Necrotizing Scleritis, Conjunctivitis, and Other Pathologic Findings in the Left Eye and Brain of an Ebola Virus–Infected Rhesus Macaque (Macaca mulatta) With Apparent Recovery and a Delayed Time of Death

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A 3.5-year-old adult female rhesus macaque (Macaca mulatta) manifested swelling of the left upper eyelid and conjunctiva and a decline in clinical condition 18 days following intramuscular challenge with Ebola virus (EBOV; Kikwit-1995), after apparent clinical recovery. Histologic lesions with strong EBOV antigen staining were noted in the left eye (scleritis, conjunctivitis, and peri-optic neuritis), brain (choriomeningoencephalitis), stomach, proximal duodenum, and pancreas. Spleen, liver, and adrenal glands, common targets for acute infection, appeared histologically normal with no evidence of EBOV immunoreactivity. These findings may provide important insight for understanding sequelae seen in West African survivors of Ebola virus disease.

Keywords. Ebola virus disease; Ebola virus; sequelae; macaque; rhesus; intramuscular; delayed death; eye; brain.

The current West Africa Ebola virus disease (EVD) epidemic was declared by the World Health Organization on 8 August 2014 as a Public Health Emergency of International Concern, as defined under the International Health Regulations [1]. Although disease incidence has recently declined dramatically, the extent of chronic clinical and pathologic manifestations in survivors is yet to be determined. Postinfection sequelae have previously been reported for filoviruses and include skin sloughing, hair loss, myalgias, parotitis, orchitis, hearing loss, and pericarditis [2, 3]. Uveitis and vision loss have been reported in the media during this outbreak [4], and other ophthalmologic and neurologic signs (convulsions, meningitis, psychotic behavior, and neck stiffness) have been described in survivors of previous outbreaks [2, 5–7]. Clinicians at Emory University (Atlanta, Georgia) recently reported uveitis and culturing of virus from the eye 9 weeks after clearance of viremia in a severely ill patient [8]. While the macaque EVD model is nearly universally lethal, understanding sequelae in rare survivors may improve understanding of disease sequelae in humans. We found only 1 published report describing pathologic findings in nonhuman primates associated with a delayed death after EBOV challenge [9]. Here, we describe pathologic changes, with particular focus on the eye and brain, in a nontreated (je, vehicle control recipient) rhesus macaque (Macaca mulatta) that developed eye swelling after surviving acute EVD.

MATERIALS AND METHODS

A 3.5-year-old female rhesus macaque was presented to the Pathology Division of the US Army Medical Research Institute of Infectious Diseases (Fort Detrick, Maryland) for a full postmortem examination after it was euthanized because of a declining clinical condition and left eye swelling 18 days after EBOV challenge. This animal was Rhe #7 in a report previously published on EBOV-related physiological changes in 9 animals [10]. Briefly, 9 adult rhesus macaques (6 males and 3 females) were challenged intramuscularly with approximately 50 plaque-forming units (PFU) of Ebola virus H.sapiens-tc/COD1995/Kikwit-9510621 (EBOV/Kik) in a 1-mL suspension. Six of the animals displayed typical courses of disease and met end point euthanasia criteria 7–10 days after infection [11, 12]. Two animals survived acute infection with no apparent sequelae prior to being euthanized.

Rhe #7 came from a commercial primate vendor. Prior to assignment for study, physical examinations and routine serologic screenings with biochemical analyses demonstrated no abnormalities. An Integrated Telemetry Systems Model T27 telemetry device (Konigsberg Instruments) was surgically implanted for continuous physiological monitoring, including placement of pressure transducers in the left ventricle, left pleural space, and descending aorta, as well as a subcutaneous electrocardiogram lead in the left anterior chest wall. Following surgical recovery, a central venous catheter was implanted for daily collection of a blood specimen while the animal was awake [10].

Blood specimens collected via the central venous catheter underwent virologic, hematologic, and chemical analyses. Complete blood counts with differential were conducted using the HemaVet 950FS (Drew Scientific, Waterbury, Connecticut), and serum chemical analysis was performed using the Piccolo General Chemistry 13 panel and Piccolo xPress analyzer (Abaxis, Union City, California). Virologic analysis was performed as

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previously described [12]. Full necropsy was performed in a biosafety level 4 necropsy suite, and a complete set of tissue specimens were collected and processed for histopathologic investigation. Tissue specimens were stained with hematoxylin and eosin, and select tissues (eye, brain, liver, spleen, lymph nodes, stomach, small intestine, and pancreas) were evaluated for EBOV antigen presence by immunohistochemical analysis as previously described [12].

RESULTS

Clinical Pathologic Findings

This animal exhibited pathophysiologic changes (temperature, respiratory rate, blood pressure, and pulse) more similar to those of the other 2 surviving animals, with a less pronounced decline in systolic pressure and increase in pulse than the animals that died during the normal time to death. Viremia was initially detected for all 9 animals between days 4 and 6 after infection. Levels for this animal and the 2 other survivors were lower than those for the nonsurvivors (<10^5 vs >10^5 PFU equivalents/mL plasma). Additionally, quantitative reverse transcription–polymerase chain reaction analysis on day 18 revealed that the circulating levels of EBOV RNA were below the limit of detection.

Severe lymphopenia occurred between days 3 and 7, with an 85% decrease in lymphocytes. The mean white blood cell (WBC) count on day 6 was approximately 1.3 times greater than baseline, with a concomitant 3-fold increase in neutrophils. Mild leukocytosis at day 6 (11.2 × 10^3 cells/µL) was followed by a steady decline in the WBC count to a value on day 10 (4.7 × 10^3 cells/µL) that was half the baseline value. By day 18, this animal developed a strong leukocytic response with concomitant neutrophilia and lymphocytosis. There was slight increase from baseline platelet values (374 × 10^3 platelets/µL) between days 0 and 3, followed by profound thrombocytopenia between days 6 and 10 (89 × 10^3 platelets/µL). By day 18, platelets had rebounded and surpassed preinfection levels (422 × 10^3 platelets/µL).

Serum levels of liver enzymes (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase

Figure 1. A, The left eye of Rhe #7 on day 18 after Ebola virus (EBOV) challenge. EBOV immunohistochemical staining revealed scattered EBOV-immunoreactive mononuclear cells (most likely of histiocytic or dendritic origin) and spindled cells (the morphology is suggestive of fibroblastic reticular cells) are present in the conjunctival subepithelium. The inset reveals mild subepithelial edema in the palpebral conjunctiva with minimal mononuclear inflammation (conjunctivitis). B, The optic nerve (ON) of the left eye of Rhe #7 on day 18 after challenge. Hematoxylin-eosin staining reveals marked thickening of and inflammation in the pia and dura mater (asterisk) surrounding the ON (peri-neuritis). The pigmented choroid layer remains intact with no evidence of inflammation; the retina (arrow) has been iatrogenically separated from the pigmented choroid during processing. C, The sclera of the left eye of Rhe #7 on day 18 after challenge. Hematoxylin-eosin staining reveals significant scleral thickening by inflammation (asterisk) that extends into the adjacent peri-ocular adipose tissue (necrotizing scleritis and steatitis). The pigmented choroid layer (arrow), not affected by inflammation, has been iatrogenically separated from the underlying sclera during processing. Also, the retina was iatrogenically removed during processing and is not present. The inset shows intense, mostly non-cell-associated EBOV immunoreactivity in the affected sclera. D, The choroid plexus of the brain of Rhe #7 on day 18 after challenge. Hematoxylin-eosin staining reveals numerous lymphocytes and plasma cells, fewer macrophages, and hemorrhage expanding the choroid plexus. The inset shows free viral antigen and EBOV-positive mononuclear cells (likely of histiocytic origin) in affected areas.
By day 18, levels of all liver enzymes had returned to normal.

**Gross Necropsy Findings**

At postmortem examination, there was left upper eyelid and conjunctiva (chemosis) swelling with no gross evidence of subconjunctival hemorrhage. The left globe had retracted slightly into the orbital cavity but appeared grossly normal. There was no evidence of the petechial rash that had been reported during days 9–11 after inoculation [11, 12]. Fibrous strands adhered to and between the pleural surfaces of the left superior and inferior lung lobes, the thoracic cavity wall, and the pericardial sac. All lung lobes had a normal size and consistency but were congested multifocally. Axillary and mesenteric lymph nodes were mildly enlarged and edematous bilaterally. All other lymph nodes appeared grossly normal. The spleen was moderately enlarged and turgid. Bilateral renal cortices were pale. Scattered petechial hemorrhages were present on the urinary bladder mucosa. Findings for the remaining organ systems, specifically the liver, a major EBOV target, were unremarkable.

**Histopathologic, Immunohistochemical, and Histochemical Findings**

The most noteworthy changes were present in the eye, brain, stomach, and pancreas. Conjunctival subepithelial connective tissue of the left eye (but not the right eye) was expanded by edema and infiltrated by increased numbers of neutrophils and macrophages. Immunoreactive fibroblast-like cells and mononuclear cells, suggestive of histiocytic origin, were easily recognized (Figure 1). Neutrophilic and necrotizing scleritis, peri-optic neuritis, and steatitis (Figure 1) were observed in the left eye but not the right eye. Strong EBOV immunostaining, cell and noncell associated, was associated with the lesions. Despite the significant scleral and peri-optic nerve changes, findings for the choroid layer and retina appeared to be unremarkable. Multiple sections of brain tissue specimens (from the frontal cortex, corpus striatum, thalamus, mesencephalon,pons, cerebellum, and medulla oblongata) were evaluated. Although varying in severity, microscopic lesions were observed and consisted of 1 or more of the following: neutrophilic and lymphoplasmacytic to necrotizing choriomeningoencephalitis (Figure 1), perivascular cuffing, glial inflammatory foci (glial nodules), and hemorrhage. Non–cell-associated EBOV immunoreactivity was common particularly in areas of necrosis, but perivascular mononuclear cells and ependymal cells lining the choroidplexus were occasionally EBOV positive. Neutrophilic gastroenteritis with apoptotic-like and necrotic cellular debris (primarily affecting villar tips of the pyloric stomach and proximal duodenum) and lymphocytic and plasmacytic and necrotizing pancreatitis were also confirmed by immunohistochemical analysis.

Rare histologic lesions were present in all lymph nodes except the axillary nodes and included follicular lymphocytosis and few tingible body macrophages. When EBOV antigen was present, immunoreactivity was seen only in lymph nodes with affected germinal centers and in marginal zones. Besides hyalized follicles, the spleen appeared histologically normal without evidence of EBOV immunoreactivity. Histologic lesions were not seen in any other hematopoietic tissues or the liver and adrenal glands, both of which are early EBOV targets. The liver was diffusely negative for EBOV by immunohistochemical investigation; viral antigen detection was not performed on the adrenal gland. Focal bladder mucosal hemorrhage was confirmed histologically. Mild lymphocytic and histiocytic pericarditis, left ventricular epicarditis, pleuritis, and subpleural interstitial inflammation were also observed.

Lillie-Twort Gram staining and periodic acid–Schiff staining were negative for bacteria and fungi in specimens of select tissues (ie, eye, brain, pancreas, stomach, and small intestine).

**DISCUSSION**

We report the clinical ophthalmologic manifestations, with supporting pathologic, macroscopic, and microscopic findings and immunohistochemical results, in an untreated rhesus macaque with recovery and delayed death following EBOV infection. Clinical abnormalities, including cutaneous maculopapular rash, viremia, lymphopenia, thrombocytopenia, and elevated liver enzyme levels are clear indications of acute EBOV infection and consistent with findings in the nonhuman primate model [9, 11, 12]. This case is noteworthy because of the resolution of typical clinical pathologic changes and the development of atypical manifestations and pathologic lesions following apparent recovery.

From the limited reports available, conjunctival injection, excess lacrimation, loss of vision, uveitis, and conjunctival hemorrhage have all been anecdotally observed, but the pathophysiology of these is unknown [6, 7]. Late EBOV-induced ocular changes, such as conjunctivitis with hemorrhage and anterior and posterior uveitis, have been described in human survivors based primarily on bio-slit lamp microscopy but not on ocular histopathologic findings [5–7], which is why this report is important. A recent case report noted panuveitis and isolation of EBOV from the anterior chamber of a survivor 9 weeks following clearance of viremia after severe illness [8]. Interestingly, early conjunctivitis and postinfection uveitis have also been noted in cases of Marburg virus infection [3, 13, 14], with postrecovery culturing of Marburg virus from the anterior chamber [13]. This is consistent with our observations and suggests that this phenomenon may be a
filovirus phenomenon and a result of viral activity and not solely due to immunologic responses.

In an EBOV therapeutic study, Larsen [9] described atypical pathologic lesions in the brain, but not the choroid plexus, and the pancreas in 6 EBOV-challenged rhesus macaques with a delayed time to death (mean, 21.7 days prior to dying from infection or being euthanized in extremis). Larsen also described EBOV immunoreactivity in the corneal epithelium, stroma, and endothelium but, unlike us, did not observe extensive scleritis, peri-optic neuritis, and ocular steatitis. Additional differences between the animals described by Larsen and our rhesus macaque include (1) the challenge dose (1000 PFU vs 50 PFU in this case), (2) the absence of clinical ocular manifestations (chemosis and conjunctivitis) despite the presence of histopathologic lesions and EBOV antigen, and (3) the use of candidate antiviral therapeutics in Larsen’s report versus an untreated/vehicle control (ie, saline for catheter patency) in our case.

Although the obvious sequelae after apparent recovery occurred in the eye and brain, gastroenteritis and necrotizing pancreatitis were also confirmed by immunohistochemical analysis. Histologic lesions were absent in the liver, adrenal gland, and spleen (all targets for EBOV infection) and were rare in lymph nodes. Additionally, mild pericarditis, left ventricular epicarditis, pleuritis, and subpleural interstitial inflammation were observed. Although interstitial pneumonia has been associated with EBOV infection [9], the pleural and subpleural locations here, coupled with epicarditis, indicate a physiologic response to the telemetry transducers, which were Surgically implanted to monitor cardiovascular function [10].

The significance and implications of EBOV and EBOV antigens in nonlymphoid targets has been under significant debate. Multiple theories have been suggested, including inability of the virus to replicate effectively in the cells of these organs, as well as adaptation or selection of the virus to these cell types over time. Perhaps the more likely explanation is that the development of an anti-EBOV immune response drives the virus into or allows the virus to remain only in immune privileged or semiprivileged sites [8, 9]. As the host or victim recovers, they eventually clear the virus. If their immune response falters, there is a rebound of the virus and, in the case of nonhuman primates, spilling of the virus from these sites. In the case reported here, the nonhuman primate was euthanized on the basis of the attending veterinarian’s assessment of the ocular lesion, combined with the difficulty of treating the animal in a biosafety level 4 environment. It is unclear whether this animal would have eventually cleared the virus or progressed to a more significant disease state. Regardless, the observations and findings reported in this article provide the first glimpse of the pathology of several major organs, especially the left eye and brain, after apparent recovery from EBOV infection without pharmacologic treatment. These findings provide an important first insight into the etiology and pathology of some of the sequelae now being reported in EBOV survivors in West Africa.

Notes

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