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COMMENTARY

Simian virus 40 and non-Hodgkin lymphoma

See pages 817 and 851

The role of DNA polymaviruses in the development of human cancer has been under scrutiny for almost four decades, especially given the potential origins of simian virus 40 (SV40). Although the spectrum of tumours induced by SV40 in rodents closely resembles that observed in human beings, a plausible mechanism of transmission has been difficult to verify. Unlike for most other oncogenic viruses (Epstein-Barr and hepatitis B viruses and human papillomavirus), the mechanism underlying cellular transformation in vivo for the polymavirus family is less well understood.

The reports by Regis Vilchez and Narayan Shivapurkar and their respective colleagues in this issue of The Lancet expand the list of human cancers associated with SV40 infection to include non-Hodgkin lymphoma. These findings complement animal studies in which polymaviruses transform B lymphocytes in culture and induce B-cell lymphomas. The new studies reaffirm that variations in the frequency of detection of polymaviruses in human cancers may reflect differences in technical approaches to virus detection, variability in the viability and preparation of samples, and definition of the requirements for confirmation of the presence of virus. Both studies used methodologically rigorous approaches (PCR amplification of large [/>500 bp] fragments, DNA sequencing, and Southern blotting) to substantiate their conclusions. However, detection of SV40-derived DNA does not necessarily mean a biologically relevant consequence in the host cell.

Koch's criteria that associate a microorganism as being causative of a particular disease are fulfilled by SV40: 1) SV40 T-Ag is often observed in diverse cancers; 2) SV40 can be isolated in pure culture on artificial media; 3) inoculation of SV40 produces diverse cancers (identical to those in human beings) in experimental animals; and 4) SV40 is recoverable, at least as DNA fragments, from some cancers. Other polymaviruses have been associated with human cancer: JC virus with medulloblastoma and BK virus with neuroblastoma. The resistance to implicate SV40, and to a lesser extent JCV and BKV, in human carcinogenesis lies in the inability to convincingly define a biologically functional role at a cellular level.

Vilchez and colleagues found that SV40 was more frequently observed in patients with diffuse large B-cell and follicular lymphomas compared with other subtypes. In that underlying molecular alterations in non-Hodgkin lymphoma subtypes are distinctive, the presence of SV40 T-Ag may represent a step in the multistage process of lymphomagenesis. T-Ag binds specifically to consensus sequences on both the retinoblastoma and p53 tumour suppressors, functionally inactivating them. Observation of formation of complexes of p53 or pRb with T-Ag would provide persuasive evidence for the pathogenic nature of this virus. Laser-capture microdissection and isolation of single cells for in-situ molecular characterisation make investigations of virus/tumour-suppressor interactions possible.

It is unlikely that presence of SV40 T-Ag in these lymphomas is sufficient to cause tumour formation. Several examples of the multistage process of viral carcinogenesis are documented. First, in Burkitt's lymphoma, the immune dysregulation associated with enhanced B-cell proliferation may allow Epstein-Barr virus to expand its targets for infection, and enhance the likelihood for cellular immortalisation. Second, hepatocellular carcinoma is rare in the West, yet virtually endemic in certain regions of Africa and Asia. Although infection with hepatitis B virus is commonly associated with this cancer, the large discrepancy in cancer risk is related to the interaction with the liver carcinogen, aflatoxin. Both the virus and aflatoxin bind to p53 and inactivate its function. Third, the frequency of human papillomavirus infection is much higher than the global incidence of cervical cancer (p53 inactivation may also be needed in cervical carcinogenesis). Thus viral infection appears to be necessary but insufficient for malignant transformation. The observations of Vilchez and Shivapurkar and their colleagues strongly support an important role for SV40 in lymphomagenesis, without resolving the question of where in the cascade of stochastic molecular events the viral infection is critical.

Timing of exposure to virus, tissue tropism, and coincident molecular alterations must all be important in determining whether cellular transformation ultimately occurs. SV40 T-Ag has been detected in tumours of at least two patients with Li-Fraumeni syndrome in whom heterozygous germline p53 mutations exist. SV40 T-Ag was detected in the tumours in which the normal p53 allele has been retained, whereas T-Ag is not found when the p53 allele loses heterozygosity. Thus the p53 status of a cell may provide a favourable milieu for subsequent SV40-associated induction of cellular transformation. Complexes of p53 and pRb with T-Ag form in other tumours. In fact, patients with diffuse large cell as well as follicular lymphomas often have detectable p53 alterations. Co-incident detection of SV40 and p53 alterations in lymphoma would be highly informative.

The seroprevalence of the polymaviruses is much lower than that of other cancer-associated viruses, yet the frequent and widespread detection of polymaviruses in various human tumour types indicate that these viruses deserve careful attention. Although seropositivity would
greatly enhance the argument for infection and causation in these and other studies, effective serologic assays for polyomaviruses are still in development. Nonetheless, the observations of Vilchez and Shivapurkar and their colleagues, and those of many other investigators, can no longer be considered serendipitous. Carefully controlled studies of brain tumours, Li-Fraumeni syndrome, bone tumours, mesothelioma, and lymphoma, with convincing in-vitro and animal data, support an important role for SV40 in carcinogenesis. Epidemiological studies to determine the distribution of the virus in global sub-populations may shed more light on its propensity for certain predisposed hosts. Studies that explore the biologically relevant interactions of the transforming T antigen with other intracellular components will generate provocative information that should ultimately explain how the presence of these viruses in certain cells actively contributes to their malignant transformation. As we close the chapter that defines polyomaviruses as tumorigenic in human beings, we can now move forward with confidence to define the molecular mechanisms by which these viruses contribute to human cancer.

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Addressing suicide as a public-health problem

See page 835

Premature death from suicide is estimated to be the tenth leading cause of death in the world, and as common as deaths from road-traffic accidents. Furthermore, in all countries there is a greater or lesser degree of stigma that attaches to suicide, so not all suicides are officially recorded as such. Epidemiological necropsy studies in several countries suggest that the proportion of suicides that are "unofficial" is very great. Apart from the loss of life, suicides mean the loss of a breadwinner and parent for the family, long-lasting psychological trauma for children, friends, and relatives, and the loss of economic productivity for the nation, so there is every reason to take suicide seriously.

In today's Lancet, Michael Phillips and colleagues describe a new attempt to estimate the current pattern and burden of suicide in China. As is the case everywhere, in China some stigma attaches to suicide, so not all such deaths are officially recorded as suicide. Moreover, like many developing countries, China does not have a complete registration system for vital data, and the official mortality figures provided to the WHO are based on data from about 10% of the population. This 10% sample is not nationally representative but is collected from locations with relatively good reporting mechanisms, thus containing an over-representation of urban areas. Since suicide in China is three times less common in urban than in rural areas, if the rate for this 10% sample is presented as the national rate, it is likely to be a serious underestimate. Attempts to compensate for this underestimation by various adjustments have resulted in rates of 30 per 100 000.

Phillips and colleagues took suicide rates for 1995-99 by 5-year age-group, sex, and region (grouping the regions as urban or rural), as provided by the Chinese Ministry of Health, and adjusted them first for regional unrepresentativeness by projecting the sex, age, and region-specific mortality rates in the vital registration data for each year to the total population for each year reported by the Statistics Bureau. Second, the researchers adjusted the rates for a general estimated rate of unreported deaths, as derived by comparing data from the Ministry of Health vital registration system with mortality estimates reported by the Statistics Bureau from its 1995 1% nationally representative sample survey.

These adjustments resulted in an estimated annual suicide rate of 23 per 100 000 and a total of 287 000 suicides deaths per year, which accounted for 3.6% of all deaths in China, and 19% of all deaths in the 15-34 age-group. Of particular interest is the sex and direction of the sex ratio and the urban-rural ratio, which contrast sharply with those in the West. Phillips and colleagues do not analyse the suicide data by reported method of suicide, but other work suggests that the relatively high rates of suicide among young rural women in South-East Asia is linked to the ready availability of pesticides that are potent poisons, so what would have been an impulsive suicidal gesture became a completed suicide.

Phillips and colleagues' estimate of 23 per 100 000 is much lower than the Global Burden of Disease estimate of 30 per 100 000 for China in 1990. The latter was based on higher estimates of uncounted deaths, a different adjustment for the urban-rural disparity, and a reassessment of a significant proportion of deaths coded as accidents or other violent deaths to suicide. Psychological necropsy studies and other work indicate
Association between simian virus 40 and non-Hodgkin lymphoma

Regis A Vilchez, Charles R Madden, Claudia A Kozinetz, Steven J Halvorson, Zoe S White, Jeffrey L Jorgensen, Chris J Finch, Janet S Butel

Summary

Background Non-Hodgkin lymphoma has increased in frequency over the past 30 years, and is a common cancer in HIV-1-infected patients. Although no definite risk factors have emerged, a viral cause has been postulated. Polyomaviruses are known to infect human beings and to induce tumours in laboratory animals. We aimed to identify which one of the three polyomaviruses able to infect human beings (simian virus 40 [SV40], JC virus, and BK virus) was associated with non-Hodgkin lymphoma.

Methods We analysed systemic non-Hodgkin lymphoma from 76 HIV-1-infected and 78 HIV-1-uninfected patients, and non-malignant lymphoid samples from 79 HIV-1-positive and 107 HIV-1-negative patients without tumours; 54 colon and breast carcinoma samples served as cancer controls. We used PCR followed by Southern blot hybridisation and DNA sequence analysis to detect DNAs of polyomaviruses and herpesviruses.

Findings Polyomavirus T antigen sequences, all of which were SV40-specific, were detected in 64 (42%) of 154 non-Hodgkin lymphomas, none of 186 non-malignant lymphoid samples, and none of 54 control cancers. This difference was similar for HIV-1-infected patients and HIV-1-uninfected patients alike. Few tumours were positive for both SV40 and Epstein-Barr virus. Human herpesvirus type 8 was not detected. SV40 sequences were found most frequently in diffuse large-B-cell and follicular-type lymphomas.

Interpretation SV40 is significantly associated with some types of non-Hodgkin lymphoma. These results add lymphomas to the types of human cancers associated with SV40.

Lancet 2002; 359: 817–23
See Commentary page B12

Introduction

Non-Hodgkin lymphoma comprises a biologically diverse group of haematological malignancies with clinical courses ranging from indolent to highly aggressive. During the past 30 years, the reported incidence and death rate of the disease have increased strikingly, nearly doubling since 1970. About 55 000 new cases of non-Hodgkin lymphoma are estimated to be diagnosed annually in the USA, and deaths related to the disorder are ranked fourth and fifth among all cancer deaths in women and men, respectively. Although the reasons for the increase in incidence are not fully understood, a substantial number of cases of non-Hodgkin lymphoma are linked to the HIV-1 epidemic. Indeed, non-Hodgkin lymphoma is a common malignancy in HIV-1-infected patients and the incidence can be up to 300 times higher than in HIV-1-negative individuals.

No obvious risk factors have emerged for non-Hodgkin lymphoma in the general population, but a viral cause has been postulated. Some cases of non-Hodgkin lymphoma in HIV-1-infected patients have been attributed to deficient immune surveillance of oncogenic herpesviruses, such as Epstein-Barr virus (EBV) and human herpesvirus 8 (HHV-8), or to chronic antigenic stimulation and defective immune regulation. EBV is suspected of having a major role in primary central-nervous-system non-Hodgkin lymphoma in HIV-1-infected patients, since most of those tumours contain EBV DNA, but it is detected less frequently (<40%) in systemic non-Hodgkin lymphoma in HIV-1-infected patients. EBV is found even less commonly in non-Hodgkin lymphoma from HIV-1-negative patients. HHV-8 is specifically associated with multicentric Castleman's disease and primary effusion lymphoma, which often occurs in a setting of profound immunosuppression.

Because EBV and HHV-8 are absent from many cases of non-Hodgkin lymphoma, other viral agents should be considered as possible causes. The small DNA-containing polyomaviruses (simian virus 40 [SV40], JC virus, and BK virus) are known to infect human beings, to have oncogenic potential, and to be associated with some human cancers. SV40 DNA sequences have been found repeatedly in some brain and bone cancers and mesotheliomas. Polyomaviruses typically establish subclinical and persisting infections in their natural host, with persistence or latency in several organs, including kidney, brain, and spleen. Studies have identified SV40, JC virus, and BK virus DNA sequences in B lymphocytes from HIV-1-infected and HIV-1-uninfected patients, suggesting that polyomaviruses are lymphotropic in man. Polyomaviruses are known to induce tumour formation in animals, including the production of B-cell lymphomas by SV40. The major types of tumours induced by SV40 in laboratory animals are the same as the human cancers found to contain SV40 DNA, with the exception of lymphomas. In animals, oncogenesis is mediated by the polyomavirus large tumour (T) antigen. The large T antigen is a multifunctional protein that stimulates host cells to enter S phase and is
required for initiation of viral DNA synthesis. Fundamental to the effects of T antigen on host cells is binding to cellular tumour-suppressor proteins p53 and members of the pRB family.2,6,7

Studies have reported the detection of SV40 DNA sequences in non-Hodgkin lymphoma from HIV-1-infected and HIV-1-uninfected patients,6,12 and the amplification of JC virus DNA sequences from systemic non-Hodgkin lymphoma of HIV-1-infected children.6 These findings suggest a possible role for polyomaviruses in lymphoproliferative disorders, but the small size of the study populations, the lack of screens for other tumour viruses, and the limited confirmation of identity of the viral sequences detected made conclusion of whether polyomaviruses were definitely associated with non-Hodgkin lymphoma difficult. We aimed to determine the frequency of detection of polyomavirus T antigen DNA sequences in non-Hodgkin lymphoma among HIV-1-infected and HIV-1-uninfected patients, to identify which one of the three polyomaviruses able to infect humans (SV40, JC virus, and BK virus) was associated with non-Hodgkin lymphoma in adult patients, and to establish clinical correlations between the presence of viral sequences and non-Hodgkin lymphoma among HIV-1-infected and HIV-1-uninfected patients. The HIV-1-infected population was included in this study because of their high incidence of non-Hodgkin lymphoma and because immunocompromised individuals are known to be at risk of development of virus-mediated neoplasms.7

**Patients and methods**

**Patients**

We studied 28 adult patients with HIV-1 infection and 35 HIV-1-uninfected patients who were diagnosed with systemic non-Hodgkin lymphoma between January, 1996, and August, 2001, at the Harris County Hospital District, the Veterans Administration Medical Center, and the Methodist Hospital, all of which are affiliated with Baylor College of Medicine, Houston, TX, USA. Additionally, the AIDS and Cancer Specimen Bank of the US National Cancer Institute, through collaboration with the Baylor Center for AIDS Research, provided non-Hodgkin lymphoma samples and clinical data from 48 HIV-1-positive and 43 HIV-1-negative adult patients diagnosed between November, 1987, and May, 2000, at different medical centres in the USA. The histological types of non-Hodgkin lymphoma among HIV-1-infected and HIV-1-uninfected patients were categorised according to WHO Classification for Neoplastic Diseases of the Lymphoid Tissues.6,11 No lymphomas of the central nervous system were included in this study.

Two types of control sample were analysed. Peripheral blood leucocytes and hyperplastic lymph nodes from 79 HIV-1-positive and 107 HIV-1-negative patients without non-Hodgkin lymphoma or any type of cancer from the Harris County Hospital District, the Methodist Hospital, and the AIDS and Cancer Specimen Bank served as the non-malignant lymphoid control samples. 26 samples of colon carcinoma and 28 of breast carcinoma from patients diagnosed with these malignancies between January and August, 2001, at the Methodist Hospital served as the cancer control group. A preliminary analysis of some non-Hodgkin lymphoma specimens was included in an earlier report.8 Institutional Review Board approval was obtained for this study.

**Procedures**

All sample processing was done in a laminar flow hood within a biosafety level 3 facility free from viruses and plasmids at the Department of Molecular Virology and Microbiology, Baylor College of Medicine. Total cellular DNA from non-Hodgkin lymphoma and control samples was extracted as previously described.8 All PCR assays were set up in the PCR Clean Rooms core facility of the Department of Molecular Virology and Microbiology at Baylor College of Medicine to avoid contamination of reaction mixtures. As a further precaution, positive-displacement pipettes and barrier-tip pipettes were used. Oligonucleotide primers used for PCR and DNA sequence analysis have been described previously.5-10 All DNA samples were tested for suitability for amplification with primers specific for a fragment of the human β-haemoglobin gene (primers PC03/KM38). Only specimens from which cellular β-globin gene sequences could be amplified were then examined for viral sequences by PCR amplification with primer sets specific for a region of the large T antigen gene (PYYlor/PYYrev), conserved among all three polyomaviruses capable of infecting humans (SV40, JC virus, and BK virus), for the EBV latent membrane protein 2a (LMP-2a) gene (TP1Q5/TP1Q3), or for a region of the HHV-8 capsid gene (KSI1/KSI2).11,12 Primers were obtained from Integrated DNA Technologies (Coralville, IA, USA).

Positive control plasmids were added to the control PCR reactions outside the core facility after tubes containing negative controls and test DNA were closed. The positive controls for polyomavirus PCR reactions were plasmid DNAs containing cloned SV40 (pSV5sp21-N), JC virus (pBRJC-MAD-1), or BK virus (pBBKV-Dunlop) genomes. The SV40 control genome contains an engineered restriction site that distinguishes it from natural isolates.5 The positive control for EBV reactions was DNA extracted from the EBV-positive Burkitt's lymphoma cell line Namalwa; that for HHV-8 was a plasmid DNA containing cloned viral sequences of the capsid antigen gene. Negative controls for PCR assays were reactions without added DNA template. PCR amplifications (45 cycles) were done with a GeneAmp PCR system 2400 thermocycler (Perkin-Elmer, Norwalk, CT, USA). High-stringency annealing temperatures specific for each primer set have been described elsewhere.5,11,12 PCR amplification products were analysed by agarose gel electrophoresis.

Probes specific for each virus were used to discriminate among amplified N-terminus T antigen polyomavirus sequences.11,12 These specific oligoprobes are 3'-labelled with a tail of dUTP-fluorescein by terminal transferase. Electrophoresed polyomavirus PCR products were transferred to a nylon membrane and the DNA was cross-linked to filters by ultraviolet irradiation for 2 min. The fluorescent hybrid was detected with an anti-fluorescein horseradish-peroxidase-conjugated antibody. Autoradiography was done at room temperature for 15 min. Additionally, representative polyomavirus PCR products were cloned into a TA cloning vector (Invitrogen, Carlsbad, CA, USA); multiple clones were screened by PCR and then sequenced with the Thermo Sequenase Radiolabeled Terminator Cycle Sequencing Kit (USB, Cleveland, OH, USA) to confirm the identity of polyomavirus-specific DNA from the tumours.

**Statistical analysis**

The necessary sample size for the study was calculated a priori from previously published reports. The presence of polyomavirus SV40 neutralising antibodies has been reported in 16% of HIV-1-infected and 11% of HIV-1-uninfected patients.10 Published estimates of detection of SV40 DNA sequences in non-Hodgkin lymphoma range from 10% to 20%.5,11,12 Therefore, we assumed a
A conservative rate of 15% for the detection of polyomavirus large T antigen sequences in non-Hodgkin lymphoma in HIV-1-uninfected patients. The rate of polyomavirus T antigen DNA in non-Hodgkin lymphoma was expected to be at least 30% among HIV-1-infected patients. On those assumptions, 120 individuals in each group (HIV-1-infected and HIV-1-uninfected patients) would be necessary to observe a difference of that magnitude assuming a power of 80%, a two-sided test, and a test significance level of 0.05. Post-hoc estimates, however, indicated that, with 75 participants, our study had more than 99% power to identify SV40 detection in non-Hodgkin lymphoma compared with control.

Statistical methods were used to address the third objective of this research. χ² analysis was used to compare the distribution of viral sequences in non-Hodgkin lymphoma between HIV-1-infected and HIV-1-uninfected patients. The t test was used to compare the mean age of patients with SV40-positive non-Hodgkin lymphoma between the two groups, and non-parametric analysis of variance was used to compare the difference in CD4 cell count among HIV-1-infected patients with systemic non-Hodgkin lymphoma. Statistical analysis was done with the SAS/PC statistical software package.

Role of the funding source

The funding sources had no role in study design; in the collection, analysis, or interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

Results

Table 1 shows the demographic characteristics of the 154 HIV-1-infected and HIV-1-uninfected patients with systemic non-Hodgkin lymphoma. The distribution of histological types of non-Hodgkin lymphoma analysed among HIV-1-infected and HIV-1-uninfected individuals was indicative of the frequency of non-Hodgkin lymphoma in these two populations of patients in general. The most common histological type of non-Hodgkin lymphoma in HIV-1-infected and HIV-1-uninfected patients. The mean CD4 cell count of HIV-infected patients at the time of diagnosis of systemic non-Hodgkin lymphoma was 165/µL (SD 185, range 2–901).

Polyomavirus large T antigen PCR products were generated from 64 of 154 (42%) samples of non-Hodgkin lymphoma, including from 25 (33%) HIV-1-infected patients.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>HIV-1-infected patients (n=76)</th>
<th>HIV-1-uninfected patients (n=78)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD, range) age (years)</td>
<td>40 (7, 28–58)</td>
<td>57 (15, 12–90)</td>
</tr>
<tr>
<td>Man/woman</td>
<td>68/8</td>
<td>46/32</td>
</tr>
<tr>
<td>B-cell neoplasms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precursor B-cell lymphoblastic leukaemia/lymphoma</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Mantle cell</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Follicular</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>Diffuse large B-cell</td>
<td>58</td>
<td>40</td>
</tr>
<tr>
<td>Burkitt's</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>Plasmacytoma</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>T-cell neoplasms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral T-cell, unspecified</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Systemic anaplastic large cell</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*Including six cases of variant Burkitt's lymphoma with atypical cytological features.

Table 1: Demographic characteristics and histological type of non-Hodgkin lymphoma in HIV-1-infected and HIV-1-uninfected patients

Table 2: Presence of polyomavirus and herpesvirus sequences in non-Hodgkin lymphoma and control samples from HIV-1-infected and HIV-1-uninfected patients

<table>
<thead>
<tr>
<th>Polymavirus</th>
<th>EBV</th>
<th>EBV and polyomavirus</th>
<th>HIV-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All cases (n=154)</td>
<td>64 (42%)*</td>
<td>42 (27%)</td>
<td>13 (7%)</td>
</tr>
<tr>
<td>HIV-1-positive (n=76)</td>
<td>25 (33%)*</td>
<td>30 (39%)</td>
<td>7 (9%)</td>
</tr>
<tr>
<td>HIV-1-negative (n=78)</td>
<td>39 (50%)*</td>
<td>12 (15%)</td>
<td>4 (5%)</td>
</tr>
<tr>
<td>Non-cancer controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph nodes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV-1-positive (n=7)</td>
<td>0</td>
<td>4 (57%)</td>
<td>0</td>
</tr>
<tr>
<td>HIV-1-negative (n=7)</td>
<td>0</td>
<td>3 (43%)</td>
<td>0</td>
</tr>
<tr>
<td>Peripheral-blood leucocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV-1-positive (n=72)</td>
<td>0</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>HIV-1-negative (n=100)</td>
<td>0</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Cancer controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon cancer (n=26†)</td>
<td>0</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Breast cancer (n=28†)</td>
<td>0</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

EBV=Epstein-Barr virus. HIV-8=human herpesvirus 8. NT=not tested. *All polyomavirus-positive specimens contained SV40-specific sequences. †Healthy adult volunteers. HIV status was not assessed.

Figure 3: Agarose gel electrophoresis and staining with ethidium bromide of PCR-amplified polyomavirus sequences (upper panel), and Southern blotting (lower panels) with probes for individual polyomaviruses

M=molecular weight markers. SV40+, JCV+, and BKV+ are positive controls for SV40, JC virus, and BK virus, respectively. NC=control (no DNA template). CA=cancer controls (colon and breast carcinoma samples).
from HIV-1-uninfected patients than in cancer control samples (39 of 78 [50%] vs 0 of 54, p<0.0001).

To confirm the presence of SV40 T antigen sequences, we further analysed samples of non-Hodgkin lymphoma in which SV40 DNA was detected. Sequence analysis of amplified products obtained from ten samples of non-Hodgkin lymphoma (six HIV-1-infected and four HIV-1-uninfected patients) showed the DNA sequences to be identical to those of the SV40 T antigen gene. The sequences associated with non-Hodgkin lymphoma lacked a 9 bp insert found in both JC virus and BK virus, proving that the sequences were not derived from either of these polyomaviruses (figure 2). Additionally, primers specific for the carboxy (C)-terminal region of the SV40 T antigen gene (TA1/TA2) yielded PCR amplification products of the expected size from 29 non-Hodgkin lymphoma samples.

Sequence analysis of PCR products from five samples of non-Hodgkin lymphoma (three from HIV-1-infected and two from HIV-1-uninfected patients) confirmed that their origin was SV40. We then compared these five lymphoma-associated T antigen C sequences with a catalogue of SV40 sequences (GenBank). One C sequence was similar to that of SV40 strain CPC/MEN, previously detected in several primary human brain cancers, and one was different from any previously reported SV40 sequence, and three (one from an HIV-1-infected patient and two from HIV-1-uninfected patients) were similar to that of SV40 strain MC-028846B—a virus first detected in a sample from a contaminated poliomyelitis vaccine from 1955. These results substantiate our belief that the T antigen gene of SV40 was present in the non-Hodgkin lymphoma specimens tested, and that detection of SV40 sequences was not the result of laboratory contamination.

We saw no significant differences in the mean age of patients with SV40-positive and SV40-negative non-Hodgkin lymphoma within the HIV-1-infected group (42 years [7-0] vs 39 years [6-6], p=0.07) or the HIV-1-uninfected group (39 years [12-9] vs 54 years [17-3], p=0.2) group. Only five of the patients with SV40-positive non-Hodgkin lymphoma were born after 1963, the last year that SV40-contaminated poliomyelitis vaccine was used in the USA.

Among HIV-1-infected patients with systemic non-Hodgkin lymphoma, there was no significant difference in the mean CD4 cell count between patients with EBV-positive and SV40-positive systemic non-Hodgkin lymphoma (190 × 10⁶ [257] vs 112 × 10⁶ [93], p=0.3). Additionally, the CD4 cell count did not differ between virus-positive (EBV and SV40) and virus-negative patients with systemic non-Hodgkin lymphoma (160 × 10⁶ [200] vs 163 × 10⁶ [166], p=0.9). Non-Hodgkin lymphoma samples were more frequently EBV-positive in HIV-1-infected than HIV-1-uninfected patients (30 of 76 [39%] vs 12 of 78 [15%], p=0.001), whereas non-Hodgkin lymphoma samples were more frequently positive for SV40 T antigen in HIV-1-uninfected than HIV-1-infected patients (39 of 78 [50%] vs 25 of 76 [33%], p=0.03).

The histological types of non-Hodgkin lymphoma positive for SV40 and EBV sequences are presented in table 3. Diffuse large B-cell lymphoma was the most frequent type of non-Hodgkin lymphoma positive for viral sequences. The rate of EBV detection in diffuse large B-cell lymphomas was not significantly different between HIV-1-infected and HIV-1-uninfected patients (p=0.1). However, the detection of SV40 T antigen sequences was significantly more common in diffuse large B-cell non-Hodgkin lymphoma from HIV-1-uninfected than from HIV-1-infected patients (p=0.003). SV40 sequences were also found frequently in follicular tumours from HIV-1-uninfected patients. Virus detection rates did not differ among tumours obtained from different sources (Houston...
of the seven Burkitt's lymphomas found to contain SV40 DNA, six were regarded as atypical variants since they displayed atypical cytological features.28 The histological types of the 11 non-Hodgkin lymphoma samples positive for both EBV and SV40 sequences were diverse large B-cell lymphomas (n=8) and Burkitt’s lymphomas (n=3). Neither EBV nor SV40 sequences were detected in the five T-cell neoplasms tested.

Discussion

This investigation showed that polyomavirus SV40 T antigen DNA sequences are significantly associated with non-Hodgkin lymphoma in HIV-1-infected and HIV-1-uninfected patients. This finding sheds new light on the possible genesis of an important group of malignant disorders. The SV40 sequences do not seem to be present simply because non-Hodgkin lymphoma cells are readily susceptible to viral infection; in that case, EBV and SV40 should be found in similar frequencies in non-Hodgkin lymphoma of HIV-1-infected and HIV-1-uninfected patients. The results also suggest that polyomavirus SV40 is not merely an opportunistic superinfection; if so, one would expect similar frequencies of SV40 detection in EBV-positive and EBV-negative non-Hodgkin lymphoma, and in other cancer samples (colon and breast) if those cell types were permissive to SV40 replication. The observation of minimal instances of co-infection with SV40 and EBV and the lack of detection of SV40 in non-malignant lymphoid samples and epithelial cancer control specimens suggest that SV40 might contribute to the development of those lymphomas in which it is present.

Overall, 42% of non-Hodgkin lymphomas tested here contained SV40 DNA sequences—a frequency similar to that found in an independent study (43%).29 This frequency is higher than reported in previous studies,30,31 and might be a consequence of characteristics of the specific populations of patients from whom specimens were obtained, the histological types of tumours tested, or variations in DNA extraction methods or PCR assay conditions. By contrast with our working hypothesis, the SV40 positivity rate detected here was significantly higher in non-Hodgkin lymphoma from HIV-1-negative patients than in those from HIV-1-infected individuals. We do not yet understand the role of HIV-1 infection in SV40 pathogenesis. Our observed non-Hodgkin lymphoma positivity rates could be a result of the particular sets of tumour specimens we obtained for this study, as well as of the fact that more non-Hodgkin lymphomas are EBV-positive in HIV-1-infected patients than in HIV-1-negative individuals. Our observations do indicate, however, that the development of SV40-positive non-Hodgkin lymphoma is not dependent on pronounced immunodeficiency in the host.

We found EBV associated with 39% of systemic non-Hodgkin lymphoma from HIV-1-infected patients and with 15% from the HIV-1-negative group, similar to rates reported previously.1 We did not detect HHV-8 sequences in non-Hodgkin lymphoma from either group of patients, in agreement with recent studies that showed lack of association between HHV-8 and non-Hodgkin lymphoma in HIV-1-infected and HIV-1-uninfected patients.32 SV40 T antigen sequences were detected frequently in diffuse large B-cell lymphomas in both groups of patients and in follicular lymphoma in HIV-1-uninfected patients. This particular association might be important, since these are the two most common histological types of lymphomas from mature B cells and account for about 50-60% of all cases of non-Hodgkin lymphoma.11 It also suggests that mature B cells could be more susceptible than precursor cells to the transforming potential of SV40.

The SV40 sequences associated with non-Hodgkin lymphoma identified here were different from those of known laboratory strains, and several examples of the C-terminal T antigen gene sequence were similar to that of an SV40 strain detected in a sample of contaminated poliovaccine from 1955.33 We know that several strains of SV40 exist,34 but whether the strains detected in non-Hodgkin lymphomas are more lymphomagenic than other strains remains to be determined.

The oncogenic potential of polyomavirus SV40 has been established in laboratory animals.35 In studies in which hamsters were inoculated intravenously with SV40, lymphomas developed among 72% of the animals in the inoculated group and none of the control group.36 The histological type was consistent with diffuse large cells, and the lymphomas were shown to be of B-cell origin because they expressed cell-surface antigen.37 More recently, a study confirmed the lymphomagenic capacity of the virus and that lymphomas represent a common malignancy induced by SV40.38 After intravenous inoculation, about a third of the animals developed more than one histological type of malignant neoplasm, with osteogenic sarcomas being most common after lymphomas.39 After intracardiac injection, malignant mesotheliomas and osteosarcomas developed in addition to lymphomas.40 These studies supported a causative role for the virus in lymphomagenesis because SV40 T antigen was expressed in all tumour cells, animals with tumours developed antibody against SV40 T antigen, and neutralisation of SV40 with specific antibody before virus inoculation prevented lymphoma development. Knowledge of these animal studies prompted us to consider a role for SV40 in human lymphomagenesis.

Polyomavirus SV40 has been associated with specific types of solid cancers in human beings, including brain tumours, osteosarcomas, and malignant mesotheliomas.41 These are the types of malignant disorders caused by the virus in laboratory animals—a finding that emphasises the predictive value of the animal studies. Recent reports provide persuasive evidence that the presence of polyomavirus SV40 is meaningful in the development of those human cancers. Immunohistochemical assays have detected the expression of T antigen in tumour cells,42,43 T-antigen protein complexed with p53 has been extracted from some cancer specimens,44 and microdissection of malignant mesothelioma samples followed by PCR assays detected SV40 DNA in tumour cells and not in adjacent non-malignant cells.45 When an antisense SV40 T antigen construct was introduced into SV40-DNA-positive malignant mesothelioma cell lines, the expression of T antigen was abrogated and growth was inhibited.46

The polyomaviruses JC virus and BK virus also have the ability to induce tumour formation in laboratory animals;47 they have been associated with some human solid tumours, in particular brain cancers;48 but much less frequently than SV40. This observation suggests that SV40 is more oncogenic in humans than are JC virus and BK virus. Up to 80% of the adult population worldwide is seropositive for these viruses, and JC virus is recognised as the causative agent of progressive multifocal leukoencephalopathy—a subacute opportunistic disease in HIV-1-infected patients. We did not detect JC virus or BK virus DNA sequences in any of the non-Hodgkin lymphoma specimens tested in this study, by contrast with a previous report of JC-virus-positive non-Hodgkin lymphoma involving HIV-1-infected children.49 These differences could reflect the age or geographic origin of the patients or a difference in oncogenic capacity among the polyomaviruses. Models of the oncogenicity of JC virus and BK virus do not indicate the development of lymphomas.47
The major source of known human exposure to polyomavirus SV40 was immunisation with SV40-contaminated poliovaccines. Inactivated and live, attenuated forms of the poliovaccine were prepared in primary rhesus monkey kidney cells, some of which were from animals naturally infected with SV40—a virus that was unknown at the time. Studies showed that residual infectious SV40 survived the vaccine inactivation treatments, and millions of people were inadvertently exposed to live SV40 from 1955 until early 1963.10 In the USA, vaccine lots received by about 20 states were estimated to have contained 0.75–0.97 mL contaminated vaccine per child, lots from about 15 states were thought to have contained 0.01–0.74 mL contaminated vaccine per child, and about 15 states were believed to have received lots that were free from SV40.10 Perhaps this distribution of contaminated vaccines influenced the differences in the rate of SV40-positive non-Hodgkin lymphoma that have been seen in recent studies. Seroprevalence studies have shown the presence of SV40 neutralising antibodies in 16% of HIV-1-infected patients and 11% of HIV-1-uninfected individuals, some of whom were born after 1963 and could not have been exposed to SV40-contaminated poliovaccines.10 Our study found that five patients with SV40-positive non-Hodgkin lymphoma were born after 1963—a finding similar to previous studies involving brain and bone cancers in which some patients with SV40-positive tumours had been born in recent decades.10 The observations suggest that polyomavirus SV40 might be causing infections in human beings long after the use of the contaminated vaccines. However, how SV40 is transmitted among humans, and the prevalence of infection, remain to be established.

In summary, our study suggests that polyomavirus SV40 is significantly associated with non-Hodgkin lymphoma in HIV-1-infected and HIV-1-uninfected patients and might have a role in the development of these haematological malignancies. Definition of a viral cofactor in the pathogenesis of these tumours could lead to new diagnostic, therapeutic, and preventative approaches.

Contributors
R A Vilchez participated in conception and study design; collection, assembly, and analysis of data; statistical analysis and interpretation of the data; and preparation of the paper. C R Madden helped with analysis of the data. C A Kozinetz assisted with conception, study design, and statistical analysis. S J Halvorson and Z S White helped with collection and assembly of data. J L Jorgensen and C J Fitch assisted with collection and histopathological diagnosis of specimens. J S Butel directed the study and was involved in study design, data analysis and interpretation, and preparation of the paper. All authors reviewed and approved the final draft.

Conflict of interest statement
None declared.

Acknowledgements
We thank the AIDS and Cancer Specimen Bank sponsored by the National Cancer Institute for providing specimens of non-Hodgkin lymphoma and lymph nodes for this study, and the staff members of the Pathology Departments at the Methodist Hospital and the Harris County Hospital District for their assistance. This study was supported in part by the Baylor Center for AIDS Research Core Support Grant Number AI32611 from the National Institute of Allergy and Infectious Diseases, and by Cooperative Agreement NCCS-58 with the National Space Biomedical Research Institute funded by the National Aeronautics and Space Administration. R A Vilchez is the recipient of the Junior Faculty Development Award from GlaxoSmithKline.

References


Clinical picture

Boerhaave’s syndrome

Kirk M Chan-Tack

A 21-year-old woman was admitted with respiratory failure, fever, hypotension, and tachycardia. She had nausea and vomiting over the previous fortnight. On pulmonary auscultation she had coarse bilateral breath sounds and bibasilar dullness. Computed tomography of the chest showed anterior mediastinal air and large bilateral pleural empyemas (figure). Chest tubes were placed for empyema drainage and broad-spectrum antibiotics were started. Endoscopy showed a 1.5 cm esophageal tear. Thoracotomy with mediastinal drainage was done. Blood cultures grew multiple pathogens, consistent with polymicrobial sepsis. The patient’s retching had precipitated esophageal rupture (Boerhaave’s syndrome). Multi-organ failure developed due to sepsis and the patient eventually died.

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Presence of simian virus 40 DNA sequences in human lymphomas

Narayan Shivapurkar, Kenichi Harada, Jyotsna Reddy, Richard H Scheuermann, Yin Xu, Robert W McKenna, Sara Milchgrub, Steven H Kroft, Ziding Feng, Adi F Gazdar

Simian virus 40 (SV40)—a potent oncogenic virus—has been associated previously with some types of human tumours, but not with lymphomas. We examined human tumours for the presence of specific SV40 DNA sequences by PCR and Southern blotting. Viral sequences were present in 29 (43%) of 68 non-Hodgkin lymphomas, and in three (9%) of 31 Hodgkin’s lymphomas. Viral sequences were detected at low frequencies (about 5%) in 235 epithelial tumours of adult and paediatric origin, and were absent in 40 control tissues. Our data suggest that SV40 might be a cofactor in the pathogenesis of non-Hodgkin lymphomas.

Lancet 2002; 359: 851–52

See Commentary page ???

Polyomaviruses are DNA viruses whose genomes encode tumour antigens. Tumour (T) antigens are multifunctional proteins that are essential for transformation by binding to and inactivation of specific host-cell proteins.1 Three polyomaviruses are associated with human infection and disease. The widely distributed JC and BK viruses are of human origin, but of low oncogenic potential; however, simian virus 40 (SV40) is a potent oncogenic virus and seems to have spread to human beings via contamination of poliovirus stocks between 1955 and 1963 as well as by other means.1

Inoculation of SV40 into hamsters results in four main types of tumours including B-cell lymphomas, mesotheliomas, and brain and bone tumours.3 Several reports have documented the association of SV40 and human tumours, especially mesotheliomas and brain and bone tumours.4 However, relatively small studies have failed to document the association of SV40 and human non-Hodgkin lymphomas for these reasons, we tested for the presence of specific SV40 T antigen sequences in non-Hodgkin lymphomas and other human tumours.

We gathered specimens after obtaining permission from the patients. We used 10 controls, all of whom had been treated in the Hospital for 3 to 6 months the month after. PCR-Southern blot screening of lymphomas for presence of SV40 T antigen sequences

Results of 12 B-cell lymphomas are illustrated. Samples 1–5 are diffuse large-cell lymphomas, samples 6–9 are follicular lymphomas, and samples 10–12 are mantle-cell lymphomas. Upper half of panels illustrates amplicons generated with the following primers: (A) SV TagF (forward) and SV TagR (reverse); (B) SV TagF (forward) and SV TagG3 (reverse); (C) SVfo2r (forward) and SVrev (reverse). Lower half of panels illustrates Southern blotting of corresponding amplicons illustrated in upper panels. + indicates positive control (mesothelioma DNA previously confirmed for SV40 T antigen sequences); − indicates negative control (no template DNA).

<table>
<thead>
<tr>
<th>Non-Hodgkin lymphomas</th>
<th>Number positive/number tested</th>
<th>Percentage (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>29/68</td>
<td>43% (31–55)</td>
</tr>
<tr>
<td>Diffuse large (B cell)</td>
<td>15/31</td>
<td>48% (30–67)</td>
</tr>
<tr>
<td>Follicular (B cell)</td>
<td>6/16</td>
<td>31% (11–59)</td>
</tr>
<tr>
<td>Mantle cell (B cell)</td>
<td>4/8</td>
<td>50% (18–84)</td>
</tr>
<tr>
<td>Burkitt’s (B cell)</td>
<td>2/2</td>
<td>100% (16–100)</td>
</tr>
<tr>
<td>Large cell anaplastic (T cell)</td>
<td>3/11</td>
<td>27% (6–61)</td>
</tr>
<tr>
<td>Hodgkin’s lymphoma</td>
<td>3/31</td>
<td>9% (2–26)</td>
</tr>
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<table>
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<th>Paediatric tumours</th>
<th>Number positive/number tested</th>
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</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>8/155</td>
<td>5% (2–10)</td>
</tr>
<tr>
<td>Wilms’ tumour</td>
<td>1/31</td>
<td>3%</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>0/27</td>
<td>0%</td>
</tr>
<tr>
<td>Hepatoblastoma</td>
<td>2/27</td>
<td>7%</td>
</tr>
<tr>
<td>Rhabdomyosarcoma</td>
<td>1/18</td>
<td>6%</td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>1/16</td>
<td>6%</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>2/11</td>
<td>18%</td>
</tr>
<tr>
<td>Ewing’s sarcoma</td>
<td>0/8</td>
<td>0%</td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>1/17</td>
<td>6%</td>
</tr>
</tbody>
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<table>
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<th>Adult carcinomas</th>
<th>Number positive/number tested</th>
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</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>5/80</td>
<td>6% (2–14)</td>
</tr>
<tr>
<td>Lung carcinomas</td>
<td>1/20</td>
<td>5%</td>
</tr>
<tr>
<td>Breast carcinomas</td>
<td>2/20</td>
<td>10%</td>
</tr>
<tr>
<td>Colon carcinomas</td>
<td>1/20</td>
<td>5%</td>
</tr>
<tr>
<td>Prostate carcinomas</td>
<td>1/20</td>
<td>5%</td>
</tr>
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<table>
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<th>Controls</th>
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<tbody>
<tr>
<td>All</td>
<td>4/40</td>
<td>0% (0–9)</td>
</tr>
<tr>
<td>Blood</td>
<td>0/12</td>
<td>0%</td>
</tr>
<tr>
<td>Lymphoid tissues</td>
<td>0/14</td>
<td>0%</td>
</tr>
<tr>
<td>Other non-malignant tissues</td>
<td>0/14</td>
<td>0%</td>
</tr>
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HIV-1 status of non-Hodgkin lymphoma cases

<table>
<thead>
<tr>
<th></th>
<th>Number positive/number tested</th>
<th>Percentage (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>6/13</td>
<td>46% (19–75)</td>
</tr>
<tr>
<td>Negative</td>
<td>10/20</td>
<td>50% (27–73)</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>14/35</td>
<td>40% (24–58)</td>
</tr>
</tbody>
</table>

Presence of SV40 T antigen sequences in lymphomas, other human tumours, and control tissues

institutional review board. Tumours and control tissues are listed in the table. Most specimens were obtained from residents of north Texas, USA. Demographic information was available for 62 of the non-Hodgkin lymphoma cases. Their mean age was 56 years (SD 15±1, range 23–89), and there were about equal numbers of men (n=33) and women (n=29). Of the 28 Hodgkin’s lymphoma patients from whom information was available, the mean age was 49 years (16–84), and there were 15 men and 13 women. The non-malignant tissues consisted of 14 blood samples from healthy donors, 14 hyperplastic lymphoid tissues (12 tonsils and two thymus glands) from patients without cancer, and 12 organs (11 kidneys and one liver) from patients with cancer. Most tissues were frozen samples stored at −70°C until used, and the remainder were archival paraffin-embedded tissues.

DNA was extracted and analysed for the presence of SV40 T antigen sequences with either two sets (for paraffin tissues) or three sets (for frozen tissues) of primers that amplified overlapping regions of the gene encoding the large T antigen of SV40. About 100 ng DNA in a total volume of 10 μL were used for each PCR reaction, which comprised 45 cycles of denaturation. To confirm the SV40 specificity of the PCR products, Southern blotting and DNA sequencing were done on the resultant amplicons. Extensive precautions were taken to prevent artefactual contamination, and positive results were independently confirmed. Statistical differences between groups were examined with Fisher’s exact test. To obtain exact binomial 95% CIs for the differences between proportions, we drew 5000 bootstrap samples from each group. The CIs were calculated with the lowest and highest 2.5% of the differences between the two groups among 5000 bootstrap samples.

As summarised in the table, 43% of the non-Hodgkin lymphomas were positive for T antigen sequences. The HIV-1 status of some of the patients with non-Hodgkin lymphoma was known, and the SV40 sequence detection rates were
similar among patients positive, negative, or indeterminate for HIV-1. In all cases, the results from the three sets of primers were completely concordant (figure). Southern blotting (figure) and sequencing confirmed that the amplicons contained DNA sequences specific for SV40, and excluded the presence of JC or BK viral sequences. By contrast with the non-Hodgkin lymphoma cases, only 9% of the Hodgkin's lymphoma cases were positive for SV40 sequences (table). However, because all of the Hodgkin's disease cases were paraffin embedded, we compared this 9% with the proportion of paraffin-embedded non-Hodgkin lymphoma cases positive for SV40 (10 of 26 [38%]; difference 29% [95% CI 7-50], p=0.013). SV40 sequences were present in three of eight non-Hodgkin lymphomas and in one of 10 Hodgkin's disease cases born after 1963, the last known year in which SV40 contamination of poliovirus stocks occurred. All 40 non-malignant tissues were negative for SV40 sequences (table). Of the solid tumours, 5% of the paediatric cases and 6% of the adult carcinomas were positive. Control tissues including some of the sites of latent polyomavirus infections (blood, lymphoid tissues, and kidney) were negative for viral sequences (table). The requirement for 45 cycles of PCR for reproducible detection suggests that the virus was present in low copy number in non-Hodgkin lymphomas. Earlier smaller studies failed to find an association between SV40 and human lymphoma.1 Possible reasons include geographical differences in the prevalence of the virus resulting from the uneven distribution of SV40 contaminated poliovirus stocks, and technical difficulties in detecting low copy number viral sequences.3 The spectra of human and hamster tumours associated with SV40 virus are identical. The finding of SV40 viral “footprints” in a large subset of non-Hodgkin lymphomas suggests that the virus may function as a cofactor in the pathogenesis of this important human tumour.

Contributors
Narayan Shivapurkar, Kerenchi Harada, and Jyvons Reddy did the laboratory analyses, Richard Sturmer, Yin Xu, Robert McKenna, Sara Milchgrub, and Steven Knoll collected the lymphoma samples, pathology reports, and demographic data. Ziding Feng did the statistical analyses, Adi Gazdar planned and supervised the study and wrote the paper.

Conflict of interest statement
None declared.

Acknowledgements
We thank Gail Tomlinsin, Shinichi Teyooka, Domenico Mastrangelo, Antiban Maitra, Asha Padar, and Rieichi Maruyama for contributing some of the non-lymphoma samples. Funding for this study was provided by a grant from the Early Detection Research Network, National Cancer Institute, Bethesda, Maryland, USA.

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Fatal allergic vasculitis associated with celecoxib
F Schneider, F Meziani, C Chartier, M Alt, A Jaeger

We report on the occurrence of a rare and as yet unforeseeable adverse reaction to treatment with celecoxib, a cyclooxygenase-2 (COX-2) selective, non-steroidal, anti-inflammatory drug. A previously healthy adult suffered fatal acute multiple organ failure presumably after diffuse allergic vasculitis with diffuse necrotic purpura. Although no conclusive proof is available, such a reaction could have been triggered by at least one of two mechanisms: an allergic reaction linked to the chemical structure of celecoxib; or an interaction of the drug with synthesis of endothelial eicosanoids leading to an imbalance between vasoactive end products, resulting in widespread rise to local thrombosis.

Lancet 2002; 359: 852–53

Celecoxib, a COX-2 selective non-steroidal anti-inflammatory drug (NSAID), is widely prescribed for the treatment of pain related to arthritis. COX-2 inhibitors have a higher safety profile than other non-selective NSAIDs, especially with regard to gastrointestinal adverse effects.1 Nevertheless, celecoxib has the side-effects of all other NSAIDs, including various mild cutaneous reactions that improve after withdrawal.2 We report the first case of fatal allergic vasculitis after treatment with celecoxib.

A 52-year-old patient was treated with a daily dose of 200 mg of celecoxib for cervicobrachial neuralgia. On the eighth day of treatment he developed a maculopapular and urticarial eruption on his thorax and arms, which became angioedematous. The patient was advised to stop taking celecoxib and was given 40 mg per day prednisolone and 10 mg per day cetirizine. In spite of this treatment, 2–3 days after celecoxib withdrawal he developed diarrhoea, hyperthermia (41°C), circulatory failure, and acute purpuric dermal necrosis during the next 12 h. He was admitted to hospital.

Blood tests in the first 6 h showed acute metabolic acidosis, hyperlactataemia, rhabdomyolysis, haemolysis, disseminated intravascular coagulation, and renal and hepatic failure. There were no signs of acute infection or chronic inflammatory disorders (C-reactive protein <10 mg/L, fibrinogen 1-98 g/L, leucocyte count 7.5×10⁹/L). We took blood, tracheal aspirate, urine, stool, cerebrospinal fluid, and skin samples: all were sterile. We noted a large transient drop in the plasma concentrations of C3 and C4, whereas the plasma histamine concentration was within the normal range. There was no dysglobulinaemia. We did a skin biopsy, on the second day of hospital admission (day 10 after celecoxib was started), and found a diffuse capillary leukocytoclastic and thrombotic vasculitis (figure), similar to that on a later colonic biopsy. We could not find any other causes for leukocytoclastic vasculitis, and excluded sepsis and immunological angitis. Extension of the skin damage was at its greatest extent at arrival covering face, scalp, body, legs, and palms. Initially, the injury was maculopapular, it became gradually purpuric and necrotic with bullae developing in some places or resembling second-degree thermal injury in other places. After 1 week, diffuse cutaneous necrosis occurred. The case was reported to the French pharmacovigilance network with a high suspicion of being caused by celecoxib. Despite maximum intensive-care support, the patient died 8 days after admission to hospital from multiple organ failure and