Association of Epstein-Barr virus with sinonasal angiocentric T cell lymphoma

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Abstract

Aim—To investigate whether non-Hodgkin's lymphomas arising in the sinonasal region or Waldeyer's ring contain the Epstein-Barr virus (EBV) genome in lesional tissue.

Method—Sections from paraffin wax blocks of 22 lymphoid proliferations arising in the sinonasal region or Waldeyer's ring were studied with EBV encoded RNAs (EBER-1 and -2) using in situ hybridisation.

Results—EBV was detected in nuclei of tumour cells of five of seven T cell lymphomas and in nuclei of two of seven diffuse, large cell immunoblastic lymphomas of B phenotype in the sinonasal region. Of tumours arising in Waldeyer's ring, two of 10 non-Hodgkin's lymphomas (both large cell) were positive, as was a single case of Hodgkin's disease. Lymphoma of other types, including Western type Burkitt's lymphoma, and nodular and diffuse small cleaved cell lymphoma, were negative.

Conclusion—EBV is highly associated with large cell lymphomas especially T cell lymphomas of sinonasal origin in the indigenous Irish population, underlining the importance of this virus in nasopharyngeal lymphomas in Northern European as well as Asian populations.

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Keywords: Epstein-Barr virus, sinonasal lymphomas, Waldeyer's ring lymphomas, in situ hybridisation.

The association between Epstein-Barr virus (EBV) and certain B cell neoplasms such as African Burkitt's lymphoma,¹ nasopharyngeal carcinoma² and lymphomas associated with congenital or acquired immunodeficiency³ is well known. Nasopharyngeal non-Hodgkin's lymphomas of B and T cell type have been associated with EBV in Asian patients.⁴ Recent studies from the USA, China, Japan, Peru, and Germany have shown a particular association with nasal/nasopharyngeal T cell lymphomas.⁵⁻⁹ Studies of Western patients have suggested that most sinonasal non-Hodgkin's lymphomas are of B cell type.⁷⁻⁹

To determine the immunophenotype and occurrence of EBV in sinonasal lymphomas in the indigenous Irish population, we studied all lymphoproliferative disorders arising in the sinonasal region or in Waldeyer's ring which were resected at the Ear, Nose and Throat Department of The Royal Victoria Eye and Ear Hospital, Dublin, between 1979 and 1994.

Methods

Between 1979 and 1994, 22 lymphoid tumours arising in Waldeyer's ring and the sinonasal region were examined at the Department of Pathology of the Royal Victoria Eye and Ear Hospital. All of the patients were from the indigenous Irish population. The patients were aged from 16 to 73 years. Fifteen were male and seven were female. None had a history of congenital or acquired immunodeficiency. The sinonasal region or Waldeyer's ring was the primary site of tumour and the cause of the patient's clinical presentation.

Tissues from each case were fixed in formalin and embedded in paraffin wax. The non-Hodgkin's lymphomas were classified according to the proposal for classification of lymphoid neoplasms (published by the International Lymphoma Study Group).¹⁰ Immunophenotyping was carried out on fixed sections using the avidin-biotin peroxidase conjugate method as described by Hsu *et al*¹¹ and the Vector Elite kit (Vector Laboratories, Burlingame, California, USA). All tumours were stained with leucocyte common antigen (CD45RB), L26 (CD20) for B cells, UCHL1 (CD45RO), CD3 for T cells, and Ki1. One case for which frozen tissue was available was stained with an antibody to CD56 (HNK1), which identifies natural killer cells. Angiocentric lymphomas were stained for Leu 7, which identifies a subset of natural killer cells. All of the above antibodies were obtained from Dako Laboratories, Copenhagen, Denmark. Normal tonsillar tissue served as a positive control for immunohistochemistry.

Negative controls for immunohistochemistry comprised omission of the primary antibody and its replacement by serum on a duplicate section of the lymphoma to be tested. Molecular studies were not performed on these tumours.

IN SITU HYBRIDISATION WITH EBER-1/2 OLIGONUCLEOTIDES

In situ hybridisation (ISH) was performed with the Dako hybridisation kit (ref Y.017), which contains a mixture of fluorescein isothiocyanate (FITC) conjugated EBV oligonucleotides (EBER-1 and -2, both 30 bases long). The slides were deparaffinised in xylene, treated with proteinase K (3 µg/ml) and dehydrated in 95% alcohol. The sections were incubated for 12 hours at 37°C with the probes. The hybridisation product was detected by the Dako ISH kit (K046) using a mouse monoclonal anti-FITC and an alkaline phosphatase conjugated polyclonal rabbit antimouse immunoglobulin. The chromogen was new fuchsin and the slides

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Clinical and pathological details

Case No.	Year of diagnosis	Sex/age (years)	Site	Diagnosis	EBV status	Outcome
Sinonasal origin						
1	1984	M/68	SN	Angiocentric T cell	Positive	DOD
2	1993	F/29	SN	Angiocentric T cell	Positive	NED
3	1992	F/59	SN	Angiocentric T cell	Negative	DOD
4	1981	M/42	SN	Angiocentric T cell	Positive	DOD
5	1988	F/34	SN	Angiocentric T cell	Positive	DOD
6	1987	M/69	SN	Angiocentric T cell	Positive	DOD
7	1991	M/71	SN	Angiocentric T cell	Negative	DOD
8	1994	M/70	SN	Angiocentric T cell	Positive	AWD
9	1994	F/55	SN	Diffuse, large, B cell	Negative	DOD
10	1994	M/74	SN/O	Diffuse, large, B cell	Negative	AWD
11	1993	F/60	SN/O	Midline destructive, B and T cell	Negative	NED
Waldeyer's ring origin						
12	1992	M/73	PN	Follicle centre, diffuse	Negative	DOD
13	1985	F/62	Tonsil	Large cell, diffuse, B cell	Positive	DOD
14	1984	M/49	Tonsil	Large cell, immunoblastic, B cell	Positive	LFTU
15	1985	F/86	Tonsil	Large cell, diffuse, B cell	Negative	DOD
16	1993	M/73	Tonsil	Large cell, diffuse, B cell	Negative	NED
17	1986	M/37	Tonsil	Large cell, diffuse, B cell	Negative	NED
18	1992	M/71	Tonsil	Large cell, diffuse, B cell	Negative	AWD
19	1992	M/16	Tonsil	Burkitt's B cell	Negative	NED
20	1979	M/70	Tonsil	Follicle centre, diffuse	Negative	LFTU
21	1988	M/67	Tonsil	Follicle centre, follicular	Negative	NED
22	1993	M/35	Tonsil	NS Hodgkin's disease	Positive	NED

SN = sinonasal; O = orbit; PN = postnasal; LFTU = lost to follow up; AWD = alive with disease; DOD = died of disease; NED = no evidence of disease; NS = nodular sclerosing.

Figure 1 Perineural and perivascular proliferation of lymphoid cells within a fibrous stroma.

Figure 2 Large atypical lymphoid cells with a polymorphous appearance are seen invading a vessel wall.

were counterstained with Harris's haematoxylin. The positive control for ISH comprised tonsillar tissue from patients with serologically confirmed infectious mononucleosis.

Results

There were distinct differences in the immunophenotype and grade of non-Hodgkin's lymphomas arising in the sinonasal region and Waldeyer's ring. Clinical and pathological details are summarised in the table.

All of the lymphomas arising in the sinonasal region were of high or intermediate grade. T cell lymphomas (cases 1 to 8) predominated. All were high grade destructive lesions in which pleomorphic atypical cells, many spindle shaped, were admixed with a population of small round lymphocytes, plasma cells and eosinophils in a fibrotic background. At first glance, these lesions were not always readily recognisable as a lymphoma. Figure 1 illustrates the fibrotic appearance of a typical biopsy specimen with many spindle shaped cells. Angioinvasion was a prominent feature (fig 2). Tumours had a high mitotic rate and focal areas of necrosis. On immunohistochemistry, most cells expressed CD3, UCHL1 and MT1. Four of eight cases studied were also Ki1 positive. Many of the small round or angulated cells were L26 positive. The single case with frozen tissue available was CD56 negative. Seven of eight T cell lymphomas contained a few scattered cells (<1%) positive for Leu 7.

In six of eight T cell tumours the cell nuclei contained EBV. The distribution of these EBV containing cells strongly suggested that they were present within the neoplastic elements and not within the B cell population. A representative case is illustrated in fig 3.

The two large cell diffuse lymphomas of B cell immunophenotype presenting in the sinonasal region (cases 9 and 10) were EBV negative. Histological examination in each of these cases revealed a diffuse, relatively monotonous lymphoid proliferation. The angiocentricity, cellular pleomorphism and

Figure 3 Nuclear positivity for EBV within an angiocentric T cell lymphoma. The distribution of EBV positive cells strongly suggests that they are present within the neoplastic population.

fibrosis seen in the T cell lymphomas were absent. The single case (case 11) which was clinically and radiologically destructive, but had a bland histological appearance and contained equal numbers of B and T lymphocytes, was EBV negative.

In contrast to the sinonasal region, the lymphomas which presented in Waldeyer's ring were all of B cell immunophenotype. They varied from low to high grade. Only two of six large cell lymphomas (cases 13 and 14) contained nuclear EBV. All of the low grade lymphomas were negative. Reed–Sternberg cells, lacunar cells and atypical large mononuclear variants were positive for EBV in the single case of Hodgkin's disease.

Discussion

We have used ISH to demonstrate the presence of the EBV genome in lymphoid proliferations arising in the sinonasal region and Waldeyer's ring. ISH is a highly sensitive method which has the advantage over DNA amplification methods in that cells containing the virus can be directly visualised. This obviates the possibility that positivity is due to EBV infected non-neoplastic lymphocytes—for example, Reed–Sternberg cells are seen to be the only cell type containing virus in the Hodgkin's disease cases. Only cells considered to be lesional contained EBV in the non-Hodgkin's lymphomas. We found that most sinonasal non-Hodgkin's lymphomas of T cell type were positive for EBV and two of six tonsillar, B cell non-Hodgkin's lymphomas were also positive. Interestingly, the single case of Western type Burkitt's lymphoma was negative.

Previous studies have found a strong association between EBV and neoplasms of the upper aerodigestive tract other than nasopharyngeal carcinoma in Asian patients. EBV

was found in five cases of nasal T cell lymphoma in Japanese patients¹⁰ and in 11 of 12 Chinese patients.⁴ Nine of the 12 were T cell lymphomas. Previous studies have suggested that lymphomas of the sinonasal region in Western patients are predominantly of B cell phenotype.^{7–9} We have found that the predominant immunophenotype of sinonasal lymphomas in Ireland is angiocentric T cell lymphoma. By contrast, all of the Waldeyer's ring non-Hodgkin's lymphomas were of B cell immunophenotype.

Our results confirm those of Weiss *et al*⁵ who found EBV in three of three sinonasal lymphomas of T cell type and two of five sinonasal lymphomas of B cell type. Harabuchi *et al*¹² also found that EBV is particularly associated with sinonasal T cell lymphomas. Two of 10 B cell Waldeyer's ring lymphomas in that series contained EBV. In a large study of angiocentric sinonasal T cell lymphoma, undertaken in the USA,¹³ EBV was present in neoplastic cells of 17 of 18 tumours.

Ng *et al*¹⁴ have demonstrated expression of natural killer cell markers in nasal lymphomas and the same group have recently proposed¹⁵ that cases of polymorphic reticulosis (another name for sinonasal T cell lymphoma) in the nasal tract containing EBV may be of natural killer cell phenotype. This hypothesis is based on CD56 immunopositivity and CD3 immunonegativity. Our results are different. In the present study all of the sinonasal T cell lymphomas mainly contained CD3 positive cells. CD56 was negative in one case tested and only rare cells expressed Leu 7 in all of the T cell lymphomas.

EBV may act as an environmental co-factor in the development of non-Hodgkin's lymphomas of the respiratory tract, as this strong association is not recognised between EBV and T cell non-Hodgkin's lymphomas at most other sites, in the absence of immunosuppression. Clinical details recorded on our patients did not include smoking history, so we are unable to state whether this was a common denominator. The findings from this study provide further evidence, in Western European patients, that EBV is present within the genome of cells considered to be lesional in sinonasal lymphomas, particularly angiocentric T cell lymphomas. These data implicate EBV in lymphoid tumorigenesis in the sinonasal region and suggest that antiviral therapy could play a role in the treatment of sinonasal peripheral T cell lymphoma.

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