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BREIF REPORT

Primary Polyomavirus Infection, Not Reactivation, as the Cause of Trichodysplasia Spinulosa in Immunocompromised Patients

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Classic human polyomaviruses (JC and BK viruses) become pathogenic when reactivating from latency. For the rare skin disease trichodysplasia spinulosa, we show that manifestations of the causative polyomavirus (TSPyV) occur during primary infection of the immunosuppressed host. High TSPyV loads in blood and cerebrospinal fluid, sometimes coinciding with cerebral lesions and neuroendocrine symptoms, marked the acute phase of trichodysplasia spinulosa, whereas initiation and maturation of TSPyV seroresponses occurred in the convalescent phase. TSPyV genomes lacked the rearrangements typical for reactivating polyomaviruses. These findings demonstrate the clinical importance of primary infection with this rapidly expanding group of human viruses and explain the rarity of some novel polyomavirus-associated diseases.

Keywords. polyomavirus; trichodysplasia spinulosa; primary infection; reactivation; dissemination; viral load; cidofovir.

Human polyomaviruses (HPyVs) are known to cause severe infection and cancer, especially in immunocompromized hosts. The polyomavirus family nowadays includes 11 novel HPyVs, along with the classic polyomaviruses JC (JCPyV) and BK (BKPyV) [1]. JCPyV and BKPyV latently infect the majority of the general population, with increasing adult seroprevalences up to 60% and 90%, respectively [2]. In immunocompromised individuals, they can reactivate and cause severe conditions, such as progressive multifocal leukoencephalopathy (PML), nephropathy, and hemorrhagic cystitis. These reactivations are often accompanied by specific viral genome rearrangements in the noncoding control region (NCCR) [3, 4]. Whether conditions related to novel HPyVs result from reactivation is unknown.

For one of the novel HPyVs, a causal relationship with trichodysplasia spinulosa (TS) has been established. TS is a rare, disfiguring skin disease of severely immunocompromised patients characterized by papules and spines on the face, alopecia of eyebrows and lashes, and thickening of the affected skin layers [5]. The typical lesions show abundant trichodysplasia spinulosa polyomavirus (TSPyV) replication and gene expression in the affected hair follicles [5].

Comparable to JCPyV and BKPyV [2, 6], TSPyV is ubiquitous, with primary infection occurring in childhood and an adult seroprevalence of approximately 75%. Unlike PML and nephropathy, the number of 35 patients with documented TS worldwide is much lower than would be expected based on the number of susceptible, latently infected, immunocompromised hosts. In the Netherlands, for example, only 3 TS cases were identified between 2010 and 2015 [5], as opposed to approximately 250 BKPyV-associated nephropathy cases within the same period [7]. Based on this discrepancy, there is reason to question the assumption that clinically manifest HPyV infections result from reactivation.

By systematically analyzing specific immunological and molecular markers of TSPyV infection in 2 adult immunosuppressed patients, we showed that the signs and symptoms of TS are caused by disseminated primary TSPyV infection accompanied by high viral loads in blood and cerebrospinal fluid (CSF). Our findings provide a logical explanation for the low number of TS cases regardless of the high TSPyV seroprevalence and increase our understanding of the novel HPyVs and their clinical manifestations.

METHODS

TSPyV-specific immunoglobulin (Ig) M, IgG, and IgG avidity measurements were performed using published Luminex and enzyme-linked immunosorbent assay methods [6, 8]. Quantitative polymerase chain reaction (PCR) was used to detect and quantify TSPyV DNA. Additional TSPyV PCR primer sets were designed and used for (direct) sequencing and cloning. Peripheral blood mononuclear cells and lymphocyte subsets were obtained by Ficoll and immunodensity centrifugation. Details regarding the methods are provided in the Supplementary data. Because all materials were obtained for diagnostic purposes, medical ethical approval was not needed, as confirmed by the institutional review board. Both patients consented to the study in writing.

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RESULTS

Case Descriptions

Patient 1, a 54-year-old immunosuppressed man treated for recurrent lymphocytic leukemia and autoimmune hemolytic anemia with chemotherapy and cyclosporin, presented with papular and follicular skin eruptions and spiny keratosis in May 2014 (Figure 1*A*). The lesions that had first been noticed on his face in December 2013 gradually extended to the neck, back, and extremities. Hair loss in his eyebrows and eyelashes was also observed. Histopathological examination showed keratotic masses expanding from the hair follicles, with widened infundibuli and inner follicular layers containing viral inclusions (Figure 1*A*). Plucked spicules and a skin biopsy were positive for TSPyV DNA (approximately 10^5 genome copies per cell; Supplementary Table 1). After diagnosis of TS, topical antiviral cidofovir cream treatment (1%) was administered twice a

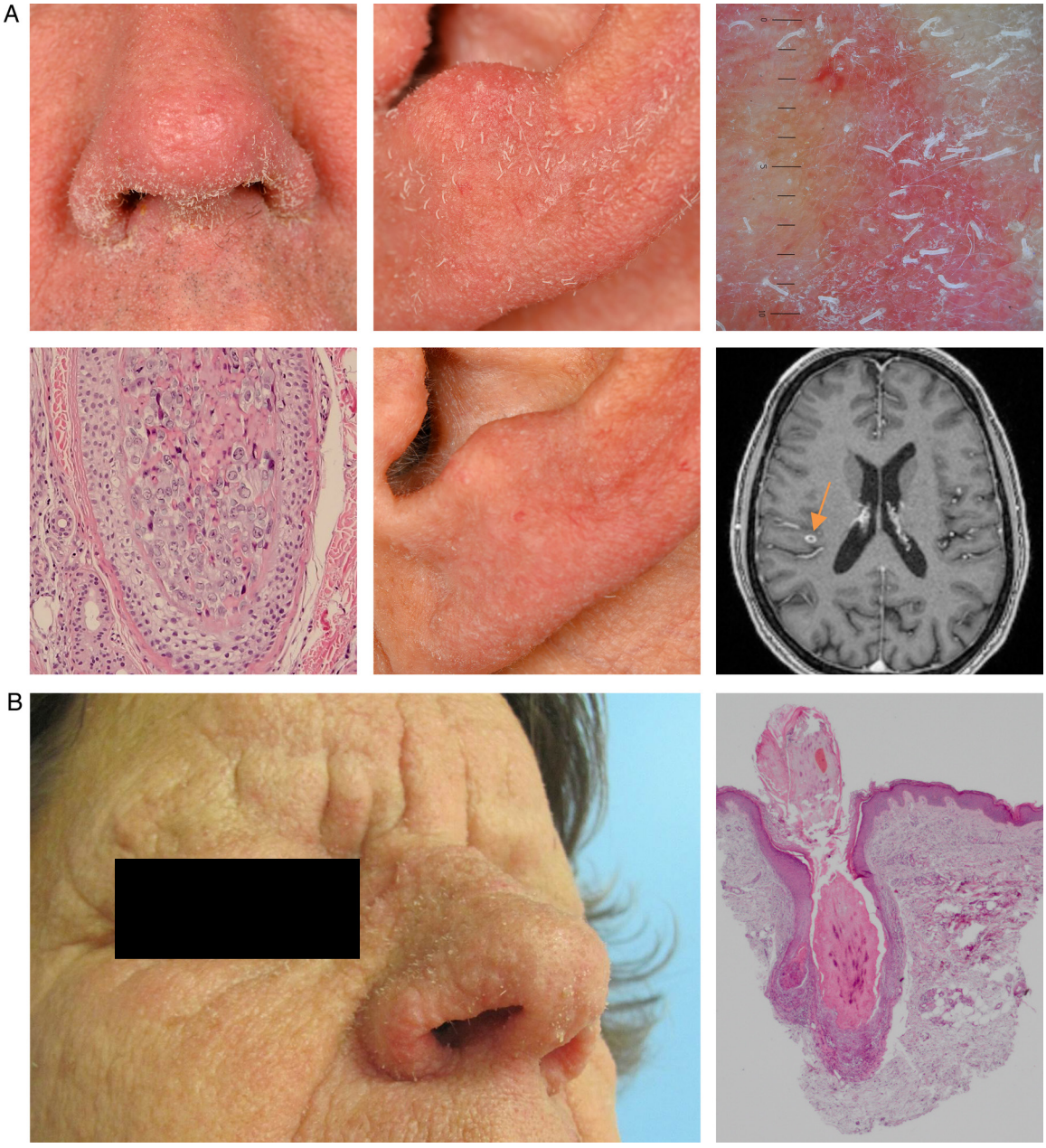

Figure 1. Clinical, histopathological, and radiological characteristics of patients 1 and 2. *A*, Papules and spicules on the nose and left earlobe of patient 1 before (*upper images*) and after (*lower image*) topical 1% cidofovir treatment. Vertical hematoxylin-eosin–stained section (×200) shows a distended hair follicle with disorganized inner root sheath cells with eosinophilic trichohyalin granules and viral inclusions (lower left image). Magnetic resonance image with contrast enhancement of the brain shows one of the cerebral lesions (*arrow*) observed in patient 1 (lower right image). *B*, Papules and spicules on the face of patient 2, accompanied by thickening of the skin and alopecia of the eyelashes and eyebrows. Histopathological examination in patient 2 shows a dilated hair follicle with keratin plugging.

day. Rapid improvement was seen in the patient's face, which was free of lesions within 4 weeks (Figure 1*A*, ear in lower image) and showed a 10^4-fold reduction in TSPyV load (Supplementary Table 1). After 14 weeks of treatment, his remaining skin was also cleared and the treatment was ended. No nephrotoxicity was observed.

By the end of 2014, when he was treated with steroids for relapsing autoimmune hemolytic anemia, he briefly received cidofovir intravenously (5 mg/kg, once per week) in an attempt to reduce systemic viral replication (see below), but no significant antiviral effect was observed. Along with the cutaneous symptoms, in late 2013 the patient had also developed irreversible blindness and papilledema and lost pituitary function, and small cerebral lesions were seen on a magnetic resonance image (Figure 1*A*). Because these lesions proved not to be progressive, cerebral localization of recurrent lymphocytic leukemia was considered unlikely. Alternatively, the patient's condition was assumed to be infectious, possibly toxoplasmosis, and he was treated accordingly. His lesions did not significantly improve with treatment, consistent with negative CSF laboratory findings for *Toxoplasma gondii*.

Patient 2, a 62-year-old woman who had received a kidney transplant in 2009, presented early in 2011 with itchy, follicular, hyperkeratotic skin lesions on her face and scattered over her body (Figure 1*B*). She had first noticed the lesions a few weeks earlier. Histopathological examination showed dilated hair follicles with keratin plugging and sparse perifollicular lymphocyte infiltration (Figure 1*B*). The initial differential diagnosis included a drug eruption, folliculitis, and atypical keratosis pilaris. Tacrolimus, part of the patient's immunosuppressive regimen, was replaced by cyclosporine, and sequential topical treatment with urea cream, adapalene gel and oral minocycline was started, but this did not improve her condition. After histopathological and clinical revision, TS was considered. A skin biopsy specimen was found to be positive for TSPyV DNA by PCR (approximately 10^4 copies per cell; Supplementary Table 1), and antiviral treatment was started with oral valgancyclovir, at a reduced dose of 450 mg once daily owing to impaired renal function. The dosage of the immunosuppressant mycophenolate mofetil was also reduced. After 5 months of treatment, the patient's skin lesions had improved markedly and her itch had resolved.

TSPyV Seroreactivity and Seroconversion

In serum and plasma samples obtained from both patients before, during, and after TS diagnosis, TSPyV-specific IgG seroresponses were detectable only after TS had erupted (Figure 2*A*; Supplementary Table 1). IgG seroresponses against the related Merkel cell polyomavirus and BKPyV were already detectable before TS (Supplementary Table 1), demonstrating the ability of these immunocompromised patients to raise comparable seroresponses. In conjunction with the appearance of TSPyV-specific IgG, a marked increase in TSPyV IgG avidity was noted (Figure 2*A*). Moreover, TSPyV-specific IgM seroresponses were detected that preceded IgG seroconversion and coincided with the first detection of TSPyV DNA in blood in both patients (see below). In addition, in CSF from patient 1, months after TS erupted, TSPyV-specific IgG seroconversion was apparent (Supplementary Table 1).

TSPyV in Blood

Analysis of serum and plasma samples collected about the time of TS diagnosis revealed the presence of high levels of TSPyV DNA (approximately 10^8 copies/mL) in the blood of both patients just before onset of TS symptoms and before seroconversion (Figure 2*A*). TSPyV DNA was also found in other materials (urine, feces, conjunctiva) (Supplementary Table 1). Because some HPyVs are known to infect lymphocytes [9], we analyzed fluorescence-activated cell sorted peripheral B-cell, T-cell, and natural killer cell fractions in patient 1 for the presence of TSPyV DNA. Mutually comparable low levels of TSPyV DNA (<1 copy per cell) were detected, much lower than in serum or plasma, suggesting that none of these cell types preferentially harbored TSPyV (Supplementary Table 1).

TSPyV in CSF

Triggered by the high levels of viremia and the concomitant development of blindness, pituitary failure, and cerebral lesions, the involvement of the central nervous system in TSPyV infection was explored in patient 1. In 2 CSF samples from patient 1, taken 1 year apart, a considerable amount of TSPyV DNA (10^5 copies/mL) was detected (Supplementary Table 1), while other pathogens, including *Toxoplasma gondii*, *Cryptococcus neoformans*, BKPyV, and JCPyV, were not found.

TSPyV Genome Rearrangements

To further explore these highly replicative, primary TSPyV infections, we analyzed the NCCR of various TSPyV isolates from both patients for the presence of specific genome rearrangements reminiscent of reactivating, classic HPyV infection. Besides a small number of 6 single-nucleotide polymorphisms, all isolates obtained from skin, blood, urine, and CSF contained genomes corresponding to the consensus TSPyV genome (Figure 2*B*) [10]. The typical NCCR duplications found in pathogenic JCPyV and BKPyV isolates were not observed, but in both urine and serum of each patient some isolates carried a 30–60-nucleotide NCCR deletion (Figure 2*C*–2*D*).

DISCUSSION

This detailed investigation of 2 TS cases revealed several previously unknown aspects of TSPyV infection, and of HPyV infections in general. The course, intensity, and maturation of the measured TSPyV-specific IgG and IgM seroresponses point to TSPyV primary infection as the cause of TS. Cross-recognition of other HPyVs is unlikely because of the established high specificity of the serological methods used [6, 8]. The level of the measured viral loads is also indicative of primary infection,

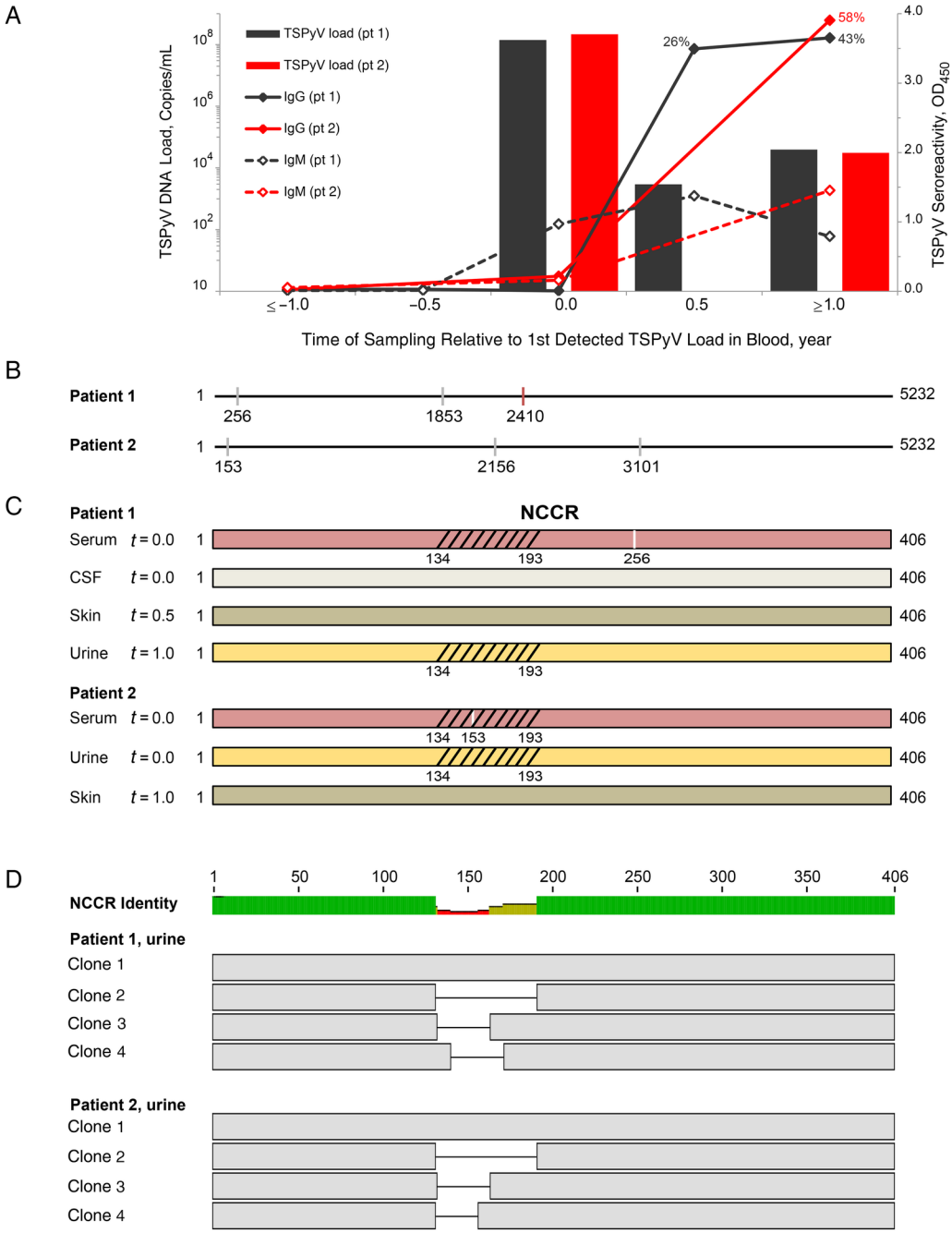

Figure 2. Detection of trichodysplasia spinulosa polyomavirus (TSPyV) DNA, TSPyV seroresponses, and TSPyV genome sequences. *A*, TSPyV DNA loads (*bars*) and seroresponses (*lines*) are shown for patients 1 (*black*) and 2 (*red*). TSPyV immunoglobulin (Ig) G seroresponses are shown in solid lines and closed symbols, IgM seroresponses in dashed lines and open symbols. Time points on x-axis are relative to the first detection of TSPyV DNA in blood samples (viremia). Percentages next to closed IgG symbols represent IgG avidity values. *B*, Linear representation of the circular, 5232 base pair–containing TSPyV genome with the position of each single-nucleotide polymorphism (SNP) compared with the consensus TSPyV sequence [10], indicated by vertical lines for synonymous (gray) and nonsynonymous (red) substitutions). *C*, Sequence analysis of the noncoding control region (NCCR) of TSPyV isolates from serum (*red*), skin (*olive*), urine (*yellow*), and CSF (*white*). Time points (*t*) refer to those on the x-axis in *A*. Vertical lines represent presence of SNP; hatching, stretch of DNA where mixed sequences were found, indicative of a deletion in the area. Numbers refer to nucleotide positions within the NCCR. *D*, Graphic representation of sequenced individually cloned polymerase chain reaction products spanning the NCCR of urine samples from both patients. Numbers refer to nucleotide positions within the NCCR. Apart from full-length, consensus sequence-containing clones (clone 1 of patients 1 and 2) [10], specific deletions were observed between nucleotides 134–193 (clone 2), 135–165 (clone 3), and 143–173 (clone 4) for patient 1, and between nucleotides 134–193 (clone 2), 135–165 (clone 3), and 134–158 (clone 4) for patient 2.

because high viral loads typically accompany primary infection. Although the number of analyzed TS cases in this study is very small, we assume that a primary TSPyV infection underlies development of TS in general. This scenario provides an explanation for the rarity of this disease, with primary TSPyV infection after childhood being rare and coincidence with severe

immunosuppression even rarer. Whether this pattern holds for other rare HPyV-related diseases, for example those caused by HPyV7 and NJPyV [11, 12], deserves further study.

The marked increase in TSPyV IgG seroreactivity after primary infection resembles that of BKPyV, another HPyV with established viremic potential [6]. From findings in immunocompetent children, it is known that low levels of viremia sometimes accompany asymptomatic primary TSPyV infection [8]. Preliminary analysis of a previously described cohort of asymptomatic, mostly TSPyV-seropositive adult kidney transplant recipients, showed the presence of low-level TSPyV viremia in one-third of the subjects, a pattern recently observed by others as well [13]. In all, it seems that TSPyV reactivation may be accompanied by low-level viremia, whereas manifest TS is accompanied by high level viremia resulting from disseminated primary TSPyV infection in a naive immunocompromised host.

Because we hardly detected TSPyV DNA in peripheral lymphocyte populations, these cells do not seem to be potential sites for (latent) TSPyV infection. The course of infection, with viremia preceding development of cutaneous lesions, argues against the skin being the main *porte d'entrée*. Because viral DNA has been detected in tonsils and nasopharyngeal swabs [14], the nasopharynx might play an important role in TSPyV infection.

To our knowledge TSPyV-DNA detection in CSF has not been reported before. We considered the intrathecal presence of large amounts of viral DNA and specific antibodies suggestive of central nervous system involvement in TSPyV infection. Whether the development of the neuroendocrinological signs and cerebral lesions concurrent with TS in patient 1 were causally related to TSPyV infection could not be assessed. A cerebral biopsy was considered too dangerous, and ocular fluid was not available for analysis. It is interesting to note that other novel HPyVs, HPyV6 and NJPyV, were recently found to be associated with (JCPyV-negative) encephalopathy [15] and blindness [12], respectively.

Rearranged NCCRs, in particular those containing the typical duplications seen in JCPyV genomes isolated from CSF samples in patients with PML [3], were not found for TSPyV. However, in both patients, a number of TSPyV isolates from serum and urine samples contained specific NCCR deletions. Whether these findings speak against or in favor of reactivation is unclear, especially because we are unaware of the nature, archetype or rearranged, of the TSPyV genomes reported in the literature [10] and GenBank, which are usually obtained from skin. Because the observed NCCR-deletions might affect transcription factor binding and promoter activity, consequences for viral transcription and replication are worth studying.

In summary, this report provides strong evidence that novel polyomavirus-associated disease is caused by primary infection later in life, in the course of immunosuppression. These findings can explain the rarity of some HPyV-associated diseases and challenge the dogma that HPyV-associated diseases are caused by viral reactivation. Whether determination of HPyV serostatus before immunosuppression could be useful to identify immunosuppressed patients at risk requires further study, as does the potential contribution of TSPyV infection to disease of the central nervous system.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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