

Investigation of Leukemia Cells From Children With Common Acute Lymphoblastic Leukemia for Genomic Sequences of the Primate Polyomaviruses JC Virus, BK Virus, and Simian Virus 40

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Background. An infectious etiology for childhood acute lymphoblastic leukemia (ALL) has long been suspected, although the characteristics of the putative childhood ALL-inducing agent(s) remain a mystery. We describe the testing of ALL leukemia cells for the presence of DNA sequences of the polyomavirus family: JC virus, BK virus, and simian virus 40 (SV40). **Procedure.** Cryopreserved leukemia cells from 25 children between 2 and 5 years of age at the time of diagnosis and classified as having "common" ALL (B-precursor ALL expressing the CD19 and CD10 surface antigens) were tested for the presence of polyomavirus se-

quences using standard PCR methods. **Results.** Human β -globin gene sequences were detected in 22 of 25 leukemia specimens. However, polyomavirus sequences were not detected in any of the 22 specimens with amplifiable DNA. **Conclusions.** The inability to detect JC virus, BK virus, and SV40 virus DNA sequences in any of the 22 specimens with amplifiable DNA suggests that these members of the polyomavirus family are unlikely to be causally associated with most childhood ALL. *Med. Pediatr. Oncol.* 33:441–443, 1999.

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INTRODUCTION

An infectious etiology for childhood acute lymphoblastic leukemia (ALL) has long been suspected (summarized by Smith et al. [1]). While there is currently considerable enthusiasm for hypotheses that associate childhood ALL with infection-related events (e.g., as presented by Greaves and by Kinlen and their colleagues [2–4]), the characteristics of the putative childhood ALL-inducing agent(s) remain a mystery [5,6], as does the timing of the infectious events that either result in or protect against leukemia [1,7–9]. Recently the polyomavirus JC virus (JCV) was noted to have several likely characteristics of an infectious agent able to cause ALL in children [10]. We describe an investigation of leukemia cells from children with B-precursor ALL for genomic sequences of JCV and for the related polyomaviruses BK virus (BKV) and simian virus 40 (SV40).

MATERIALS AND METHODS

Leukemia Cell Specimens

Cryopreserved leukemia cells (cell concentration 1–2 $\times 10^7$ /ml) from 25 children between 2 and 5 years of age at the time of diagnosis and classified as having "common" ALL [B-precursor ALL expressing the CD19 and CD10 surface antigens (>90% positive cells)] were selected for testing. This age group was selected because of prior epidemiologic evidence suggesting that cases from

the peak age of childhood ALL incidence would most likely be related to an infectious etiology [10]. The age range for cases at diagnosis ranged from 2.3 years to 4.9 years, and the average and median ages were 3.3 years and 3.1 years, respectively. This group of 25 children included 24 white children and 1 black child and included 17 males and 8 females, which is consistent with the expected demographics for ALL in this age range.

PCR Amplification and Identification of Polyomavirus Genomic Sequences

DNA for polyomaviruses was detected using previously described primers and probes and PCR methods

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TABLE I. Synthetic Oligonucleotide Primers and Probes Used for Detection of BKV, JCV, and SV40 by PCR [11,14]

PEP-1	5'-AGTCTTTAGGGTCTTCTACC-3'
PEP-2	5'-GGTGCCAACCTATGGAACAG-3'
BEP-1	5'-TTTTTTGGGTGGTGTGAGTGTGAGAAT-CTGCTGTTGCT-3'
JEP-1	5'-CTTTTTAGGTGGGGTAGAGTGTGGGATC-CTGTGTTTTCA-3'
SV.For3	5'-TGAGGCTACTGCTGACTCTCAACA-3'
SV.Rev	5'-GCATGACTCAAAAACTTAGCAATTCTG-3'
SV40 probe	5'-GGAAAGTCCTTGGGGTCTTCTACC-3'

(Table I) [11–14]. In brief, DNA was extracted from 100 μ l of bone marrow mononuclear cells using an equal amount of 400 μ g/ml proteinase K. From each digest, 10 μ l was tested in each of three separate PCR amplification reactions. PCR for BKV and JCV used a common primer set, PEP-1 and PEP-2, amplifying a 218-bp segment of the T-antigen region of each virus (Table I) [11]. PCR for SV40 used SV.For3 and SV.Rev, amplifying a 105-bp T-antigen segment (Table I) [14]. To confirm the adequacy of DNA from the specimens for PCR, the human β -globin gene was amplified using GH20 and PC04, which amplify a 268-bp fragment [13]. The PCR amplifications were conducted in a 96-well thermocycler using 1.25 units of Taq polymerase per 50 μ l reaction. After 10 min at 94°C for activation of the polymerase, 40 cycles of amplification were employed (1 min each at 94°C for denaturing, 52°C for annealing, and 72°C for extension), followed by a 5-min extension period after the last cycle [13]. Amplification products were detected with biotinylated probes in the Amersham enhanced chemiluminescence system, using probe PC03 for the human β -globin gene, probe BEP-1 for BKV, probe JEP-1 for JCV, and an SV40 probe (Table I) [11,13]. Negative controls were included at the specimen-processing step using K562 cells. All negative controls were β -globin-positive with negative results in viral assays.

RESULTS AND DISCUSSION

Human β -globin DNA was detected in 22 of 25 leukemia cell specimens. Genomic sequences from JCV, BKV, or SV40 were not detected in any samples. The probability of zero positive results among 22 leukemia specimens is less than 10% if the true prevalence of association with JCV (or BKV or SV40) is 10% or greater and is less than 1% if the true prevalence is 20% or greater. These negative results are unlikely to be due to assay characteristics; previous studies in this laboratory using the same methods detected BKV and JCV at high prevalence in urine samples from HIV-infected and uninfected homosexual men with a detection limit of

1–10 copies of polyomavirus genome per reaction [12,13]. The negative findings are also unlikely due to the specimens tested. Fresh frozen tissue is considered optimal for PCR testing, and β -globin was successfully amplified from most specimens. In addition, because cancer is a clonal disease, all tumor cells should have contained the virus genome, if present, and the number of viral copies present in each sample should have been much greater than the 1–10 copy sensitivity of the PCR assays. Although the possibility of remote infection prior to cancer diagnosis, a so-called hit and run mechanism, cannot be excluded, this is an unlikely mechanism for polyomavirus-induced tumorigenesis. In animal models of polyomavirus-induced tumorigenesis, there is generally continued expression of polyomavirus T-ag and maintenance of viral DNA sequences throughout tumor development [15,16]. At any rate, these data provide strong evidence that the vast majority of childhood ALL cases are not associated with an infectious process involving polyomaviruses that results in the maintenance of viral genomic sequences in leukemia cells.

The epidemiologic findings suggesting a relationship between patterns of childhood infection and ALL remain persuasive. Although the results in this report suggest that JCV, BKV, and SV40 are unlikely to be causally associated with most childhood ALL, the search for a virus etiologically associated with the development of ALL should continue.

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