VIROLOGY

Detection of JC virus in two African cases of progressive multifocal leukoencephalopathy including identification of JCV type 3 in a Gambian AIDS patient

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Progressive multifocal leukoencephalopathy (PML) is a fatal demyelinating central nervous system (CNS) infection, affecting mainly oligodendrocytes, but also occasional astrocytes. In the USA, Europe and Asia, PML is caused by the human polyomavirus JC virus (JCV) and in autopsy series occurs in about 4–7% of AIDS patients. In Africa, the prevalence of PML in AIDS patients is uncertain and the causative agent is unknown. This study reports immunocytochemical and PCR confirmation of PML in the CNS of an AIDS patient dying in Uganda, East Africa (case 1). In a Gambian patient infected with HIV-2 who died 3 months after onset of AIDS/PML in Germany (case 2), it was possible to confirm the identity of the virus by DNA sequencing of the PCR amplified JCV product. This African genotype of the virus (type 3) showed an unusual re-arrangement of the regulatory region, and could be distinguished at several sites from East African and African-American JCV strains described previously. This study has confirmed that PML is a complication of African AIDS as it is in Europe and the USA, and that JCV type 3 is pathogenic in African AIDS patients. Furthermore, the finding of an African genotype of JCV in a patient dying in Germany suggests that in this individual JCV represented a latent infection acquired in Africa.

Introduction

Infection with JC virus (JCV) is thought to be endemic throughout the world [1–3]. No known symptoms are associated with primary infection in children, but the virus persists in the kidneys of a large segment of the population and *c.* 40% are excreting JCV in the urine at any one time [2, 4]. PCR amplification of DNA extracts of urinary cells has been used to group JCV coding regions into four major genotypes. Types 1, 2 and 3 are European, Asian and African, respectively [2]. The fourth type, found to date only in the USA, is very similar to type 1 and may reflect an initial recombination event between types 1 and 3 [5]. The portion of the genome used for genotyping includes the

coding regions, introns and intergenic regions, as well as the non-re-arranging regulatory region to the early side of *ori* (4854 bp). The regulatory region to the late side of *ori* shows an 'archetypal' configuration (267 bp) in urinary strains which is re-arranged by deletion and duplication in 'progressive multifocal leukoencephalopathy-type' (PML-type) JCV strains from the central nervous system (CNS). This unstable portion of the genome is not included in the genotype analysis [6, 7].

As many as 4–7% of AIDS patients in Europe and the USA die with PML [8–10]. Although PML was first described nearly 40 years ago [11], and has been reported on all other continents, PML was not identified in Africa until its appearance in a Ugandan AIDS patient with multiple opportunist infections [12]. Light microscopic study of routine pathological sections showed multifocal, subcortical demyelinated lesions with typical enlarged, hyperchromatic oligodendrocytes. The viral agent was not identified. Since then a case of PML in a Gambian dying in Germany has been described [13]. Additional cases of PML in

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Africans dying in Belgium have been observed (H. Taelman, personal communication). Recently, four PML cases were identified in Abidjan [14], but the agent was not further characterised.

In the present studies, immunocytochemical confirmation of the pathological diagnosis of PML in the Ugandan case (case 1) employed a rabbit polyclonal antibody reactive with capsid proteins of both JCV and SV40. SV40-specific monoclonal antibodies (MAbs) and JCV-specific PCR confirmed that this case of PML was associated with JCV infection. Verification of the African PML agent as JCV came from the DNA sequence of the VP1 gene amplified from the brain of the Gambian who died in Germany (case 2). Furthermore, the sequence of this VP1 fragment identified it as a type 3 strain of JCV. The regulatory region (promoter-enhancer) showed a style of rearrangement unlike any reported previously. This study demonstrates that in both East and West Africa PML is due to JCV virus, and that the African genotype (type 3), can be the pathogenic agent.

Materials and methods

Brain tissue

The source of PML brain tissue used in these studies has been described. Case 1 occurred in a Ugandan AIDS patient [12]. In case 2, PML was found in a Gambian patient infected with HIV-2 and dying in Germany after residence there for 12 months [13]. PML was the sole manifestation of AIDS, with onset 3 months before death. Blocks from the subcortical white matter and the cerebellum were available for study. A case of PML in the macaque brain (MSP-190) [15, 16] was used as an SV40-positive, JCV-negative control. Three cases of human PML from the USA known to be due to JCV were studied in parallel [17]. For PCR, DNA from PML tissue was extracted as described previously [18]. Briefly, after deparaffination at room temperature, digestion buffer (100–200 µl) was added which included proteinase K 200 µg/ml. After digestion overnight at 56°C, the extract was boiled for 10 min.

Double-label immunocytochemical method

Capsid proteins VP1-3 of JCV were detected in deparaffinised PML brain sections with a polyclonal rabbit antibody to JCV by a modification of the method described previously [19, 20]. Alternatively, a rabbit polyclonal antibody raised against SV40 (Lee Bio-molecular, San Diego, CA, USA) could be used for JCV detection because of cross-reactivity between these closely related primate polyomaviruses. A double-label method combined the detection of VP1-3 with detection of glial fibrillary acidic protein (GFAP) with a MAb (clone G-A-5, Boehringer-Mannheim, Indianapolis, IN, USA). The staining was performed as described

previously [20], except that the secondary antibodies were a biotinylated goat anti-rabbit IgG (Kirkegaard and Perry, Gaithersburg, MD, USA) mixed with a goat anti-mouse IgG. This was followed by streptavidin alkaline phosphatase mixed with mouse peroxidase-antiperoxidase (PAP). The detection system used Vector Red I followed by 3,3'-diaminobenzidine (DAB) with NiCl₂. This change reverses the original colour pattern [20]. With this method reactive astrocytes expressing GFAP appeared black, while JCV-infected oligodendrocytes were stained red.

Virus detection with MAbs to SV40 VP1

Immunoreactivity of MAbs to VP1 of SV40 in tissue sections was detected by a modification of the method described previously [21, 22]. Briefly, the method used biotinylated goat anti-mouse IgG, followed by streptavidin-peroxidase (Vector Laboratories, Burlingame, CA, USA). The slides were developed with a 3,3'-diaminobenzidine (DAB) solution (DAB 0.05%, hydrogen peroxide 0.01%, nickel chloride 0.04%) followed by dehydration in graded alcohols and coverslip mounting.

PCR amplification

Primers used for DNA amplification from deparaffinised sections of case 1 included JCV-specific primers for the T-antigen gene JTP-1 and 2 [18] combined with a JCV-specific probe (JTP-1.1) for detection of the 141-bp amplified product by Southern blot. SV40-specific primers included STP-1 (2699-2720, 5'-CAGGTT-CAGGGGAGGTGTGGG-3') and STP-2 (2876-2855, 5'-GATGGTGGGGAGAAGAACATGG-3'). The SV40-specific probe, STP-1.1 (2760-2784, 5'-TGGCTGATTATGATCATGAACAGAC-3') detected the 178-bp product. To ensure virus-specific amplification, primers for JCV were mismatched to the homologous SV40 sequence at two 3'-terminal nucleotides in both primers. Primers designed for the specific detection of SV40 were similarly mismatched to JCV. Numbering of the JCV genome throughout is that of JCV (Mad1) [23].

Primers used for Case 2 included JLP-1 and 4 yielding a 129-bp fragment in the VP1 gene [2]. The entire 610-bp V-T intergenic region [7] was amplified in two segments, primers VPV-5 and 6 amplified the 3' end of the T-antigen gene and primers VPV-9 and 10 amplified the 3' end of the VP1 gene [24]. The primer VPV-6, which replaced VPV-8 used previously [24], is located at nucleotide position 2764-2742 (5'-GAAGCAGAAGACTCTGGACATGG-3').

Primers JTP-5 and 6 amplify a 295-bp region around the zinc-finger motif of T antigen. The primer sequences were: JTP-5 (3621-3642) 5'-CTTTGTTTGGCTGCTACAGTAT-3' and JTP-6 (3915-3896) 5'-GCCTTAAGGAGCATGACTTT-3'.

Primers JSP-1 and 2 were used for amplification of the large T-antigen intron (423 bp) including mutations near splice sites found in African type 3 strains. Primer sequences were: JSP-1 (4390-4411), 5'-AC-CAGGATTCCCATTTCATCTGT-3' and JSP-2 (4812-4791), 5'-GTTGCTCATCAGCCTGATTTTG-3'.

Primers for regulatory region amplification were JRR-1 and 8 and JRR-25 and 26 [24]. JRR-1 and 8 amplify a 295-bp fragment that includes the archetypal regulatory region. JRR-25 and 26 amplify a short fragment including the non-re-arranging region to the early side of *ori* and containing three typing sites as described [7]. Primers JRR-25 and 28 [25] amplify the entire regulatory region (363 bp) on both sides of *ori*, and were used to confirm the results with primer pairs JRR-1 and 8 and JRR-25 and 26.

Reaction conditions for PCR have been described [2, 26, 27]. Briefly, PCRs were performed with UITma DNA polymerase with 3'-5' proofreading activity (Perkin-Elmer Cetus, Norwalk, CT, USA) in a standard PCR buffer containing 1.5 mM Mg²⁺. Brain extracts were run at 1 µl and 5 or 10 µl/reaction. Following an initial denaturation for 1.5 min at 94°C, the reaction was initiated by addition of DNA polymerase in 10 µl of buffer at 80°C (hot start). Reactions were run for 50 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min with a final extension at 72°C for 10 min, except that annealing for primers JRR-25 and 28 was at 62°C.

For cycle sequencing of gel-purified PCR products, the Excel kit (Epicentre, Madison, WI, USA) was used with the same primers for DNA amplification end-labelled with ³³P-ATP (Amersham, Arlington Heights, IL, USA) as described previously [27]. The initial denaturation of 1 min at 95°C was followed by 30 cycles of 30 s at 95°C for denaturation and 1 min at 60°C for annealing and elongation.

Results

PML in a Ugandan AIDS patient (case 1)

Multifocal demyelinated lesions were found in the subcortical white matter, with enlarged, hyperchromatic oligodendrocytes. Many of these lesions occurred at the grey-white matter junction, as is typical for PML [20]. Demyelinated lesions stained with LFB/H&E are shown in Fig. 1A. This African case lacked the giant, bizarre astrocytes which characterise many, but not all, PML cases [28]. To confirm the identity of the agent and the pathogenesis of these lesions, immunocytochemical studies were performed with a rabbit polyclonal antibody to JCV capsid proteins. A double-label method detecting both polyomavirus capsid proteins and the astrocyte marker GFAP was utilised. This method revealed an intense glial reaction in demyeli-

nated foci containing infected oligodendrocytes with polyomavirus capsid proteins present (Fig. 1B and C). Sometimes this glial hypertrophy extended throughout the white matter of the entire section. The double-label method demonstrated that these reactive astrocytes were not expressing JCV capsid antigens, but were sometimes closely apposed to infected oligodendrocytes (Fig. 1B) as has been described in other PML brains [20].

As this polyclonal antiserum is not specific for JCV, but cross-reacts with SV40 – which is known to cause PML in the macaque [29] and, reportedly, in man [30] – a series of MAbs raised against SV40 VP1 was used to characterise further the antigenic nature of the infecting agent. Twelve antibodies that immunoprecipitate SV40 VP1 [31] were tested for reactivity on formalin-fixed, paraffin-embedded CNS sections from PML in the macaque brain. Five of the antibodies to SV40 capsid protein VP1 (designated BE8, BH3, CE5, CG9, FG8) reacted strongly with viral proteins in and around the demyelinated lesions of macaque PML. All five MAbs were completely unreactive with JCV-infected lesions in three known cases of human PML from the USA. This agrees with their failure to react with JCV in Western blots (W. A. Scott, personal communication). Significantly, these antibodies were also unreactive with the Ugandan PML tissue. These results indicate that the polyomavirus in this case of Ugandan PML was more closely related to JCV than it was to the macaque PML agent, SV40.

PCR primers specific for the T-antigen gene of JC virus, JTP-1 and 2, amplified a band of the appropriate size from the DNA extract (Fig. 2A) and on Southern blots the band hybridised with a JCV-specific probe (JTP-1.1) (Fig. 2B). SV40-specific primers amplified the expected product from macaque PML brain, but not from African PML. Following Southern blotting, the band from macaque brain hybridised with the SV40-specific probe, STP-1.1 (not shown).

PML in a Gambian AIDS patient (case 2)

Only the extracts from the cerebellum could be amplified with JCV primer pairs. Reactions with 1 µl of extract were always stronger than those with 5 or 10 µl, suggesting the presence of an inhibitor in the extracts.

The JCV genome in case 2 was identified as type 3 by comparison with the prototypes for two PML strains, type 1 (Mad1) and type 2 (GS/B), and a consensus of three East African (Tanzanian) urinary strains (Fig. 3). Also included in the sequence analysis were African-American urinary strains #311 and #312 [26]. Comparisons of strains within the 610-bp VT-intergenic region by amplification of two overlapping fragments with primers VPV-5 and 6 and VPV-9 and 10 showed

Fig. 2. Characterisation of PCR amplified products from case 1. **A**, Products amplified with primers JTP-1 and 2. Lane 1, Ugandan PML brain extract; 2, known JCV-positive PML brain extract; 3, brain extract of monkey PML; 4, control brain extract; 5, PCR buffer alone; 6, size markers. **B**, Southern blot of above gel. Only 141-bp products from human PML hybridised with the JCV-specific probe JTP-1.1 (lanes 1 and 2).

only two differences from the type 3 consensus, one of which was shared with African-American type 3 strains (Fig. 3B). This genotype assignment was confirmed in the fragment amplified by JLP-1 and 4 (Fig. 3A) and in the three typing sites to the early side of *ori* (Fig. 3D), where no deviations from type 3 consensus were found. However, at position 5117 the Gambian strain #313 shows the African-American 'T' in contrast to the 'A' in the Tanzanian consensus sequence.

The T-antigen intron is included in the fragment amplified by JSP-1 and 2 (Fig. 3C). The consensus type 3 sequence is mutated from Mad1 and GS/B on

the acceptor and on the donor side [26]. At position -4 on the acceptor side (4430), #313 shares the mutation (A → C) common to all type 3 strains. However, at position +4 on the donor side (4767), #313, like the African American strain #311, does not share the mutation of C → T occurring in the Tanzanian consensus sequence.

In the fragment amplified by primers JTP-5 and 6, the Gambian JCV strain showed the same mutation at nucleotide 3768 (A → T) changing a Gln codon to Leu as described previously in strains of type 3. This non-conservative amino acid change is located in the zinc finger region, two positions before the first zinc-coordinating Cys residue.

The nature of the regulatory region re-arrangement was confirmed by two independent reactions with primers JRR-1 and 8 and JRR-25 and 28. The latter fragment includes the three typing sites to the early side of *ori* identified previously, and a fourth described here. According to the system previously devised, the Gambian regulatory region re-arrangement can be described as [1-160]T[226-238][120-160]T[226-...] [25]. The 'T' in this notation indicates that this nucleotide could represent either position 161 or 225 in the unre-arranged archetypal sequence. This re-arrangement is shown schematically in Fig. 4, where it is compared to representative types found in other PML brains.

Discussion

The first reported case of PML in Africa came from an East African (Ugandan) AIDS autopsy series. Routine pathology revealed multifocal demyelinated lesions with typical enlarged oligodendrocytes characteristic of PML in Europe and the USA [12]. The agent was not further characterised. The present study used a combination of antibodies to show that the agent is JCV-like, rather than SV40-like. In the immunocytochemical method employed here, a cross-reactive polyclonal antiserum was used in combination with SV40-specific MAbs. The polyclonal rabbit antiserum revealed polyomavirus infection of oligodendrocytes in subcortical white-matter CNS lesions with extensive reactive gliosis. As polyclonal rabbit antisera raised to JCV also react with the closely related SV40 virus, the activity of anti-SV40 MAbs specific for the capsid protein VP1 was tested. These did not react with the African PML agent in infected tissue sections, nor with JCV in the brain of

Fig. 1. Pathology of Ugandan PML (case 1). **A**, Subcortical white matter with small demyelinated lesions at the grey-white matter interface (LFB/H&E) (×12). **B**, JCV-infected oligodendrocytes (red reaction product) in early subcortical lesion closely apposed to reactive astrocytes expressing GFAP (black reaction product). Virus capsid proteins were detected with a polyclonal rabbit antiserum, followed by biotinylated goat anti-rabbit IgG, and streptavidin-alkaline phosphatase. GFAP was detected by a MAb by the peroxidase-antiperoxidase method (×307). (Method modified from refs. 19 and 20). **C**, JCV-infected oligodendrocytes at the margins of a demyelinated lesion with reactive astrocytosis at the center (×120).

A. JLP Fragment **B. Typing sites within the VT- intergenic region**

	JLP-1 & 4	VPV-9 & 10	VPV-5 & 6
	1804 1818 1837 1843 1850 1869 1870	2177 2224 2227 2245 2260 2266 2274 2278 2293 2311 2320 2356 2368 2369 2386 2428 2429 2455 2462 2464 2482 2494 2502 2524 2587 2592 2596 2621 2661 2663 2671 2712 2726	
Mad1	T G T G A G G	T A C C A G C G C G G C T T T A G A G G C C A A T A G C A A T A T	
GS/B	T C C T G G A	C G T T T A C A G T A T T C C G G G G A C T G G C G G C T A G T A	
Type 3	C C T T A C A	C G T T T A C G C T A C C T C A A G C A T T G A T T T T T G G G A	
#311	T C T T A C G	C G T T T A C G C T A C T T C G A G C A T T G G T T T T T G G G A	
#312	C C T T A C A	C G T T T A C G C T A C T T C A A G C A T T G A T T T T T G G G T	
#313	C C T T A C A	C G T T T A T G C T A C T T C A A G C A T T G A T T T T T G G G A	

C. Typing sites within the T-antigen intron

D. Typing sites left of *ori*

JSP-1 & 2															JRR-25 & 28				
	4430	4432	4434	4438	4443	4477	4479	4481	4484	4522	4534	4714	4724	4767	4782	5017	5026	5039	5117
Mad1	A	A	A	C	A	G	A	A	A	G	T	G	T	C	T	A	C	C	T
GS/B	A	A	C	A	G	G	C	A	T	G	C	A	T	C	T	T	T	G	T
Type 3	C	A	A	A	A	C	A	G	T	A	C	A	T	T	T	T	T	C	A
#311	C	A	A	A	A	C	A	G	T	A	C	A	T	C	T	T	T	C	T
#312	C	A	A	A	A	C	A	G	T	A	C	A	T	T	T	T	T	C	T
#313	C	G	A	A	A	C	A	G	T	A	C	A	A	C	A	T	T	C	T

Fig. 3. Typing sites which identify the Gambian JCV strain (case 2) as type 3 (strain #313). Sequence of #313 compared to a consensus of three Tanzanian complete genomes (#308, #309, #310) [26] and two African-Americans' complete genomes (#311, #312) and representatives of other genotypes: Mad1, prototype for type 1 strains (European); GS/B, prototype for type 2 strains (Asian). Typing sites listed here include those identified previously, as well as sites in which #313 differs from all other strains. Positions at which strains #311, #312 and/or #313 differ from the type 3 Tanzanian consensus are enclosed in boxes. Numbering follows that of Frisque *et al.* [23]. **A**, sequence at typing sites in the JLP-amplified fragment; **B**, typing sites within the V-T intergenic region; **C**, typing sites within T-antigen intron; **D**, four typing sites left of *ori* in the non-re-arranging regulatory region.

three cases of PML from the USA. In contrast, the MAb reacted with antigen in tissue sections from a case of PML due to SV40 in the macaque.

A single immunodominant epitope in SV40 VP1 was reactive with all five MAbs [31]. Amino acid relationships among the primate polyomaviruses in this VP1 sequence are shown in Table 1. A change of Gln to Pro within this epitope is sufficient to prevent MAb reactivity with JCV VP1. On the other hand, BKV is identical to SV40 in this region, and would probably be reactive with these same antibodies.

This approach should allow identification of the PML agent in most formalin-fixed biopsy or autopsy CNS material. Moreover, this method can retrospectively distinguish JCV and SV40 polyomaviruses in those PML cases diagnosed pathologically, but not confirmed virologically by DNA specific in-situ methods or PCR. This approach would be particularly useful for cases in which the tissue is unsuitable for PCR amplification because of prolonged formalin fixation.

JCV DNA was amplified by PCR from tissue of case 1 with JCV-specific primers, but not with a primer

Fig. 4. Regulatory region re-arrangement in the Gambian strain (#313). Regulatory region re-arrangements in JCV genomes from PML brain are highly variable and each is unique [25, 32]. The configurations observed among 40 re-arranged PML-type regulatory regions could be roughly divided into three categories called 'long duplicate', 'short triplicate' and 'D-retaining'. These are represented here by strain #117 (type 1), #222 (type 2) and #209 (type 2), respectively [25]. The 'D-retaining' configuration is similar to archetype, but with one or more small deletions elsewhere in the regulatory region. Note that #313 has the 'C' at position 133 which distinguishes type 3 strains [24]. The re-arrangement found in the regulatory region of strain #313 is unlike any of those analysed previously.

Table 1. Comparison of amino acid sequences in the immunodominant epitope in VP1 of SV40 with other primate polyomaviruses

Polyomavirus	Amino acid sequence								
SV40* (aa. 313–321)	Arg	Thr	Gln	Arg	Val	Asp	Gly	Gln	Pro
BKV† (aa. 313–321)	–	–	–	–	–	–	–	–	–
JCV (aa. 305–313)	–	–	Pro	–	–	–	–	–	–
LPV	Leu	Met	Pro	Gln	Ile	Gln	–	–	–
SA-12	NA‡								

SV40, simian virus 40; BKV, K virus; JCV, JC virus; LPV, monkey lymphotropic papovavirus; SA-12, baboon polyomavirus.

*See ref [23] for amino acid sequences of VP1.

†BK virus, dashes indicate identities to the SV40 amino acid sequence.

‡NA, not available. Elsewhere in the genome SA-12 is most closely related to BKV [33].

pair specific for SV40. This 141-bp product hybridised with the specific DNA probe following Southern blotting, confirming the identity of the product as authentic JCV sequence. The PCR product was not sequenced.

The second PML case was reported in an HIV-2-infected AIDS patient from West African (Gambia) dying with HIV-2 infection after 1 year's residence in Germany. JCV infection in the brain of this patient was confirmed by PCR amplification and DNA sequencing of the product. The JCV in the brain of this patient was the same African genotype (type 3) which had been identified in the urine of Tanzanian AIDS patients [24, 26]. This indicates that although the patient died after developing PML in Germany – where he lived 9 months prior to onset, and where JCV type 1 is likely to predominate, – he carried with him the JCV type 3 characteristic of his native Africa.

The regulatory region in PML-type viruses is usually highly re-arranged, each in a unique way [25], but the regulatory region re-arrangement of JCV in the HIV-2-infected Gambian patient was different from any pattern previously observed in PML-type JCV strains in the USA or elsewhere. Three general patterns have been described [25, 32]. The most common, the 'long duplicate', has *c.* 60–70 base pairs (region D) deleted, with the flanking sequence usually duplicated in tandem. A variation on this pattern has a similar deletion, but with shorter flanking regions which are triplicated or quadruplicated ('short triplicate'). A less common pattern, 'D-retaining', comprises strains which retain region D, but sustain one or two small deletions elsewhere with little, if any, duplication. This configuration is very close to the original archetypal configuration found in the urinary tract isolates. The unusual re-arrangement in the Gambian case is the inverse of the common patterns described previously. In the Gambian genome the sequence usually deleted in other strains (region D) is partially duplicated, and a flanking region, usually duplicated or triplicated, is deleted. Therefore, we have called this new and unusual re-arrangement the 'D-duplicating' pattern. It is not known whether this is a general feature of PML associated with HIV-2 infection in Africa. It could also reflect differences in JCV type 3 strains, although

no other type 3 PML infections have been described. Alternatively, it could be a genetic effect in the African host which alters regulatory region re-arrangement or virus growth in the CNS. Regardless of the mechanism, this finding extends the range of JCV regulatory region re-arrangements associated with PML brain.

The coding region sequence of JCV DNA from case 2 (strain #313) shows several mutations which distinguish it from the consensus sequence of East African (Tanzanian) strains, and from two strains obtained from African-Americans [26]. At other sites (e.g., 2368 and 5117), #313 follows the sequence of the two African-American strains, #311 and #312. Three additional sites at which the African American sequences differ from the Tanzanian consensus (i.e., 1061, 3006 and 4076) [26], were not sequenced in this study. Thus, whether this West African strain follows the African-American sequence at all sites where they differ from the Tanzanian consensus remains to be established. If so, it would support the suggestion that these sites may represent East-West African differences in type 3 strains, and that most African-American type 3 strains follow the West African sequence, reflecting their origins in West African populations.

These findings make it unlikely that SV40 or other non-human primate polyomaviruses are causes of PML in Africans. The baboon polyomavirus SA-12 infects naturally the chacma baboon [33]. Immunological cross-reactivity of SA-12 with SV40 and JCV, as well as BK virus, has been demonstrated [34], and preliminary sequence studies show a very close relationship of the SA-12 early region and regulatory region with the DNA sequence of BKV [33]. SA-12 is not known to infect man and has never been associated with PML in man or other primates. Therefore, it seems unlikely that this agent is the cause of any human PML in Africa.

Another primate polyomavirus, monkey lymphotropic papovavirus (LPV), which infects certain B-lymphoblastoid cell lines of monkey or human origin, is not closely related immunologically to SV40, BKV or JCV [35], nor is its amino acid sequence similar [35]. Up

to 30% of the human population may have antibodies to LPV or a related agent [36]. However, like SA-12, it has never been associated with PML, either in man or in other primates and no neurotropic character has been reported.

Is the incidence of PML lower in African AIDS patients than in AIDS patients in Europe and the USA? The only large series reported from Africa has been the recent Abidjan study of 271 HIV-positive autopsied patients dying with HIV-related disease in which four cases (1.5%) were found histologically to have PML [14]. On the other hand, PML was found in six (5.2%) of 115 African AIDS patients dying in Belgium who were thoroughly investigated neuropathologically (cerebral biopsy or autopsy) (H. Taelman, personal communication). The numbers observed in Abidjan are low compared to the 4–7% found in most series in the USA and Europe [8–10], suggesting that PML in Africa may be less common, but is not rare.

In this study immunocytochemical methods confirmed the presence of SV40 in macaque brain tissue in an infection similar to that of JCV in the human PML brain. The MAbs and PCR methods will be ideal for demonstrating SV40 capsid proteins and viral DNA, respectively, in suspected PML lesions of macaque brain in animals with SAIDS. It appears that the relationship of SV40 to SIV in the macaque is analogous to the relationship of JCV to HIV-1 (and HIV-2) in man. The pathogenesis of neither human nor macaque PML is well understood, but the simian disease may provide a model for better understanding the human infection.

In conclusion, this study confirmed that the African agent of PML is JCV, and, in the case of a Gambian dying in Germany, that it represents the type 3 genotype previously found in urine of Tanzanian AIDS patients and African-Americans. Thus, genotyping demonstrates that in this case the likely source of the CNS agent was a latent or persistent virus infection acquired in Gambia, rather than a recent infection by the European type (type 1) in a naive individual.

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