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The EBV action in tonsils and adenoids

L.H. Endo^{a,b,*}, E. Sakano^a, L.A. Camargo^c,
D.R. Ferreira^a, G.A. Pinto^b, J. Vassallo^b

^aDepartment of Otorrhinolaryngology, State University of Campinas, São Paulo, Brazil

^bDepartment of Pathologic Anatomy, State University of Campinas, São Paulo, Brazil

^cDepartment of Otorrhinolaryngology of São Paulo University of Ribeirão Preto, Brazil

Abstract. The bacteria involved in tonsil disease have been well studied, but we cannot say the same for the viruses. The method to detect virus make this approach difficult to study. Epstein–Barr Virus (EBV) infection usually occurs in early childhood and can persist in palatine and pharyngeal tonsil lymphocytes. EBV has been closely associated with the undifferentiated form of nasopharyngeal carcinoma (NPC) in its effect. Nevertheless, the presence of EBV in non-neoplastic lymphoid tissue of the nasopharynx and tonsil has rarely been investigated. Our objective was to study the frequency of EBV in tonsils and adenoids and to define the correlation between EBV and adenoid hyperplasia.

In this study, we looked for EBV in adenoid and tonsil tissue of 165 patients (2 and 15 years old) by in situ hybridization (ISH) for EBER 1/2 RNA. Resection of the adenoids was done for relief of upper respiratory tract obstruction, and the tonsils were resected because of recurrent tonsillitis and/or hyperplasia with upper airway obstruction. We divided the adenoid samples in two groups: one group 12–24 months old (average 18 months old) and the second group, 25 months to 15 years old. Tonsils were obtained from 85 patients, 3–13 years old (mean age 5.6 years) who underwent surgery due to recurrent tonsillitis or hyperplasia.

EBV was demonstrated in lymphoid cells of 11 (34.3%) out of 32 adenoids for the first group and 36 (72%) out of 48 children of the second group. EBV was found in the respiratory epithelial cells of adenoid in one case.

Children under 24 months of age can be infected by EBV, and this virus might be responsible for obstructive hyperplasia. Tonsils are less affected by EBV than the adenoids, suggesting that the EBV is more attracted to the adenoid tissue than the tonsillar tissue. © 2003 Elsevier B.V. All rights reserved.

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1. Introduction

Recurrent and chronic infection and obstructive hyperplasia are the most common diseases affecting the tonsils and adenoids in the pediatric population. Tonsils and

* Corresponding author. Rua Guilherme da Silva 281, apto 111 Cambuí, Campinas, 13025-070, São Paulo, Brazil. Tel.: +55-19-32535602; fax: +55-19-32510125.

E-mail address: luiza_endo@hotmail.com (L.H. Endo).

adenoids are continually exposed to antigens, and its lymphoid component accounts for the increase in size of the tissue.

The hyperplasia of the tonsils and adenoids, without a specific etiological factor, is called benign idiopathic hyperplasia (BIH). Some authors have demonstrated a strong relation between viral infection [1,2,3,4] and recurrent pharyngotonsillitis. There are many viruses involved in the etiopathogenesis of pharyngotonsillitis: adenovirus, parainfluenza, rhinovirus, herpes simplex, virus respiratory syncytial, Epstein–Barr virus (EBV), influenza, coxsackie A, coronavirus, and citomegalovirus [5,6]. In spite of the high prevalence of viral infection in adenotonsillar tissue, the method to detect the virus makes this approach difficult in routine practice.

EBV can cause the pharyngotonsillitis with exudates in 19% of cases [6]. EBV has been closely associated with the undifferentiated form of nasopharyngeal carcinoma (NPC) in its function. Nevertheless, the presence of EBV in non-neoplastic lymphoid tissue of the nasopharynx and tonsil has been rarely investigated [7].

This virus is B-lymphotrophic and has the ability to transform memory B-cells into blastic cells, with permanent proliferation. Endo et al. [8] have used in situ hybridization (ISH) in tonsillar tissue of patients with recurrent pharyngotonsillitis (RT) and BIH and demonstrated EBV in both groups, suggesting its participation in the pathogenesis of recurrent infections and lymphoid hyperplasia.

We begin our investigation by looking for EBV in tonsils and adenoid tissue and verify its correlation with recurrent tonsillitis and hyperplasia.

The in situ hybridization (ISH) is a fast test that allows detecting EBV in paraffin-embedded tissue [9], and it is considered to be the gold standard for identifying EBV in Hodgkin's disease.

1.1. Purpose

The aim of this study was to detect the frequency of EBV by in situ hybridization in surgically removed tonsils and adenoids, to look for differences between them, and to define the correlation between EBV and adenoid hyperplasia.

2. Materials and methods

We studied 165 samples of adenoid and tonsil tissue. The resection of adenoids was done to relieve upper respiratory tract obstruction. There were 80 adenoid samples. They were divided in two groups: one group with 32 children from 12 to 24 months of age (mean, 18 months) and the second group with 48 children from 25 months to 15 years old.

Tonsils were obtained from 85 patients, from 3 to 13 years old (mean, 5.6 years). They underwent surgery (tonsillectomy with or without adenoidectomy) due to recurrent tonsillitis (42 patients) or hyperplasia with upper airway obstruction (43 patients).

The removed adenoids and tonsils were fixed in 10% formalin and embedded in paraffin. Tissue sections 5 μ m thick were stained with hematoxylin and eosin for histological analysis and others submitted to the in situ hybridization (ISH). The ISH was carried out with the EBER probes from Novocastra, which consists of a mixture of oligonucleotides EBER (EBER 1 and EBER 2, of 30 base length). The EBER probe was labeled with fluorescein isothiocyanate (FITC). The detection of the hybridization

products was achieved by using the Novocastra universal detection system. The positive control of the reaction was a previously tested EBV-positive nasopharynx carcinoma. The slides were analyzed on a standard optical microscope, by only one observer.

The intensity of positiveness to EBV was classified as mild, moderate, and strong.

We have compared the results obtained from adenoid tissue from children under 24 months of age and older than 25 months, both EBV positive, and tonsillar tissue and adenoid tissue, both EBV positive.

3. Results

3.1. Adenoid tissue

In the first group (12–24 months of age) with 32 children, 11 were EBV positive (34.3%), and 21 were EBV negative (65.6%). In the second group (25 months to 15 years old) with 48 samples, 35 were EBV positive (72.9%) and 13 were EBV negative (27%). We found positive cells inside and outside the germinative center. EBV was found in respiratory epithelial cells of adenoid in one case. In relation to intensity, we classified 68 cases (85%) as mild, 6 cases as moderate (7.5%), and 6 cases as strong (7.5%) positiveness to EBV. Considering all adenoid samples, we obtained 46 positive cases (57.5%).

3.2. Tonsillar tissue

Among the 85 tonsil samples, 25 cases (29.4%) were EBV positive, and 60 patients (70.5%) were EBV negative. The intensity was found mild in 21 cases (84%), moderate in 1 case (4%), and strong in 3 cases (12%).

4. Discussion

The adenoid, being an immunologic organ, enlarges with recurrent infections by virus, bacteria, allergy, and also unknown stimuli. This last condition is known as idiopathic benign hyperplasia.

It is well known that EBV has a tropism for the oral and nasopharynx regions, and that is associated with lymphoproliferation. EBV is carried by more than 90% of the population worldwide. The role of oropharyngeal epithelial cells as a reservoir of EBV was already suggested. In a previous study by our group, we used in situ hybridization to detect EBV in patients with recurrent tonsillitis and idiopathic benign hypertrophy, and we found the virus in both groups.

Many authors [10,11,12,13] have described the presence of EBV in the upper airway tract, using different methods, such as ELISA, PCR (polymerase chain reaction), immunohistochemical, and in situ hybridization. Niedobitek et al. [14,15] have shown that EBV attacks the B lymphocytes, which are the primary target of infection in patients with infectious mononucleosis. Takimoto et al. [16] have observed a small number of B lymphocytes in the nasopharynx that work as a reservoir to EBV, comparing tissue samples from patients with suspected nasopharyngeal carcinoma and non-neoplastic tissue samples from adenoid. Ikeda et al. [10] have studied normal tonsils and have concluded that the tonsillar lymphocytes have the function of a reservoir and a place of EBV replication. In spite of the suggestion that EBV may be involved in adenoid hypertrophy,

there are few large-scale studies on the presence of EBV in adenoid and tonsillar tissue simultaneously.

It is very interesting to note the high level of positiveness of EBV in adenoids, when it is compared with tonsil tissue. We know that adenoid tissue grows mainly between 3 and 5 years of age. Adenoid hyperplasia under 24 months of age is uncommon, and this fact is not yet very well studied. For this reason, we have divided our patients into two groups; one with patients between 12 and 24 months and the other, with patients older than 25 months. We found 34.3% of adenoid tissue with EBV-positive cells in the first group. Would this hyperplasia be the one called “idiopathic benign hyperplasia,” due in part to the proliferative action of EBV?

The adenoid tissue of the group of children above 25 months of age presented a high percentage (72.9%) of EBV-positive cells, almost twice as many, suggesting that it becomes easier to be infected with aging.

Comparing the positiveness to EBV between adenoids and tonsils, we have verified that adenoids are more frequently positive for EBV (57.5%) than tonsils (29.4%). Kobayashi et al. [13] found a similar percentage of EBV positive cells in tonsils. Some authors have demonstrated that EBV is equally distributed in the Waldeyer’s ring; however, this is not in agreement with our findings. Considering the intensity of positiveness, there were no significant differences. EBV was found in the tonsillar parenchyma and in the parafollicular region, or inside the germinal center. It was also found in the adenoid epithelium, though rarely.

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