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Is there a relationship between the detection of human herpesvirus 8 and Epstein—Barr virus in Waldeyer's ring tissues?

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Summary

Objective: Human herpesvirus 8 (HHV-8) and Epstein—Barr virus (EBV) are human pathogens associated to a number of neoplasms, including tumors of the Waldeyer's ring. Both viruses have been previously detected by *in situ* methods in tonsils and adenoids from children. HHV-8 was found in 6.8% of the cases and EBV in about one third of the cases. As they belong to the same *γ-herpesvirus* subfamily and share some biological characteristics, it is of medical interest to further explore their possible relationship in the Waldeyer's ring, an issue not yet addressed in the specialized literature. The purpose of the present study is to compare the presence of EBV by *in situ* hybridization (ISH) in tonsils and adenoids from children up to 14 years of age in cases previously shown to be positive and negative for HHV-8.
Methods: Paraffin wax-embedded sections consisting of 38 tonsils and two adenoids from 40 patients were analyzed. HHV-8 was detected by ISH, using the T1-1 probe for the viral mRNA. EBV was also detected by ISH, using the EBER probe. Both probes and the detection systems were provided by Novocastra.
Results: HHV-8 was detected in 19 tonsils and one adenoid. The other 19 tonsils and one adenoid taken from the HHV-8-negative group were selected by pairing age and

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gender of patients with the HHV-8-positive group. In both groups EBV was detected in 13 cases and was negative in other 7.
Conclusion: Although both viruses are related in many aspects, some biological and epidemiological features differ. This is reflected in the present results, as EBV is similarly detected in the groups negative and positive for HHV-8, favoring different mechanisms of spread.

1. Introduction

Human herpesvirus 4, best known as Epstein—Barr virus (EBV) and the human herpesvirus type 8 (HHV-8, also known as Kaposi sarcoma herpesvirus, KSHV) belong to the *γ-herpesviridae* subfamily [7,8,12,13]. Both are clinically relevant, as they represent pathogens associated with human non-neoplastic and neoplastic diseases. Medical importance of these viruses is reflected by the ever-growing list of studies available. EBV is associated with Hodgkin's disease in half of the cases (even more frequently in children), with polyclonal lymphoproliferations and with malignant non-Hodgkin's lymphomas, especially in patients infected by the human immunodeficiency virus (HIV). In the Waldeyer's ring, EBV is found in all cases of undifferentiated nasopharyngeal carcinomas and T/NK lymphomas. HHV8 has been associated with Castleman's disease and neoplasms, such as Kaposi's sarcoma (KS), and pleural effusion lymphoma. KS is described to occur in African infants, even prior to the HIV epidemic, and which now represents one of the leading cancers in HIV+ children [6,15]. Research on viruses that have oncogenic potential, especially in children, are important both to alert physicians to the possibility that such lesions may appear in other regions of the world, especially if a supervening immune suppression takes place, and for the development of prophylactic or therapeutic measures.

In previous studies addressing the detection of members of the *Herpesviridae* family in tonsils and adenoids using *in situ* histomolecular techniques, it was demonstrated that the Epstein—Barr virus (EBV) was present in about 30% of the tonsils and adenoids [9—11]. In contrast, in none of the 36 cases studied by us could herpesviruses 1 and 2 (HSV-1/2) be found [18].

More recently, we investigated tonsils and adenoids from 293 patients for the presence of human herpesvirus 8 (HHV-8) and found that 6.8% were positive [5]. Virus was mainly found in lymphoid cells, and more rarely in epithelial cells, as it was also observed for EBV [9—11]. In that study, we were able to support the evidence of an oral route of contamination by HHV-8, as it is the case for EBV and HSV-1/2 [5,6,15].

Thus, EBV and HHV-8, but not HSV-1/2, seem to share the organs of the Waldeyer's ring as a site of prolonged residency, according to these findings. However, two differences are noticeable: first, EBV is more frequently found in adenoids and tonsils, perhaps reflecting the more widespread distribution of this virus in the general population. In addition, EBV was detected in 29.4% of tonsils from children aged 2—14 years and in 33% of adenoids from children aged 1—2 years, whereas HHV-8 was detected in 19/181 (10.4%) tonsils and in just one out of 162 adenoids (0.06%) [5,9,10]. Then, a clear difference in the preference of both lymphoid organs seems to exist. On the other hand, in both cases, younger children present more frequently EBV and HHV-8 positive cells in comparison with older ones [5,9]. A selected list of similarities and differences between EBV and HHV-8 are summarized in Table 1.

Table 1 Summary of similarities and differences between EBV and HHV-8

Similarities
Both belong to the *γ-herpesviridae* subfamily and share close similarities in their genomes
Latent infection of lymphoid and epithelial cells of the Waldeyer's ring
Implication in the pathogenesis of lymphoproliferative disorders
Increasing evidence of similar transmission by saliva and, in some extent, also by genital secretions
Detection of infected cells by *in situ* hybridization in the Waldeyer's ring tend to decrease with age
Differences
Viral and cell receptors interactions responsible for internalization of the viruses differ
HHV-8 does not immortalize lymphoid B-cells *in vitro*, as does EBV
HHV-8 seems to have a net predilection for the tonsils and not for adenoids; EBV does not show this preference
EBV is ubiquitous, infecting more than 90% of the population worldwide; HHV-8 infects predominantly populations of poorer regions of the planet
Environmental and constitutional factors probably differ, as detection of infected cells by *in situ* hybridization in the Waldeyer's ring are unrelated (present study)