The prevalence of Epstein-Barr Virus-positive lymphoid cells in nasal mucosa: an extremely rare event*

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Abstract

Background: The prevalence of EBV (Epstein-Barr virus)-positive lymphoid cells is unknown. Because EBV is implicated in the etiology of extranodal NK/T-cell lymphoma, nasal type (nasal ENKL), the presence of EBV-positive lymphoid cells (EPLs) in nasal mucosa specimens is expected. This study evaluated the presence of EBV-positive lymphoid cells in the nasal mucosa of 420 patients who had undergone surgical resection of lesions of the nasal cavity due to nasal septal deviation, chronic paranasal rhinosinusitis, chronic hypertrophic rhinosinusitis, nasal polyps, allergic rhinitis, papillomas, and cysts.

Methodology: Three representative 1.0-mm-diameter core biopsies were taken from one paraffin-embedded donor tissue block per case and subsequently arranged in new recipient paraffin blocks with a trephine. EBV in situ hybridization study was performed to detect EPLs.

Results: None of the cases demonstrated EPLs.

Conclusion: The presence of EPLs in the nasal mucosa is an extremely rare event in immunocompetent individuals. Therefore, the detection of EPLs in nasal biopsy specimens should prompt the pathologist to perform further testing to exclude the possibility of nasal ENKL.

Key words: Epstein-Barr virus, nasal mucosa

Introduction

Epstein-Barr virus (EBV) is implicated in the etiology of nasopharyngeal carcinoma (NPC) and extranodal NK/T-cell lymphoma, nasal type (nasal ENKL). Although nasal ENKL may also involve the nasopharynx, where most NPCs arise, it usually occurs in the anterior nasal cavity. Although EBV-positive lymphocytes (EPLs) have been found in normal nasopharyngeal mucosa and adenoids, the prevalence of EBV infection in non-neoplastic lymphoid cells of the nasal mucosa in immunocompetent, low-risk individuals has not been studied well. Considering that nasal ENKL is almost always EBV-positive and occurs mostly in the nasal cavity, the presence of EPLs in nasal mucosa specimens is expected. We were interested in determining the prevalence of EPLs in the nasal mucosa of low-risk populations. Therefore, we used an EBV in situ hybridization technique to determine the presence of EPLs in the nasal mucosa tissues of 420 patients who had undergone surgical resection of lesions of the nasal cavity due to nasal septal deviation, chronic paranasal rhinosinusitis, chronic hypertrophic rhinosinusitis, nasal polyps, allergic rhinitis, papillomas, and cysts.

Materials and methods

Study population

We reviewed the H&E slides and clinical and pathologic reports of 420 patients who had undergone surgical resection for nasal septal deviation, chronic paranasal rhinosinusitis, chronic hypertrophic rhinosinusitis, nasal polyps, allergic rhinitis, papillomas, and cysts between January 1999 and October 2004 at the Gil Medical Center. The source of mucosal tissue in patients with septoplasty was the anterior portion of inferior concha. Mucosa
was not taken from the septum. Clinicopathologic parameters, such as age, gender, histologic diagnosis, and type of operation, were evaluated.

Tissue microarray method
Three representative 1.0-mm-diameter core biopsies were taken from one paraffin-embedded donor tissue block per case and subsequently arranged in new recipient paraffin blocks with a trephine (Quick-Ray, UNITMA). Serial sections from the tissue microarray blocks were used in the EBV in situ hybridization. An adequate case was defined as that in which the nasal mucosa occupied all three cores.

EBV in situ hybridization
In situ hybridization for EBV-encoded RNA (EBER) was performed on the Bond Max Immunohistochemical Stainer® (Leica Microsystems), using fluorescein-conjugated oligonucleotide probes (EBER Probe, Leica Biosystems, Newcastle, UK) according to the following protocol. First formalin-fixed, paraffin-embedded tissue sections were deparaffinized, and the slides were incubated with enzyme for 15 minutes, after which the fluorescein-conjugated EBER probe was placed on the slides and incubated for 1 hour at 37°C. After incubation with the EBER probe, the tissue sections were incubated with a mouse anti-fluorescein antibody for 15 minutes, post-primary antibody for 8 minutes, and polymer for 8 minutes, followed by colorimetric development with DAB. We used EBV-positive diffuse large B-cell lymphoma as a positive control (Figure 1).

Results
Clinical characteristics
This study included 420 patients who had undergone surgical resection for nasal septal deviation (n = 182), chronic paranasal rhinosinusitis (n = 85), chronic hypertrophic rhinosinusitis (n = 77), nasal polyps (n = 47), allergic rhinitis (n = 20), papillomas (n = 5), and cysts (n = 4). Surgical operations included endoscopic sinus surgery (n = 45), conchotomy (n = 98), polypectomy (n = 23), Caldwell operation (n = 4), septoplasty (n = 41), submucosal resection (n = 11), and unspecified (n = 198). The mean age was 31.6 years (range, 6 to 80 years), and most patients were male (n = 302, 71.9%).

EBV in situ hybridization
The results of the in situ hybridization analysis failed to demonstrate EPL in any of the 420 cases (Figure 2).

Discussion
This study evaluated the presence of EPLs in the nasal mucosa of 420 immunocompetent individuals by constructing tissue microarray (TMA) blocks. No EPLs were found in the nasal mucosal tissues of any patient. Although EPLs are known to occur in the nasopharynx, adenoids, and lymph nodes (2-4,6,7), the presence of EPLs in the nasal mucosa has not been evaluated thoroughly. Tao et al. reported EPLs in 11 out of 13 nasal polyps in Chinese patients (8). However, Kozak et al. failed to observe EPLs in Canadian patients (9). The increased frequency of EBV infection in the nasal polyps of Chinese patients suggests that the natural history of EBV infection may vary according to environmental and/or genetic factors (8). Our study included 47 cases of nasal polyps, but none of them showed EPLs. Numerous etiologies for nasal polyps have been proposed, with allergic and infectious

Figure 1. Representative scanning image of tissue microarray slide in patients with diffuse large B-cell lymphoma (EBV in situ hybridization). The tumor cells were diffusely positive for EBV in one case (Inset x200).

Figure 2. Representative scanning image of tissue microarray slide in immunocompetent populations with nasal mucosa (EBV in situ hybridization). The lymphoid cells were negative for EBV (EBV in situ hybridization) (Inset x200).
causes predominant (10-12). Weill considered a viral etiology for nasal polyps nearly 63 years ago (13). However, viral culture of nasopharynx biopsy specimens (16). Although these studies were performed using post-nasal space biopsy specimens or nasopharynx biopsy specimens (16). Although these studies were performed using post-nasal space biopsy specimens or nasopharynx biopsy specimens, we can infer from the results that EPLs in the nasal mucosa, if present, may be widely distributed rather than clumped in aggregates. Further studies using whole blocks of nasal mucosa specimens will be needed to demonstrate the presence of EPLs.

Despite these limitations, the present study highlights an important point: the presence of EPLs in the nasal mucosa is a very rare event in immunocompetent individuals. In the nasopharynx, EPLs can be found occasionally and are mainly B-cells.

Therefore, the presence of EPLs in the nasopharynx does not necessarily indicate nasal ENKL. In contrast, even if a few scattered EPLs are identified in nasal mucosa biopsy specimens, thorough evaluation should be performed, regardless of whether the EPLs are T-, NK- or B-cells, to exclude the possibility of nasal ENKL.

Because there are currently no diagnostic criteria for nasal ENKL that define the cut-off numbers of EBV-infected NK/T cells, the possibility of so-called ‘in situ’ or indolent nasal ENKL has not been proposed yet as a provisional disease entity in cases in which small numbers of NK/T cell are EBV-positive (14).

In conclusion, the presence of EPLs in the nasal mucosa is a very rare event in immunocompetent individuals. Therefore, further evaluation is necessary when EPLs are detected in nasal biopsy specimens.

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Authorship contribution
SYH reviewed slides, constructed tissue microarray blocks and wrote this manuscript. SP reviewed slides and wrote this manuscript.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

References

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