysts and appear frequently with a granular core and sometimes are concentrically laminated. (Morgan and Johnson, 1974; El Labban, 1979; Babburi et al., 2015; Sarode et al., 2016). RBs, appear almost exclusively within the epithelial lining, only rarely being seen in the fibrous capsule of odontogenic cysts (Morgan and Johnson, 1974; Jacob, 2010; Babburi et al., 2015).

RBs are known to be found in odontogenic cysts but there is no consensus about their origin. Various theories and backgrounds have been proposed to describe the pathogenesis of RBs. (Babburi et al., 2015; Sarode et al., 2016). The origin has been suggested to be related to the odontogenic epithelium and to be a certain form of keratin. On the other hand, they have been suggested to originate from thrombosed venules as they contain hemoglobin. Sakamoto et al. (2012) has suggested RBs to contain amyloid being a consequence of abnormal

Corresponding Author: Sirke Virkkunen, Haartmaninkatu 4, FI-00290 Helsinki, Finland. e-mail: sirke.virkkunen@helsinki.fi
www.hh.um.es. DOI: 10.14670/HH-18-602

©The Author(s) 2023. **Open Access.** This article is licensed under a Creative Commons CC-BY International License.

epithelial differentiation and the presence of hemoglobin. An inflammatory origin has also been suggested (Rushton, 1955; Morgan and Johnson, 1974; Browne and Mathews, 1985; Sarode et al., 2016).

Our aim was to examine by immunohistochemistry the expression of bacterial products and virulence factor LPS in periapical inflammatory granulomas and radicular cysts to elucidate the role of LPS in these lesions (Fig. 3). LPS is a major component of the cell membrane of Gram-negative bacteria. It is an eventual endotoxin and can induce a strong proinflammatory response involving mammalian host defense mechanisms against it. Infection is triggered by inflammatory cell recognition of LPS present on the surface of the bacterium (Schumann et al., 1990).

Materials and methods

Tissue material

We collected all 728 periapical lesions from the pathology archives of Helsinki Biobank to examine their inflammatory reactions; the histology of these samples was re-evaluated by S.V. and J.H. We conducted a pilot study on 40 samples to reveal the LPS expression in these lesions. We then collected all samples diagnosed as radicular cysts with RBs (N=25). Altogether we studied 70 samples of which 25 were diagnosed with RBs. The use of the tissue samples was approved by the Ethics Committee of Helsinki University Hospital (diary number 466/2020), the study protocol was approved by the Helsinki University Hospital Research Board.

Immunohistochemistry

For LPS immunostaining, we used anti-LPS antibody from Escherichia coli (ab35654, Abcam). The staining was performed using the Envision Flex-kit (K8000, Agilent Techonolgies, Singapore), and primary antibody diluted 1:100 in Dako Real antibody diluent was applied to the sections. After incubation, Horse Radish Peroxidase (HRP) labeled polymer as secondary

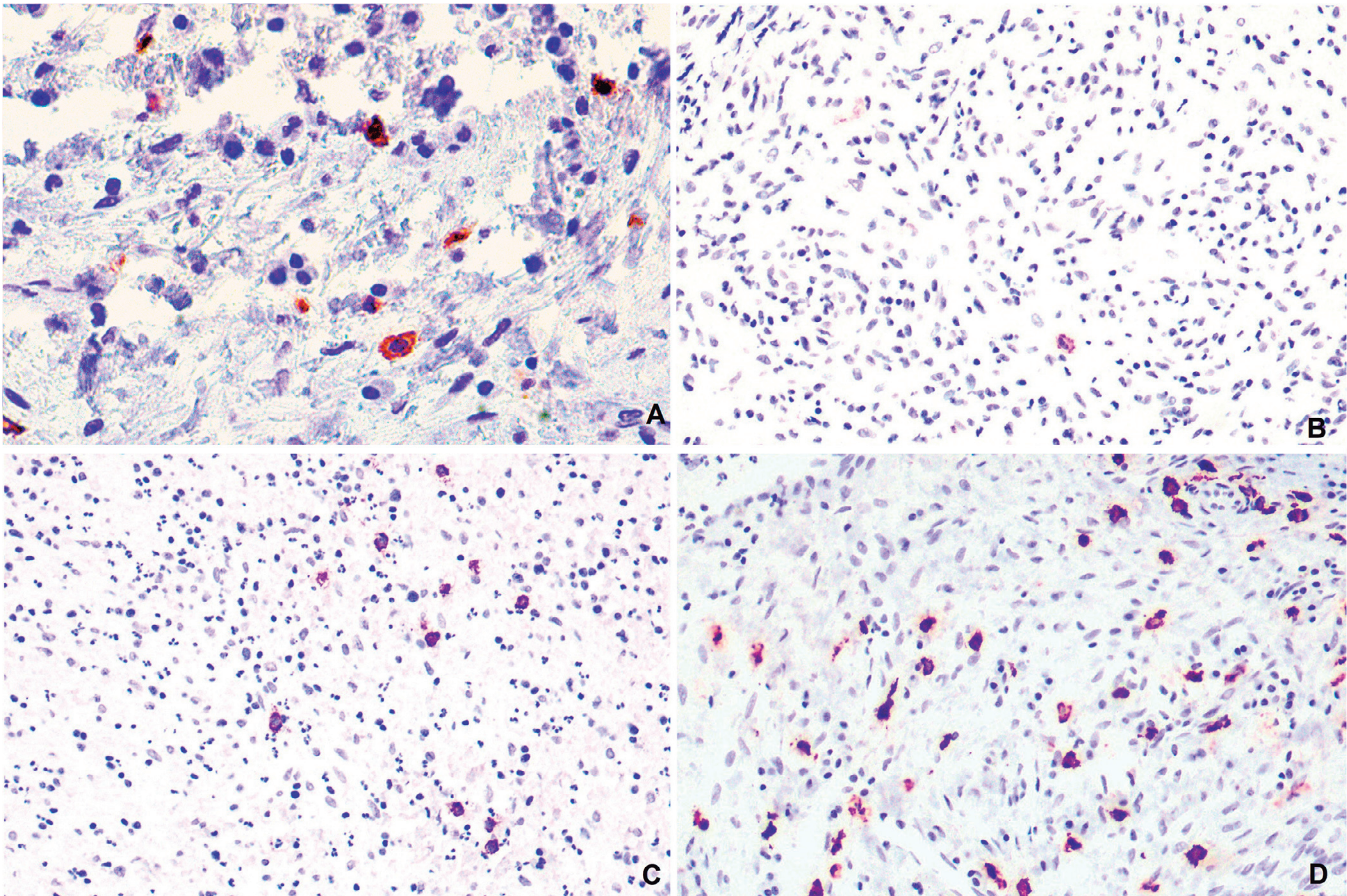

Fig. 1. LPS, lipopolysaccharide. Cytoplasmic LPS positivity in histiocyte-type cells and other inflammatory cells **(A)**. Staining intensity: mild **(B)**, moderate **(C)**, strong **(D)**. A, x 600; B-D, x 200.

antibody was applied to the sections. Rinsed slides were placed in Romulin AEC chromogen (Biocare, CA, USA) for visualization. Sections were counterstained with Mayer´s hematoxylin, dried, and mounted.

Scoring of immunohistochemical stainings

We scored the LPS positivity in the cells of the cyst capsule and epithelium according to the amount of positive cells: 0, no positivity; 1, only a few positive cells, 2; clusters of positive cells; 3, abundant positive cells (Fig. 3). RBs were scored as negative or positive. In two of the samples, the material was scarce, and we could not perform immunohistochemistry.

Results

Cyst capsule

In the cyst capsule, granular cytoplasmic LPS positivity was detected in histiocyte-type cells and other inflammatory cells (Fig. 1A). The scoring is presented in Figure 1B-D. Additionally, immunopositivity was detected in the capsule in calcification foci (data not shown).

In histiocyte-type cells and inflammatory cells, positivity was detected in 65 out of 70 samples (92.9%). The scoring results are presented in Table 1.

Table 1. Scoring of immunohistochemistry in inflammatory cells and Rushton bodies.

Samples (No. of RC, n=70)	IHC Score	N	% of all samples
Positive intensity in inflammatory cells	0	3	4,3
	1	29	41,4
	2	28	40
	3	8	11,4
NA		2	2,9
Rushton bodies (n=25)	0	0	0
Positivity in RBs	1	25	100

RC, radicular cyst; IHC, immunohistochemistry; RB, Rushton body. Staining intensity scores in RCs were as follows: 0, no staining; 1, mild staining; 2, moderate staining; 3, strong staining. Positivity was shown in histiocyte-like and inflammatory cells. NA, not applicable. Staining scores in RBs were as follows: 0, no staining; 1, positive staining.

Cyst epithelium

In the cyst epithelium, LPS immunopositive RBs were detected in 25 out of 70 samples (35.7%). All the RBs seen in our material showed immunopositivity for LPS (Fig. 2A).

Discussion

In this study, we show positive immunostaining for LPS antibodies in the hyaline structures of RBs. Our theory is that RB formation is triggered by bacteria and lesions undergo calcification in the epithelium due to the host response. Calcified structures in the cyst capsule showed positivity for LPS antibodies as well, supporting our theory of RBs being of inflammatory origin and induced by bacteria.

RBs have been suggested to originate from the odontogenic epithelium and some studies have suggested RBs to be only hematogenous or both hematogenous and epithelial in origin. None of these theories has been so far convincingly confirmed. Rushton believed that hyaline bodies originated from the odontogenic epithelium, as they contained cysteine and were probably a certain kind of keratin (Rushton, 1955). On the contrary Wertheimer (1966) pointed out that the resemblance was incomplete despite histochemical similarities to keratin. Morgan and Johnson (1974) suggested that hyaline bodies are secretory products of the odontogenic epithelium, and this hypothesis was confirmed by Philippou et al. (1990), although the pathogenesis for this phenomenon remains unknown. Our study partially supports this theory, as we believe that RBs might result from epithelial secretion as a host response induced by bacterial product.

Sarode et al. suggested in 2016 that RBs are related to inflammation eventually affecting the basement

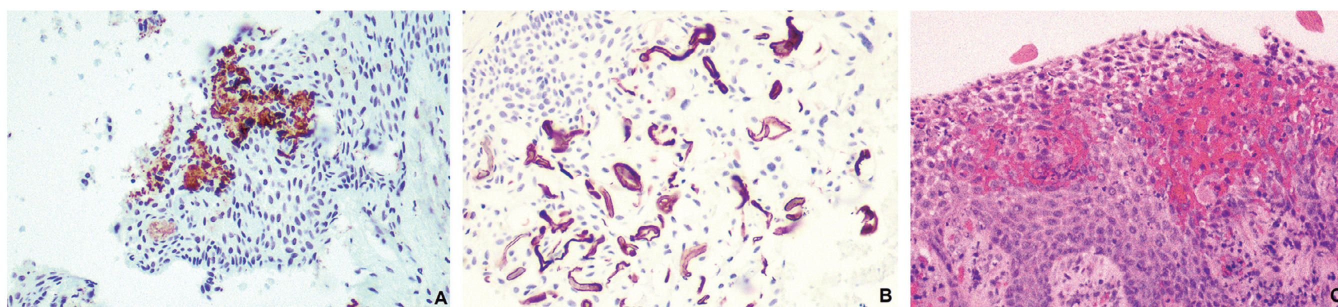

Fig. 2. LPS, lipopolysaccharide; RB, Rushton body; HE, hematoxylin and eosin. **A.** LPS positivity in RBs. **B.** RBs in HE-staining. **C.** Hemorrhage within epithelium showing similar appearance to RBs in HE-staining. x 200.

membrane and odontogenic cyst epithelium. This theory is additionally supported by our results, as we found all RBs to be positive for LPS. Furthermore, Sarode et al. (2016) proposed that the granular variant of RBs is a product of red blood cells that transmigrate inside the epithelium induced by inflammation. Browne and Mathews (1985) suggested that RBs originate as a cellular reaction to extravasated serum. This viewpoint is justified since we detected hemorrhage inside the epithelium, as seen in Figure 2C. The homogeneous variant was explained by Sarode et al. (2016) as osmotic pressure-induced cystic fluid exudates and transudates that undergo calcification in the cystic epithelium.

Jensen and Erickson (1974) demonstrated that RBs contain neither keratin nor show any structure similarity to secondary enamel cuticle. On the other hand, Sakamoto et al. (2012) reported that hyaline bodies are amyloids by nature and suggested that both keratin and hemorrhage are needed for genesis of hyaline bodies. To our knowledge, no one has shown that RBs are amyloid and Congo red positive so we sid not perform the staining for either.

RBs are claimed to be seen only in odontogenic cysts and especially in radicular cysts that arise from inflammation (Morgan and Johnson, 1974; Jacob, 2010; Babburi et al., 2015). Here, we show that RBs were all immunopositive for LPS. Additionally, we showed LPS positivity in other calcifications in the cyst capsule. Since RBs are only seen in odontogenic, particularly inflammatory cysts, we suggest that certain bacteria involved cause immune responses to produce calcification and develop into hyaline bodies as a product of host response. This enables us to suggest that the granular variant could be a form of maturing RB which has not yet been totally calcified.

Limitations of this study

The sample size of RBs was limited, although we collected all RB containing cysts among the 728 cysts found in the archives and periapical granulomas were re-evaluated.

Conclusion

Based on our results, we suggest that RBs and other calcifications detected in odontogenic cyst epithelium and capsules are induced by bacterial LPS, as a part of an inductive host response to produce calcified structures. Thereby, RC as host response may calcify bacterial LPS in the form of RBs to battle against infection. In future studies we are planning to study other bacteria and their virulence factors and presence in periapical lesions.

Acknowledgements. We thank Pia Saarinen for technical assistance.

Funding. This study was supported by Finnish Female dentist's association and HUS research grant Y780022044.

Conflict of interest. All authors have contributed to the work, and we declare no conflict of interest.

References

Babburi S., Rudraraju A.R., Aparna V. and Sowjanya P. (2015). Rushton bodies: an update. J. Clin. Diagn. Res. 9, ZE01-ZE03.

Browne R.M. (1975). The pathogenesis of odontogenic cysts: a review. J. Oral Pathol. 4, 31-46.

Browne R.M. and Mathews J.B. (1985). Intra-epithelial hyaline bodies in odontogenic cysts: An immunoperoxidase study. J. Oral Pathol. 14, 422-428.

El Labban N.G. (1979). Electron microscopic investigation of hyaline bodies in odontogenic cysts. J. Oral Pathol. 8, 81-93.

Jacob S. (2010). Rushton bodies or hyaline bodies in radicular cysts: a morphologic curiosity. Indian J. Pathol. Microbiol. 53, 846-847.

Jensen J.L. and Erickson J.O. (1974). Hyaline bodies in odontogenic cysts: Electron microscopic observations. J. Oral Pathol. 3, 1-6

Latoo S., Shah A.A., Jan S.M., Qadir S., Ahmed I., Purra A.R. and Malik A.H. (2009). Radicular cyst. JK Science 11, 187.

Morgan P.R. and Johnson N.W. (1974). Histological, histochemical and ultrastructural studies on the nature of hyaline bodies in odontogenic cysts. J. Oral Pathol. 3, 127-147.

Mosqueda-Taylor A., Irigoyen-Camacho M.E., Diaz-Franco M.A. and Torres-Tejero M.A. (2002). Odontogenic cysts. Analysis of 856 cases. Med. Oral 7, 89-96.

Philippou S., Ruhl G.H. and Mandelartz E. (1990). Scanning electron microscopic studies and X-Ray microanalysis of hyaline bodies in odontogenic cysts. J. Oral Pathol. Med. 19, 447-452.

Rushton M.A. (1955). Hyaline bodies in the epithelium of dental cysts. Proc. R. Soc. Med. 48, 407-409.

Sakamoto K., Khanom R., Hamagaki M. and Yamaguchi A. (2012). Ectopic production of hair keratin constitutes Rushton's hyaline bodies in association with hematogenous deposits. J. Oral Pathol. Med. 41, 637-641.

Sarode G.S., Sarode S.C., Tupkari J.V., Deshmukh R. and Patil S. (2016). Pathogenesis of Rushton bodies: A novel hypothesis. Med. Hypotheses 93, 166-169

Schumann R.R., Flaggs G.W., Gray P.W., Wright S.D., Mathison J.C., Tobias P.S. and Ulevitch R. J. (1990). Structure and function of lipopolysaccharide binding protein. Science 249, 1429-1431.

Srinath S., Iyengar A. and Mysorekar V. (2014). CD 44 Expression in dentigerous cyst, radicular cyst and ameloblastoma, by immunohistochemical analysis. IOSR J. Dent Med. Sci. 13, 80-83.

Wertheimer F.W. (1966). A histologic comparison of apical cuticles, secondary dental cuticles and hyaline bodies. J. Periodontol. 37, 91-94.

Accepted March 1, 2023