Prevalence of the A and B types of Epstein-Barr virus DNA in nasopharyngeal carcinoma biopsies from Southern China

Xiaoyi Chen, Stuart deV. Pepper and John R. Arrand

Cancer Research Campaign Laboratories, Paterson Institute for Cancer Research, Christie CRC Cancer Centre, Manchester M20 9BX, U.K. and Zhanjiang Medical College, Zhanjiang, Guangdong, People's Republic of China

Epstein-Barr virus (EBV) exists in the human population in two genetic forms, usually referred to as type A and type B. Although many earlier studies had indicated that the A type was generally predominant, there were suggestions that the B type may exhibit a preferential tropism for nasopharyngeal epithelial cells. This study examines the prevalence of the two forms of EBV DNA present in nasopharyngeal carcinoma biopsies obtained from the high incidence area of Southern China. The results obtained by Southern blot or polymerase chain reaction analyses show that in this patient group the A type of EBV is predominant.

Epstein-Barr virus (EBV) is one of the most ubiquitous infectious agents of man, being found in all human communities (Epstein & Achong, 1986). The virus is associated with several different diseases, perhaps the most notable of which are Burkitt’s lymphoma and nasopharyngeal carcinoma (NPC). Over 80000 new cases of these two tumours are reported annually (Parkin et al., 1984) and they both exhibit a characteristic geographical distribution. Analysis of the genomes of viruses isolated from different regions of the world and from patients with the different EBV-associated diseases has shown that all isolates are closely related (Bornkamm et al., 1980) and that there do not appear to be disease-specific subtypes (Bornkamm et al., 1984). Nevertheless, examination of the EBV nuclear antigen (EBNA) 2 (Addlinder et al., 1985; Dambaugh et al., 1984; Zimber et al., 1986), EBNA-3 (Rowe et al., 1989) and EBER (Arrand et al., 1989) encoding regions of EBV has identified two distinct families (A and B) with distinct DNA sequence and protein antigenic variation. The two subtypes, sometimes also known as EBV-1 and EBV-2 (Sample et al., 1990; Yao et al., 1991), differ in biological properties in an in vitro transformation assay (Rickinson et al., 1987) although they both seem to contribute with equal efficiency to the pathogenesis of Burkitt’s lymphoma (Young et al., 1987).

In addition to its well known tropism for B lymphocytes, EBV can be found in the malignant epithelial cells of NPC (Wolf et al., 1973). In normal individuals viral DNA can be detected in mucosal epithelial cells (Sixbey et al., 1984, 1986) including those of the nasopharynx (Lees et al., 1992) and it has been suggested (Sixbey et al., 1989) that the B type of EBV may be more prevalent in mucosal epithelial cells, whereas A type virus tends to be dominant in circulating lymphocytes.

The equal oncogenic potential of the A and B types of EBV in the pathogenesis of Burkitt’s lymphoma coupled with the proposed increased frequency of B type virus in nasopharyngeal epithelium raises the hypothesis that progression of a normal EBV-containing epithelial cell to a clonal NPC may lead to a higher frequency of B type virus genomes in NPC samples.

To examine this possibility we examined DNA from biopsies of NPC obtained from patients living in the NPC high risk region of Southeast China for the presence and type of EBV DNA present in the tumour. Initially a ‘classical’ Southern blot analysis was performed using EBNA-2 type-specific probes. A second series of experiments employed polymerase chain reaction (PCR) analysis.

Sixteen NPC biopsies obtained at the Otorhinolaryngology Institute, Shanghai, People’s Republic of China were transported, frozen, to Manchester. DNA was extracted from the biopsies as previously described (Tugwood et al., 1987) and digested with BamHI. Duplicate samples were fractionated by electrophoresis on 0.8% agarose gels and transferred to Hybond (Amersham) membranes. The blots were then hybridized with 32P-labelled DNA from either the type A-specific pM-BamH2 (Polack et al., 1984) or type B-specific pJ-HKA7 (Addlinder et al., 1985) probes. DNA from the lymphoid cell lines BL74 and Chep BL were used as positive controls, containing, respectively, EBV DNA of type A or type B (Arrand et al., 1989). The results from nine of the tumour samples are shown in Fig. 1. With the exception of the specimen in lane 8, the
specific probes hybridized to a single band of length 6 to 7 kb (Zimber et al., 1986). Of the 16 NPC biopsies analysed in this way only one contained type B EBV DNA whereas the other 15 were type A.

The NPC sample in Fig. 1, lane 8 exhibited a banding pattern different to all the other samples indicating that the EBV DNA in this particular specimen contained an extra \textit{BamHI} site within the EBNA-2 region. Restriction enzyme polymorphisms within NPC-derived EBV DNA have been classified by Lung et al. (1990), who have observed several conserved families of variation at this level of analysis. However the difference observed here does not appear to correlate with any of the consistent polymorphisms observed by Lung et al. (1990) and is probably an example of the variation inherent in the world-wide population of wild-type EBV (see e.g. Dambaugh et al., 1980; Harris et al., 1984; Rymo et al., 1979).

In a second series of experiments, biopsies were obtained from nine NPC patients from Guangdong Province. DNA was extracted in Zhanjiang and sent to Manchester for characterization. The integrity of the DNA samples was inadequate for conventional Southern blot analysis but was suitable for PCR assays.

From the known sequences of the EBNA-2 regions of the B95-8 (type A) (Baer et al., 1984) and AG876 (type B) EBV genomes (Dambaugh et al., 1984), oligonucleotide primers which would be specific for the A or B type of EBV were selected and synthesized. These sequences are shown in Fig. 2.

The specificity of the PCR system was demonstrated using DNA from lymphoid cell lines known to contain EBV DNA of either type A or type B (Arrand et al., 1989) as templates for the PCR reactions. Following agarose gel fractionation of the products, ethidium bromide staining revealed the expected 194 bp product showing tight type-specificity depending on the primer pair used for the amplification (Fig. 3). The A or B type of amplified DNA was confirmed by Southern blot analysis using the pM-BamH2 and pJ-HKA7 type-specific probes (not shown). This system was used to examine the nine Guangdong DNA samples and revealed that they all contained EBV DNA of type A (data not shown). In addition, 12 of the Shanghai samples shown to contain

---

**Fig. 1.** Southern blot analysis to determine the type of EBV DNA present in NPC biopsies from Shanghai. DNA (10 ng) from each sample was treated as described in the text. Lanes 1, Chep BL DNA; lanes 2, BL 74 DNA; lanes 3 to 11, NPC biopsy DNA. Lanes 12 contain markers; the positions and sizes (kb) are (from a shorter exposure) indicated. Panels (a) and (b), respectively, show the results following hybridization with the A or B type specific probes. The weak cross-hybridization of the type B probe to high concentrations of type A target DNA (b, lane 2) has been observed before (Zimber et al., 1986) and is due to partial identity between the sequences (Addinger et al., 1985).

---

**Fig. 2.** The sequences of the PCR amplified region of EBV DNA used for the typing studies. The sequence of the B95-8 type A strain (Baer et al., 1984) is shown on the upper line and the corresponding region of type B strain AG876 is shown below. Identical bases in AG876 are indicated by a dash and altered bases are marked. The sequences are within the EBNA-2 coding region and begin at position 49591 in the numbering system of Baer et al. (1984). The 5' primers E3A and E3B are indicated within a box as is the 3' primer mixture E2L. This latter was synthesized as the complementary strands of the B95-8 and AG876 sequences shown using an equimolar mixture of the appropriate nucleotides (C and T) at the appropriate positions to yield a mixture of perfectly matched primers for both type A and type B DNA. Oligonucleotides were prepared using a DuPont Coder 300 synthesizer and purified on a Nensorb Prep (DuPont) column according to the supplier's instructions.
Fig. 3. PCR typing of EBV DNA present in lymphoid cell lines of known EBNA-2 type. Approximately 10^6 cells were washed in PBS and resuspended in 200 μl of 50 mM-KCl, 10 mM-Tris-HCl pH 8.3, 2.5 mM-MgCl₂, 0.45% NP40, 0.45% Tween 20. To this was added 1.2 μl 10 mg/ml proteinase K and the suspension was incubated at 37 °C for 1 h, at 95 °C for 10 min, then 0.5 μl of this solution was subjected to 40 cycles of PCR using the primer pairs (a) E2A and E2C or (b) E2B and E2C. Lanes 1, no DNA; lanes 2, Ramos cells (EBV-negative); Lanes 3, P3HR-I cells (EBV-positive but deleted for EBNA-2); lanes 4, Raji cells (type A); lanes 5, B95-8 cells (type A); lanes 6, BL16 cells (type B). Lanes M contain DNA markers, the sizes of which are indicated in bp. Reactions, in a final volume of 20 μl contained 200 μM each dNTP, 100 ng of appropriate primers and 1.5 units Taq polymerase (Promega) using the buffer supplied with the enzyme. PCR cycles consisted of denaturation at 94 °C for 1.5 min, annealing at 59 °C (E2A pair) or 45 °C (E2B pair) for 1.5 min and elongation at 74 °C for 2.5 min. Reactions were overlaid with 20 μl of paraffin oil. Reaction products were fractionated by electrophoresis on 4% (3% low melting point +1% normal) agarose gels and visualized under u.v. light.

type A DNA by Southern analysis were confirmed using this method.

In this study, as in others (Young et al., 1987; Yao et al., 1991), none of the samples showed evidence of dual infection by both types of EBV. Only one out of 25 NPC biopsies from the high incidence area of mainland China contained EBV DNA of type B, indicating that, as seems also to be the case in Burkitt’s lymphoma, the predominant form of EBV which is associated with the disease is type A. Thus the study provides no evidence to support the contention of there being a higher frequency of B type virus in nasopharyngeal epithelium leading to a predominance of EBV type B-associated NPC. These data are in marked contrast to those of Chang (quoted in Spring et al., 1989) which suggested that the P3HR-1 type (type B) of EBV was more prevalent in Southern China in both NPC patients and in normal individuals. The proportion of type A virus found in the present study of Chinese samples concurs with the overwhelming predominance of this type observed in several other geographical regions (Zimber et al., 1986; Yao et al., 1991). Only in areas of Africa and Papua New Guinea does the frequency of B type EBV appear to be significantly greater (Zimber et al., 1986; Young et al., 1987). The current viewpoint is that, at least in the case of Burkitt’s lymphoma, the ratio of A type to B type virus in the tumour reflects the prevalence of infection within the general population. On this basis it may be expected that the frequency of B type EBV in Southern Chinese populations will be similar to that found in Western communities.

These data reinforce the indications from earlier studies (Bornkamm et al., 1984) that there do not appear to be disease-specific subtypes of EBV. This conclusion has relevance for the development and use of anti-EBV vaccines as prophylactic measures against EBV-associated diseases (Epstein, 1976). Since the predominant form of EBV found in all clinically relevant situations appears to be type A, a vaccine which has this type as its basis should be effective in most instances.
We are grateful to Dr Jin-Quan Jiang for supplying some of the NPC biopsy specimens used in this work and to Dr M. Mackett for assistance with oligonucleotide synthesis. This study was supported by the Cancer Research Campaign.

References


(Received 17 September 1991; Accepted 11 October 1991)