Short papers

Simian virus 40 and human pleural mesothelioma

C Mulatero, T Surentheran, J Breuer, R M Rudd

Abstract

***Background*—An aetiological role for Simian virus 40 (SV40) in malignant mesothelioma has been suggested from studies in the USA and the UK but results have been conflicting. A study was undertaken to look for evidence of SV40 in stored tissue samples from pleural mesotheliomas.**

***Methods*—DNA was extracted from paraffin embedded tissue. The presence of DNA was established by amplification of a 250 bp product from the betaglobin gene. Primers PYV.F and PYV.R were used in a concentration of 50 per mol each per reaction to amplify a 172 bp fragment of a conserved region of SV40 that codes for a portion of large T antigen that is common to SV40 and other polyoma viruses.**

***Results*—Twelve of the 17 samples contained amplifiable betaglobin DNA. None of the samples (0/12, 95% CI 0 to 26.5%) was positive for the polyoma large T antigen.**

***Conclusions*—These results do not lend any support to the hypothesis that SV40 infection may be aetiologically relevant to the increasing incidence of mesothelioma in the UK.**

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Keywords: human mesothelioma; Simian virus 40 (SV40); polyoma virus; DNA

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There has been considerable interest in recent reports of the identification of SV40 virus in samples of mesothelioma and other rare tumours.[1–4] This virus contaminated Salk polio vaccine grown on monkey kidney culture from 1954 to 1961 and various other vaccines used in smaller quantities, including early samples of oral polio vaccine, although commercial Sabin vaccine was free from contamination.[5] The incidence of mesothelioma in the UK is continuing to increase and is expected to do so until 2020.[6] This increase can be adequately explained on the basis that the peak use of asbestos without respiratory protection was around 1970, but it has been suggested that the SV40 virus could have an aetiological role.[1 2]

We sought evidence of SV40 in stored samples from patients with mesothelioma presenting to our institution between 1995 and 1996.

Table Results of reactions for betaglobin and polyoma in samples from 17 mesotheliomas

Patient no. (M/F)	*Year of birth*	*Cell type*	*β- globin PCR*	*Polyoma PCR*
1 M	1920	Epithelioid	Positive	Negative
2 M	1944	Epithelioid	Positive	Negative
3 M	1922	Epithelioid	Positive	Negative
4 M	1931	Epithelioid	Positive	Negative
5 M	1955	Biphasic	Positive	Negative
6 M	1942	Epithelioid	Positive	Negative
7 M	1939	Epithelioid	Positive	Negative
8 M	1927	Sarcomatoid	Positive	Negative
9 M	1935	Epithelioid	Positive	Negative
10 M	1930	Biphasic	Positive	Negative
11 M	1930	Biphasic	Positive	Negative
12 M	1924	Biphasic	Positive	Negative
13 M	1937	Biphasic	Negative	Negative
14 M	1927	Sarcomatoid	Negative	Negative
15 M	1925	Epithelioid	Negative	Negative
16 M	1926	Epithelioid	Negative	Negative
17 F	1924	Epithelioid	Negative	Negative

Methods

Paraffin sections of pleural biopsy tissue from 17 patients with a diagnosis of pleural mesothelioma, confirmed histologically with supportive immunohistochemistry, were analysed. All patients gave a history of exposure to asbestos. Further demographic details and results are shown in table 1. DNA was extracted with phenol chloroform after five days of proteinase K digestion and precipitated with ethanol. The presence of DNA was established by amplification of a 250 bp product from the betaglobin gene. Subsequently, 200 ng of DNA was amplified in 50 µl of reaction mix containing buffer (100 M Tris HCl, 500 mM KCl; Perkin Elmer), magnesium chloride (1.5 mM), dNTPs (200 µM each), amphilitaq polymerase (1 unit). Primers PYV.F and PYV.R were used at a concentration of 50 per mol each per reaction to amplify a 172 bp fragment of a conserved region of SV40 that codes for a portion of large T antigen that is common to SV40, BK, and JC viruses.[3] A positive plasmid control (100 copies per cell) and a negative control were included with each amplification. The reaction mix was denatured at 94°C for one minute and then subjected to 40 cycles of 94°C for one minute, 52°C for one minute, 72°C for one minute, and a final cycle of 72°C for 10 minutes. Primers were assayed against plasmid containing SV40 large T antigen with human placental DNA. The sensitivity of detection was estimated at 1–10 genome copies or 1 copy per cell when plasmid was diluted in human placental DNA. Reactions which were negative for polyoma and betaglobin were spiked with 1000 copies of plasmid containing

the gene for polyoma large T antigen and reamplified to detect inhibitors. The electrophoresis method was standard agarose gel electrophoresis at 80–100 V with ethidium bromide staining.

Results

Twelve of the 17 samples contained amplifiable betaglobin DNA. None of the samples (0/12, 95% CI 0 to 26.5%) was positive for the polyoma large T antigen. None of the PCR reactions was inhibitory.

Discussion

SV40 is an oncogenic virus in animal models and recently there has been interest in the possibility that it may be relevant to the increasing incidence of human mesothelioma.[1 2] Previous studies from the USA[3] and the UK[4] have identified evidence of SV40 virus in mesothelioma samples. Like Pepper *et al*,[4] we used archival material and found it possible to extract DNA from the majority of paraffin embedded tissue samples. We used the PYV.F and PYV.R primers because they are more sensitive for detection of SV40-like DNA sequences than primers specific for SV40.[3] The sensitivity of the assay was estimated at one copy of target sequence per cell. This is comparable to the sensitivity reported by Strickler *et al*[7]; others have not reported sensitivity.[3 4] The sensitivity of the assay is the same as that of an assay which detects papillomavirus in cervical carcinoma from similar material prepared in the same way in the same laboratory, and we are therefore confident that our results were truly negative.

Carbone and colleagues[3] reported SV40-like DNA sequences in 29 of 48 mesotheliomas (60%, 95% CI 45 to 74%). Pepper *et al*[4] found PYV positivity in six of nine mesotheliomas (67%, 95% CI 30 to 93%) and, of these, SV40 specific large T antigen was positive in four (44%, 95% CI 14 to 79%). While the number we tested was small, the 95% confidence interval (0 to 26.5%) does not overlap that reported in the two series with positive results for SV40. We can therefore be reasonably confident that our results represent a true difference from the positive results reported previously. A larger series from the USA found negative results in all of 48 betaglobin positive mesothelioma samples (95% CI 0 to 7.4%) using two SV40 primer sets with an analytical sensitivity of 1–10 genome copies.[7] The explanation for the conflicting findings is not obvious, but possibilities include false positive results due to laboratory contamination of samples, differences in methods used to harvest DNA from paraffin embedded tissue, and geographical variation in frequency of SV40 infection.

Human SV40 infection by natural routes has not been demonstrated and transmission via contaminated polio vaccine has been postulated as an explanation for the presence of SV40 in tumour tissue. However, patients with childhood ependymomas and choroid plexus tumours in which SV40 has also been identified were too young to have received the contaminated polio vaccine,[2] and some of the mesothelioma patients reported positive were too old for it to be likely that they would have received the vaccine.[3 4] It has been estimated that 62% of the US population under 60, including 90% of those under 20, had been immunised by potentially infected vaccine by the time the problem was recognised.[5] A recent retrospective cohort study in the USA found no evidence of an excess of mesotheliomas or other tumours such as ependymomas and osteosarcomas suggested to be possibly linked with SV40 in birth cohorts likely to have received SV40 contaminated polio vaccine.[8] The theoretical possibility remains that SV40 may operate on the pleura only as a co-carcinogen with asbestos, which could give rise to a more limited impact on the incidence of mesothelioma. However, our results do not lend any support to the hypothesis that SV40 infection may be aetiologically relevant to the increasing incidence of mesothelioma in the UK.

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