MAJOR ARTICLE

High Prevalence of Anelloviruses in Vitreous Fluid of Children With Seasonal Hyperacute Panuveitis

Saskia L. Smits,[1,2] Anu Manandhar,[3] Freek B. van Loenen,[1,4] Marije van Leeuwen,[2] G. Seerp Baarsma,[4,5] Netty Dorrestijn,[4,5] Albert D. M. E. Osterhaus,[1,2] Todd P. Margolis,[6] and Georges M. G. M. Verjans[1]

[1]Department of Virology, and [2]Viroclinics Biosciences B. V., Erasmus Medical Center, Rotterdam, the Netherlands; [3]Tilganga Institute of Ophthalmology, Gaushala, Bagmati Bridge, Kathmandu, Nepal; [4]Rotterdam Eye Hospital, and [5]Rotterdam Ophthalmic Institute, Rotterdam, the Netherlands; and [6]F. I. Proctor Foundation and Department of Ophthalmology, University of California, San Francisco

Seasonal hyperacute panuveitis (SHAPU) is a potentially blinding ocular disease occurring in Nepal that principally affects young children. Random amplification of partially purified vitreous fluid (VF)–derived nucleic acid revealed the presence of human anelloviruses in VF of SHAPU patients. In a comparative study of patients with different ocular pathologies, SHAPU patients were at highest risk of harboring anelloviruses in their eyes. The majority of SHAPU patients had multiple anelloviruses in their VF. The ocular anellovirus load in SHAPU and non-SHAPU patients did not differ and no SHAPU-specific anellovirus variant was detected. Analysis of paired serum and VF samples from SHAPU and non-SHAPU patients showed that the anellovirus detected in VF samples most likely originated from the systemic viral pool during viremia, potentially through breakdown of the blood-ocular barrier. The detection of anelloviruses in VF samples of uveitis patients, profoundly so in SHAPU patients, is imperative and warrants elucidation of its clinical significance.

Seasonal hyperacute panuveitis (SHAPU) is a sight-threatening ocular disease characterized as an acute diffuse fulminant ocular inflammation [1–5]. The disease was first diagnosed in Nepal about 35 years ago and has only been reported in Nepal. SHAPU preferentially affects young children [2, 5], who present with sudden onset of unilateral ocular redness, loss of vision, and leukocoria due to massive exudation in the vitreous cavity [2]. A hypopyon and dense fibrinoid reaction in the anterior chamber are commonly observed [2]. Early therapeutic vitrectomy of SHAPU patients may preserve some vision and anatomical integrity of the globe. Most affected eyes, however, lose all functional vision. SHAPU is commonly reported during the autumn months, with no sex predilection [2, 4]. The etiological cause of SHAPU is unknown. However, SHAPU patients in Nepal relate a recent history of close contact with white moths, whose appearance in a 2-year cycle coincides with the onset of new SHAPU cases [2, 4]. A pathogenic role of white moths in SHAPU has been postulated, but never proven [2].

Uveitis is a heterogeneous group of ocular diseases characterized by inflammation of the uvea either caused by potential autoimmune responses (endogenous uveitis) or initiated by an intraocular infection (exogenous uveitis). The pathology of exogenous uveitis (eg, bacterial endophthalmitis and herpetic uveitis) involves both the cytopathic effect of the infectious agent and a local pathogenic immune response. Endophthalmitis is an acute panuveitis that commonly occurs after intraocular surgery, particularly cataract surgery [6]. Patients present with severe pain and redness of the conjunctiva and episclera and a hypopyon is often observed. Various communal bacteria and fungi are identified as initiating pathogens [6]. Because of similarities in clinical symptoms and incidental detection of bacteria in ocular fluids, SHAPU

Received 26 September 2011; accepted 3 January 2012; electronically published 5 April 2012.

Correspondence: Georges M. G. M. Verjans, MSc, PhD, Department of Virology (Room Ee1720a), Erasmus Medical Center, Dr. Molewaterplein 50, 3015 GE Rotterdam, the Netherlands (g.verjans@erasmusmc.nl).

The Journal of Infectious Diseases 2012;205:1877–84

DOI: 10.1093/infdis/jis284

has also been referred to as seasonal endophthalmitis [2]. However, whereas neutrophils predominate in the pathogenesis of endophthalmitis, the ocular infiltrating cells in SHAPU resemble those of viral uveitis, mainly consisting of activated lymphocytes and macrophages [5,7,8]. Varicella-zoster virus has been detected in aqueous humor of a patient with presumed SHAPU [9].

Infectious diseases, both emerging and reemerging, pose a continuous health threat and disease burden on humanity. Many (re)emerging viruses have been identified in recent years, including human immunodeficiency virus, hepatitis C virus, H5N1 avian influenza A virus, and severe acute respiratory syndrome coronavirus [10]. New technologies have been developed to increase virus identification, such as virus microarrays, sequence-independent amplification, and sequencing of viral nucleic acids, which has already resulted in identification of novel viruses [11]. Yet, our knowledge of viruses that infect humans is still incomplete, and many acute and chronic diseases with unknown etiology may be caused by as yet unidentified viruses.

The aim of the current study was to determine the potential role of a viral infection in SHAPU. Vitreous fluid (VF) samples of SHAPU patients and disease controls were subjected to detailed polymerase chain reaction (PCR) and sequence analyses to detect the presence of viruses in diseased eyes.

MATERIALS AND METHODS

Clinical Specimens

VF samples were obtained from patients with SHAPU (n = 32) and patients suffering from retinal detachment (n = 18). VF samples of patients with endophthalmitis (n = 21) and nonbacterial/fungal infectious uveitis (eg, herpesvirus- or *Toxoplasma gondii*–induced posterior uveitis; n = 29) were included as non-SHAPU infectious controls. All VF samples were surplus specimens collected during diagnostic or therapeutic vitrectomy. The SHAPU VF samples were obtained from patients treated at the Tilganga Institute of Ophthalmology (Kathmandu, Nepal). The remaining VF samples were obtained from patients enrolled at the Rotterdam Eye Hospital (the Netherlands) and the F. I. Proctor Foundation (San Francisco, California). To ensure anonymity, clinical samples were assigned a sample number with no clinical identifiers attached. Additionally, paired serum and VF samples were collected prospectively, with written informed consent obtained from the patients and/or parents at the Tilganga Institute of Ophthalmology from 8 additional SHAPU patients and 8 non-SHAPU posterior uveitis patients (Table 1). All study procedures were performed in compliance with relevant laws and institutional guidelines and in accordance with the ethical standards of the Declaration of Helsinki.

Sequence-Independent RNA and DNA Virus Screening of VF Samples

Nineteen VF samples, obtained from endophthalmitis (n = 9) and SHAPU (n = 10) patients, were available for virus discovery studies using random PCR amplification of nucleic acids [12–15]. Large-scale molecular RNA and DNA virus screening, based on host nucleic acid depletion, sequence-independent amplification, and sequencing of partially purified

Table 1. Patient Characteristics and Anellovirus Prevalence in Paired Serum and Vitreous Fluid Samples of Nepalese Uveitis Patients

Patient ID	Age (y), Sex	Clinical Diagnosis	TTV[a]	TTMDV[a]	TTMV[a]
SV-1	2.5, Female	SHAPU	Pos/Pos	Neg/Neg	Pos/Pos
SV-2	10, Male	SHAPU	Pos/Pos	Pos/Neg	Pos/Pos
SV-3	2, Male	SHAPU	Pos/Pos	Pos/Pos	Pos/Pos
SV-4	1.5, Male	SHAPU	Pos/Pos	Pos/Pos	Pos/Pos
SV-5	7, Female	SHAPU	Pos/Pos	Neg/Pos	Neg/Neg
SV-6	3, Female	SHAPU	Pos/Pos	Pos/Pos	Pos/Pos
SV-7	10, Male	SHAPU	Pos/Pos	Pos/Pos	Pos/Pos
SV-8	2, Male	SHAPU	Pos/Pos	Pos/Pos	Pos/Pos
NV-1	35, Female	Endophthalmitis	Pos/Pos	Neg/Neg	Neg/Pos
NV-2	45, Female	Endophthalmitis	Pos/Pos	Neg/Neg	Neg/Neg
NV-3	74, Female	Endophthalmitis	Pos/Pos	Neg/Pos	Neg/Pos
NV-4	38, Male	Endophthalmitis	Neg/Pos	Neg/Neg	Neg/Pos
NV-5	50, Female	Endophthalmitis	Neg/Pos	Neg/Neg	Neg/Pos
NV-6	45, Female	Idiopathic panuveitis	Neg/Pos	Neg/Pos	Neg/Neg
NV-7	29, Female	Sarcoid uveitis	Neg/Pos	Neg/Pos	Neg/Pos
NV-8	39, Female	Herpetic retinitis	Neg/Pos	Neg/Neg	Neg/Pos

Abbreviations: SHAPU, seasonal hyperacute panuveitis; TTV, torque teno virus; TTMDV, torque teno midi virus; TTMV, torque teno mini virus.

[a]Polymerase chain reaction result for the indicated anelloviruses in paired vitreous fluid (before forward slash) and serum (after forward slash) of the indicated uveitis patient.

VF-derived RNA and DNA, was performed on VF samples diluted 1:4 in phosphate-buffered saline, essentially as described previously [12–15].

Human Torque Teno Virus–Specific PCR Analyses

Total nucleic acid was extracted from an aliquot (~50 μL) of the VF samples using the MagNA Pure LC total nucleic acid isolation kit and the MagNA Pure LC isolation station (Roche), according to the manufacturer's instructions. A highly conserved genomic area located just downstream of the TATA box, corresponding to nucleotides (nt) 99–227 in the prototype torque teno virus (TTV) isolate (TA278), nt 34–170 in the prototype torque teno midi virus (TTMDV) isolate (MD1-073), and nt 178–303 in the prototype torque teno mini virus (TTMV) isolate (CBD231), was amplified using a nested PCR, as described previously [16]. Classification of the anellovirus amplicons into the 3 distinct genera human *Alphatorquevirus*, *Betatorquevirus*, and *Gammatorquevirus* was based on assigning sizes to PCR fragments separated by electrophoresis and detected using a 3130XL genetic analyzer (Applied Biosystems) [17].

A human TTV–specific TaqMan assay was developed using primers NG779, NG780, and NG785 [16] and probe VS594 (5′-CCGAGGGCGGGTGCCG-3′) labeled with 6-carboxy-fluorescein and 6-carboxy-tetramethyl-rhodamine at the 5′ and 3′ ends, respectively. Reactions were carried out in a 25-μL format with 1× TaqMan Universal PCR Master Mix (Roche); 0.25 μL primers NG779 (10 μM), NG780 (10 μM), and NG785 (20 μM); 0.125 μL probe VS594 (20 μM); and 2.5 μL isolated nucleic acid. The conditions used for amplification were 50°C for 2 minutes, followed by 95°C for 10 minutes, and 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 60 seconds on an ABI PRISM 7000 (Applied Biosystems). A cloned TTV amplicon standard was also taken to define the amount of TTV copies/mL VF.

DNA Sequencing and Sequence Analyses

Amplicons were purified using the MSB HTS PCRapace/C kit (Invitek GmbH), according to the manufacturer's instructions. Sequencing was performed on purified amplicons using Big Dye Terminator V3.1 cycle sequencing kit (Applied Biosystems) and a 3130XL or 3700 genetic analyzer (Applied Biosystems). All obtained sequences were screened for homology with known sequences deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank/) using the Basic Local Alignment Search Tool (BLAST) by blastn and tblastx [18]. The analysis of randomly amplified amplicons from VF focused on virus discovery, that is, sequences were of interest when homology to viruses that infect mammals was observed.

Phylogenetic Analysis

Multiple alignments were created using ClustalX (2.0.10) [19]. Phylogenetic analyses were carried out with Molecular Evolutionary Genetics Analysis (MEGA), version 4.1 beta [20], in a manner similar to that used in previous TTV studies [21, 22]. Phylogenetic trees were calculated using neighbor-joining with *p* distance, and bootstrap analysis was performed with 1000 replicates.

Statistical Analyses

Data between different conditions were compared using the Fisher exact test (for binomial data) and Mann–Whitney test (for numeric data). Differences were considered significant at $P < .05$.

RESULTS

Molecular Virus Screening of VF Samples of SHAPU and Endophthalmitis Patients

Nineteen VF samples were obtained from patients with endophthalmitis (n = 9) and SHAPU (n = 10) to determine the potential presence of viral pathogens. The VF-derived RNA and DNA were subjected to random PCR amplification and subsequent sequencing of approximately 80 amplicons (average length = ~350 bp) per VF-derived nucleic acid sample. The analysis focused on identification of viruses infecting mammals. Potential viral nucleic acid sequences were detected in 1 endophthalmitis sample and 2 SHAPU VF samples. An approximately 630-bp amplicon with high homology to human herpesvirus 5 was identified in the endophthalmitis sample. A 424-bp amplicon with homology to a TTMV was identified in 1 of the SHAPU samples; in the second SHAPU sample 17 amplicons (ranging in size from approximately 200 bp to 550 bp) with homology to different parts of the TTMV and TTMDV genomes were identified (data not shown).

TTVs (family Anelloviridae) are small nonenveloped viruses with a circular, single-stranded DNA genome of negative polarity between 2.1 kb and 3.9 kb long. The family Anelloviridae contains 9 genera: *Alpha-*. *Beta-*, *Gamma-*, *Delta-*, *Epsilon-*, *Eta-*, *Iota-*, *Theta-*, and *Zetatorquevirus* [23]. The *Alpha-*, *Beta-*, and *Gammatorquevirus* genera contain anelloviruses that infect humans. TTV infections are commonly acquired during early childhood during which the virus establishes a chronic productive infection with long-lasting detectable viremia [23]. TTVs are endemic worldwide and can be detected in blood and various tissues in the body, including cerebrospinal and bronchioalveolar lavage fluid samples [23]. TTV (genus *Alphatorquevirus*) is classified into at least 29 genotypes with nucleotide divergence of >30%, consisting of 5 major genetic groups (groups 1–5) with a sequence divergence of >50% [23–25]. For TTMV (genus *Betatorquevirus*), highly divergent groups have been proposed [21, 26, 27], and for TTMDV (genus *Gammatorquevirus*), approximately 33% sequence diversity has been observed across full-length genomes [28].

Prevalence of TTV, TTMDV, and TTMV in VF Samples of SHAPU Patients and Disease Controls

To corroborate the finding of TTVs in the VF of SHAPU patients, a nested PCR specific for differential detection of 3 human anelloviruses from the genera *Alpha-*, *Beta-*, and *Gammatorquevirus* was performed on a set of VF samples obtained from SHAPU patients (n = 32) and non-SHAPU infectious disease control patients with endophthalmitis (n = 21) and infectious uveitis (n = 29). VF obtained from patients with retinal detachment (n = 18) was used as noninfectious control samples (Figure 1).

All VF samples from retinal detachment patients were negative for TTV, TTMDV, and TTMV. Among infectious uveitis patients, TTV and TTMV was detected in 5 of 29 (17%) and 3 of 29 (10%) VF samples, whereas TTMDV was not detected. No correlation between the presence of the anelloviruses and the initiating herpesvirus and parasite nor the clinical picture was found (data not shown). Among endophthalmitis patients, 10 of 21 (47%) were positive for TTV, 3 of 21 (14%) were positive for TTMDV, and 6 of 21 (28%) were positive for TTMV. The VF of 30 of 32 SHAPU patients contained either 1 or multiple anelloviruses. The viruses TTV, TTMDV, and TTMV were detected in 29 of 32 (91%), 19 of 32 (59%), and 22 of 32 (69%) SHAPU VF samples. The prevalence of all 3 viruses in VF of SHAPU patients was significantly higher compared with non-SHAPU patients (Figure 1). Notably, 18 of 32 (56%) VF samples of SHAPU patients were positive for all 3 TTVs. Among the non-SHAPU patients, only 2 endophthalmitis cases also had all 3 anelloviruses in their VF sample (data not shown).

Next, we performed real-time PCR analysis to compare the intraocular loads of TTV in SHAPU (n = 21) and non-SHAPU patient (n = 15) samples (Figure 2). The TTV loads were not significantly different between both patient cohorts, with a median viral load of 432 TTV genome equivalent copies per milliliter VF. It should be noted that the TTV-specific real-time PCR assay was less sensitive compared with the nested PCR described above. As such, viral loads by real-time PCR could only be determined in 65% (SHAPU cases) and 45% (non-SHAPU cases) of the initially TTV-positive VF samples (Figure 2 and data not shown).

Phylogenetic Analyses of Ocular-Derived TTV Sequences of SHAPU Patients

To obtain more insight into the presence of the anelloviral genomes in VF samples of SHAPU patients, the genotype of ocular-derived TTV from SHAPU and non-SHAPU patients was determined. Because SHAPU has only been described in Nepal, we hypothesized the existence of a specific SHAPU-inducing TTV variant. Genomes of TTV isolates share low DNA sequence homology [16]. However, a short stretch (~130 bp) of the intergenic nontranslated region is identified as being highly conserved among all human TTV isolates and is used to both demonstrate TTV infections and to genotype clinical isolates [16]. To test our hypothesis, we cloned and sequenced VF-derived amplicons (~8 clones per VF sample) from this particular genomic region from 10 SHAPU patients. Multiple different TTV variants (average 2.3 variants, range 1–5) were observed in 7 of 10 SHAPU samples. A neighbor-joining phylogenetic tree was created using the ocular-derived SHAPU TTV amplicons and corresponding sequences of TTV, TTMDV, and TTMV strains previously deposited in the GenBank database (Figure 3). The SHAPU-associated TTV sequences showed 70%–100% similarity to each other, but they did not belong to the same phylogenetic clade. Moreover, SHAPU TTV sequences from the same sample often did not group together, suggesting that patients are infected with multiple different TTV variants or even genotypes.

TTV Analyses of Paired Serum and VF Samples of Nepalese Patients With Those of SHAPU and Non-SHAPU Patients

The increased TTV prevalence in SHAPU patients may be owing to differences in the geographic origin of the non-SHAPU patients included as disease controls. Whereas the

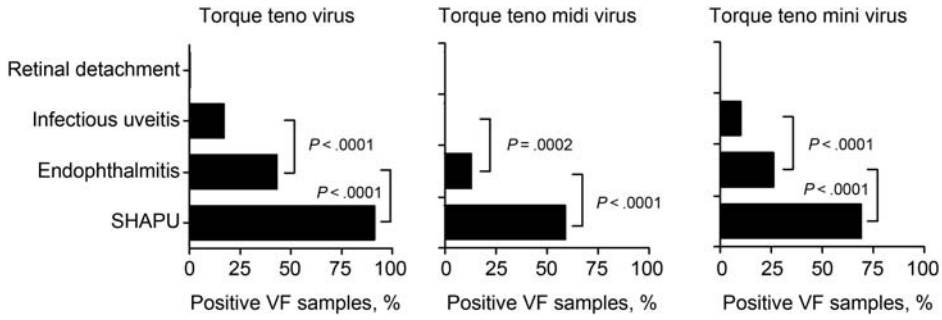

Figure 1. Prevalence of anelloviruses in vitreous fluid (VF) of uveitis patients. The percentage of anellovirus-positive VF samples for torque teno virus (*left panel*), torque teno midi virus (*middle panel*), and torque teno mini virus (*right panel*) for the indicated patients with ocular disease are shown. Fisher exact test was used to compare differences between patient groups. Abbreviation: SHAPU, seasonal hyperacute panuveitis.

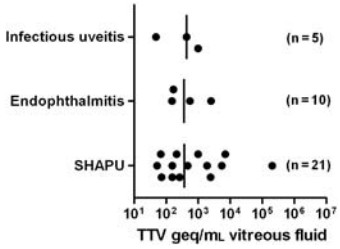

Figure 2. Torque teno virus (TTV) load in vitreous fluid (VF) of uveitis patients. The TTV load in TTV polymerase chain reaction (PCR)–positive VF samples of patients (numbers of patients assayed are shown in parentheses) with the indicated ocular diseases was determined by real-time quantitative PCR (qPCR) and presented as TTV genome equivalent copies per milliliter VF (geq/mL). The horizontal bars indicate the median TTV load. The detection limit of the TTV-specific qPCR assay was 10 geq/mL. Abbreviation: SHAPU, seasonal hyperacute panuveitis.

SHAPU patients were recruited in Nepal, all non-SHAPU patients were either Dutch or US citizens. To investigate this potential confounding factor, paired serum and VF samples of additional SHAPU patients (n = 8) and non-SHAPU patients (n = 8, including 5 endophthalmitis patients) were prospectively collected at the Tilganga Institute of Ophthalmology (Table 1).

The paired serum and VF samples of the Nepalese SHAPU and non-SHAPU patients were analyzed using the nested anellovirus PCR. The prevalence of the 3 anelloviruses in VF from both patient groups was similar to those observed in the first patient cohort analyzed (Figure 1), confirming the high prevalence of anelloviruses in SHAPU VF samples (Table 1). In the serum samples, however, no significant difference in the detection of TTV, TTMDV, and TTMV was observed between the patient groups. The prevalence of anellovirus viremia was very high in both patient groups (Table 1). All patients had TTV, three-quarters had TTMV, and about half had TTMDV viremia at time of sampling. In SHAPU patients, the presence of anelloviruses in the paired VF and serum samples correlated. Except for 1 SHAPU case (patient SV-2), the anellovirus PCR disparate VF/serum pairs consisted always of an anellovirus PCR-negative VF sample in a viremic patient (Table 1).

Ocular inflammation is commonly associated with blood-ocular barrier perturbation, specifically, increased barrier permeability [29]. The matching anellovirus PCR data of the paired VF and serum samples suggest that the anelloviruses detected in VF may have originated from circulating anellovirus in viremic patients. To address this option, we genotyped the VF- and serum-derived TTV pool of 8 SHAPU and 4 non-SHAPU patients. Multiple different TTV variants were detected in the serum (average ~2.6 variants, range 1–5) and VF (average ~3 variants, range 1–5) samples. The same TTV variant was detected in serum and VF of 6 of 8 SHAPU

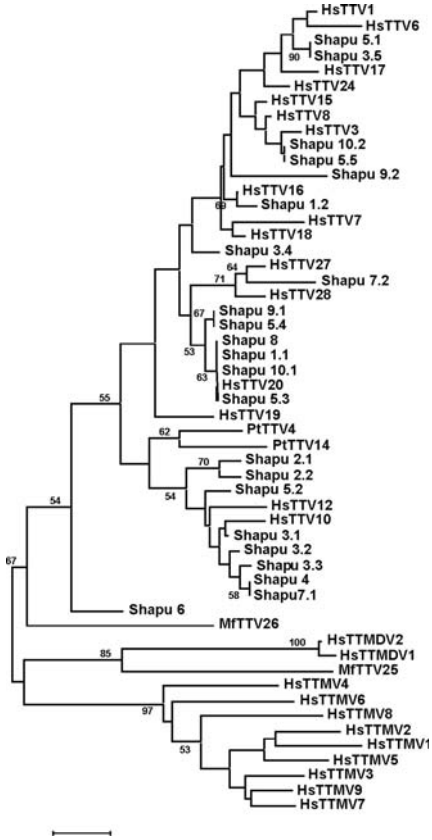

Figure 3. Phylogenetic analysis of vitreous fluid (VF)–derived torque teno virus (TTV) variants from patients with seasonal hyperacute panuveitis (SHAPU). A phylogenetic tree of a fragment (71–76 bp) of the nontranslated region of TTV recovered from VF was generated using MEGA 4.1 with the neighbor-joining method with *p* distance and 1000 bootstrap replicates. Significant bootstrap values are shown. Bar info is 0.05 nucleotide change. HsTTVx, human torque teno virus genotype x; PtTTVx, *Pan troglodytes* torque teno virus genotype x; MfTTVx; *Macaca fuscata* torque teno virus genotype x; HsTTMDVx, human torque teno midi virus genotype x; HsTTMVx, human torque teno mini virus genotype x. Sequences of TTV variants in VF-derived samples from individual SHAPU patients, arbitrarily noted as, eg, SHAPU 4, or, in the case of multiple TTV variants, eg, SHAPU 3.1–3.5, are shown.

patients and 2 of 4 non-SHAPU patients (data not shown), suggesting that the anellovirus detected in the VF samples may have originated from the systemic TTV pool, potentially through breakdown of the blood-retina barrier. Due to the relatively low sensitivity of the TTV TaqMan assay, the TTV load in only 2 paired VF and serum samples could be determined; this was too low to be analyzed statistically (data not shown).

DISCUSSION

The aim of the current study was to determine whether viruses might be causally involved in the potentially blinding

ocular disease SHAPU. Using sequence-independent amplification of nucleic acids and sequencing, the presence of anelloviruses in VF samples from patients with SHAPU was demonstrated. This finding was corroborated by nested PCR assays showing a significantly higher prevalence of the human anelloviruses TTV, TTMDV, and TTMV in eyes of SHAPU patients compared with disease controls. Since the discovery of TTV and related anelloviruses approximately 15 years ago, many investigations have been carried out to unravel their epidemiological, clinical, and pathogenic properties. Anellovirus has been associated with several diseases, including multiple sclerosis, rheumatic diseases, cancer, systemic lupus erythematosis, non-A–E hepatitis, and asthma [30–37]. Herein, we present data showing the presence of anelloviruses in VF of patients with ocular diseases, indicating a novel association between these viruses and uveitis, particularly in SHAPU patients.

Analyses of the clinical parameters and patient characteristics available for review revealed 2 major differences between SHAPU and non-SHAPU patients. SHAPU patients were usually children <10 years of age who underwent a therapeutic vitrectomy commonly within the first week of disease, the treatment of choice to preserve visual acuity in SHAPU patients [1, 2]. Non-SHAPU patients, however, were all adults and were first treated symptomatically using antibiotics, antivirals, and/or corticosteroids. Therapeutic vitrectomy was applied as a second treatment option, usually multiple weeks after onset of disease (data not shown). In herpesvirus- and *T. gondii*–induced infectious uveitis, the intraocular viral and parasitic load is inversely correlated with duration of disease, mainly owing to effectiveness of the local immune response evoked upon intraocular infection [2, 38–42]. Because significant differences in intraocular viral loads in TTV PCR-positive SHAPU and non-SHAPU VF samples were not observed, it is unlikely that disease duration influences the detection of anellovirus. The high incidence of SHAPU in young children contrasts with the increased risk of uveitis with increasing age [43–45]. Children may be more prone to ocular infection with anelloviruses than are adults, as was previously described for other viral diseases such as the 2009 pandemic H1N1 influenza A virus [46].

The absence of anelloviruses in VF samples of retinal detachment patients suggests an association between ocular inflammation and ocular anellovirus infection. Ocular inflammation causes perturbation of the blood-ocular barrier, specifically barrier permeability, in many models of retinal disease [29]. Because anellovirus infections cause a chronic plasma viremia in most infected individuals [23], detection of anelloviruses in VF samples may be the result of passive entry of blood-derived anelloviruses into the vitreous cavity due to breakdown of the blood-retina barrier in uveitis patients. Indeed, most of the SHAPU and non-SHAPU patients with anellovirus-positive VF also had TTV, TTMDV, and/or TTMV viremia. Moreover, the median TTV load in VF was relatively low compared with that previously described in serum of viremic individuals [23]. These observations, in conjunction with the phylogenetic analysis of TTV in the paired serum and VF samples, suggest that the anelloviruses in VF samples of both SHAPU and non-SHAPU patients have most likely originated from the systemic TTV pool upon inflammation-induced disruption of the blood-ocular barrier. However, exogenous sources of the anelloviruses in VF cannot be ruled out. For example, the VF sample of SHAPU patient SV-2 was TTMDV PCR positive in the absence of a detectable TTMDV viremia (Table 1).

In both scenarios, the epidemiological association between epidemics of SHAPU and the seasonal presence of white moths may be a key factor to explain the higher prevalence of anelloviruses in SHAPU patients. Ocular-penetrating caterpillar and tarantula hairs have been shown to induce severe ocular inflammation [47, 48]. Notably, SHAPU patients document close contact with white moths [1, 2, 4], and moth hairs have incidentally been detected in corneas or even in the anterior chamber of SHAPU patients (A. Manandhar, unpublished observation). Cornea-penetrating moth hairs could cause intraocular inflammation, allowing passive entry of anelloviruses into the vitreous cavity from the bloodstream. Alternatively, the ocular-penetrating hairs may be contaminated with anelloviruses, resulting in direct inoculation of the virus into the eye. This scenario, although less likely, is plausible because anelloviruses have also been detected in river water, sewage, and polluted superficial waters worldwide [49, 50].

In conclusion, the data presented demonstrate a high prevalence of TTV, TTMDV, and TTMV in VF samples of SHAPU patients. The novel anellovirus disease association reported here does not prove that these viruses are causally involved in the pathogenesis of uveitis and SHAPU in particular, but it cannot be excluded. Ultimately, experimental infection studies in appropriate animal models are required to determine the pathogenic role of anelloviruses in the development of uveitis.

Notes

Acknowledgments. This work was supported by the European Community's Seventh Framework Programme (FP7/2007–2013) under the project European Management Platform for Emerging and Re-emerging Infectious Disease Entities (EMPERIE; EC grant agreement number 223498) and the Virgo Consortium. A. D. M. E. O. and S. L. S. are part-time chief scientific officer and senior scientist of Viroclinics Biosciences B.V. (Rotterdam, the Netherlands), respectively.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Byanju RN, Pradhan E, Rai SC, Sapkota YD. Visual outcome of vitrectomy in seasonal hyperacute pan uveitis. Kathmandu Univ Med J (KUMJ) **2003**; 1:121–3.
2. Malla OK. Sudden blindness in Nepalese children. Kathmandu Univ Med J (KUMJ) **2005**; 3:4–5.
3. Manandhar A, Paudel G, Rai CK, Rai SK, Gurung R, Ruit S. Seasonal hyper acute pan uveitis—recent scenario in Nepal. Nepal Med Coll J **2008**; 10:196–8.
4. Shrestha E. A profile and treatment outcome of seasonal hyper-acute panuveitis. Nepal J Ophthalmol **2010**; 2:35–8.
5. Upadhyay MP, Rai NC, Ogg JE, Shrestha BR. Seasonal hyperacute panuveitis of unknown etiology. Ann Ophthalmol **1984**; 16:38–44.
6. Callegan MC, Gilmore MS, Gregory M, et al. Bacterial endophthalmitis: therapeutic challenges and host-pathogen interactions. Prog Retin Eye Res **2007**; 26:189–203.
7. Coupland SE. The pathologist's perspective on vitreous opacities. Eye (Lond) **2008**; 22:1318–29.
8. Albert D, Jakobiec F. Principles and practice of ophthalmology. Philadelphia: WB Saunders Company, **1994**:397–405.
9. Kathil P, Biswas J, Gopal L. Demonstration of varicella zoster virus in a case of presumed seasonal hyperacute panuveitis. Indian J Ophthalmol **2005**; 53:270–2.
10. Dong J, Olano JP, McBride JW, Walker DH. Emerging pathogens: challenges and successes of molecular diagnostics. J Mol Diagn **2008**; 10:185–97.
11. Delwart EL. Viral metagenomics. Rev Med Virol **2007**; 17:115–31.
12. Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci U S A **2005**; 102:12891–6.
13. Smits SL, van Leeuwen M, Kuiken T, Hammer AS, Simon JH, Osterhaus AD. Identification and characterization of deer astroviruses. J Gen Virol **2010**; 91:2719–22.
14. Smits SL, van Leeuwen M, van der Eijk AA, et al. Human astrovirus infection in a patient with new-onset celiac disease. J Clin Microbiol **2010**; 48:3416–8.
15. van Leeuwen M, Williams MM, Koraka P, Simon JH, Smits SL, Osterhaus AD. Human picobirnaviruses identified by molecular screening of diarrhea samples. J Clin Microbiol **2010**; 48:1787–94.
16. Ninomiya M, Takahashi M, Nishizawa T, Shimosegawa T, Okamoto H. Development of PCR assays with nested primers specific for differential detection of three human anelloviruses and early acquisition of dual or triple infection during infancy. J Clin Microbiol **2008**; 46: 507–14.
17. Duan R, de Vries RD, van Dun JM, et al. Acyclovir susceptibility and genetic characteristics of sequential herpes simplex virus type 1 corneal isolates from patients with recurrent herpetic keratitis. J Infect Dis **2009**; 200:1402–14.
18. Altschul SF, Madden TL, Schaffer AA, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res **1997**; 25:3389–402.
19. Larkin MA, Blackshields G, Brown NP, et al. Clustal W and Clustal X version 2.0. Bioinformatics **2007**; 23:2947–8.
20. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol **2007**; 24:1596–9.
21. Biagini P, Uch R, Belhouchet M, et al. Circular genomes related to anelloviruses identified in human and animal samples by using a combined rolling-circle amplification/sequence-independent single primer amplification approach. J Gen Virol **2007**; 88:2696–701.
22. Okamoto H, Takahashi M, Nishizawa T, et al. Genomic characterization of TT viruses (TTVs) in pigs, cats and dogs and their relatedness with species-specific TTVs in primates and tupaias. J Gen Virol **2002**; 83:1291–7.
23. Maggi F, Bendinelli M. Human anelloviruses and the central nervous system. Rev Med Virol **2010**; 20:392–407.
24. Hijikata M, Takahashi K, Mishiro S. Complete circular DNA genome of a TT virus variant (isolate name SANBAN) and 44 partial ORF2 sequences implicating a great degree of diversity beyond genotypes. Virology **1999**; 260:17–22.
25. Peng YH, Nishizawa T, Takahashi M, Ishikawa T, Yoshikawa A, Okamoto H. Analysis of the entire genomes of thirteen TT virus variants classifiable into the fourth and fifth genetic groups, isolated from viremic infants. Arch Virol **2002**; 147:21–41.
26. Biagini P, Gallian P, Attoui H, et al. Genetic analysis of full-length genomes and subgenomic sequences of TT virus-like mini virus human isolates. J Gen Virol **2001**; 82:379–83.
27. Takahashi K, Hijikata M, Samokhvalov EI, Mishiro S. Full or near full length nucleotide sequences of TT virus variants (types SANBAN and YONBAN) and the TT virus-like mini virus. Intervirology **2000**; 43:119–23.
28. Ninomiya M, Takahashi M, Shimosegawa T, Okamoto H. Analysis of the entire genomes of fifteen torque teno midi virus variants classifiable into a third group of genus Anellovirus. Arch Virol **2007**; 152:1961–75.
29. Chang JH, McCluskey PJ, Wakefield D. Toll-like receptors in ocular immunity and the immunopathogenesis of inflammatory eye disease. Br J Ophthalmol **2006**; 90:103–8.
30. Bando M, Takahashi M, Ohno S, et al. Torque teno virus DNA titre elevated in idiopathic pulmonary fibrosis with primary lung cancer. Respirology **2008**; 13:263–9.
31. Figueiredo CP, Franz-Vasconcelos HC, Giunta G, et al. Detection of torque teno virus in Epstein-Barr virus positive and negative lymph nodes of patients with Hodgkin lymphoma. Leuk Lymphoma **2007**; 48:731–5.
32. Gergely P Jr, Blazsek A, Danko K, Ponyi A, Poor G. Detection of TT virus in patients with idiopathic inflammatory myopathies. Ann N Y Acad Sci **2005**; 1050:304–13.
33. Maggi F, Pifferi M, Fornai C, et al. TT virus in the nasal secretions of children with acute respiratory diseases: relations to viremia and disease severity. J Virol **2003**; 77:2418–25.
34. Nishizawa T, Okamoto H, Konishi K, Yoshizawa H, Miyakawa Y, Mayumi M. A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology. Biochem Biophys Res Commun **1997**; 241:92–7.
35. Pifferi M, Maggi F, Andreoli E, et al. Associations between nasal torque tenovirus load and spirometric indices in children with asthma. J Infect Dis **2005**; 192:1141–8.
36. Umemura T, Alter HJ, Tanaka E, et al. Association between SEN virus infection and hepatitis C in Japan. J Infect Dis **2001**; 184:1246–51.
37. zur Hausen H, de Villiers EM. TT viruses: oncogenic or tumor-suppressive properties? Curr Top Microbiol Immunol **2009**; 331:109–16.
38. De Groot-Mijnes JD, Rothova A, Van Loon AM, et al. Polymerase chain reaction and Goldmann-Witmer coefficient analysis are complimentary for the diagnosis of infectious uveitis. Am J Ophthalmol **2006**; 141:313–8.
39. Garweg JG, De Groot-Mijnes JD, Montoya JG. Diagnostic approach to ocular toxoplasmosis. Ocul Immunol Inflamm **2011**; 19:255–61.
40. Klaren VN, Peek R. Evidence for a compartmentalized B cell response as characterized by IgG epitope specificity in human ocular toxoplasmosis. J Immunol **2001**; 167:6263–9.
41. Milikan JC, Kuijpers RW, Baarsma GS, Osterhaus AD, Verjans GM. Characterization of the varicella zoster virus (VZV)-specific intraocular T-cell response in patients with VZV-induced uveitis. Exp Eye Res **2006**; 83:69–75.
42. Peek R, Verjans GM, Meek B. Herpes simplex virus infection of the human eye induces a compartmentalized virus-specific B cell response. J Infect Dis **2002**; 186:1539–46.
43. Baatz H, Scharioth GB, de Ortueta D, Pavlidis M. Age distribution of patients presenting with uveitis. Open Ophthalmol J **2007**; 1:23–4.

44. Edelsten C, Reddy MA, Stanford MR, Graham EM. Visual loss associated with pediatric uveitis in English primary and referral centers. Am J Ophthalmol **2003**; 135:676–80.
45. Gritz DC, Wong IG. Incidence and prevalence of uveitis in northern California; the Northern California Epidemiology of Uveitis Study. Ophthalmology **2004**; 111:491–500.
46. Girard MP, Tam JS, Assossou OM, Kieny MP. The 2009 A (H1N1) influenza virus pandemic: a review. Vaccine **2010**; 28:4895–902.
47. Conrath J, Hadjadj E, Balansard B, Ridings B. Caterpillar setae-induced acute anterior uveitis: a case report. Am J Ophthalmol **2000**; 130:841–3.
48. Sengupta S, Reddy PR, Gyatsho J, Ravindran RD, Thiruvengadakrishnan K, Vaidee V. Risk factors for intraocular penetration of caterpillar hair in ophthalmia nodosa: a retrospective analysis. Indian J Ophthalmol **2010**; 58:540–3.
49. Diniz-Mendes L, Paula VS, Luz SL, Niel C. High prevalence of human torque teno virus in streams crossing the city of Manaus, Brazilian Amazon. J Appl Microbiol **2008**; 105:51–8.
50. Haramoto E, Kitajima M, Katayama H, Ohgaki S. Real-time PCR detection of adenoviruses, polyomaviruses, and torque teno viruses in river water in Japan. Water Res **2010**; 44:1747–52.