Short Report: Potential for Autoimmune Pathogenesis of Rift Valley Fever Virus Retinitis

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Abstract. Rift Valley Fever (RVF) is a significant threat to human health because it can progress to retinitis, encephalitis, and hemorrhagic fever. The timing of onset of Rift Valley Fever virus (RVFV) retinitis suggests an autoimmune origin. To determine whether RVFV retinitis is associated with increased levels of IgG against retinal tissue, we measured and compared levels of IgG against healthy human eye tissue by immunohistochemical analysis. We found that serum samples from RVFV-exposed Kenyans with retinitis (n = 8) were slightly more likely to have antibodies against retinal tissue than control populations, but the correlation was not statistically significant. Further investigation into the possible immune pathogenesis of RVFV retinitis could lead to improved therapies to prevent or treat this severe complication.

Rift Valley Fever virus (RVFV) is a an emerging member of the family *Bunyaviridae* and genus *Phlebovirus* that is transmitted in sub-Saharan Africa, Egypt and the Arabian Peninsula.[1,2] Transmission is most frequently detected when large epizootic/epidemic outbreaks occur after periods of unusually heavy rainfall. As such, the virus poses a threat to economic stability as well as to human health. Recent outbreaks have been documented in Saudi Arabia (2000) and the Horn of Africa (2006–2007).[3–9]

The most common severe complication of human RVFV infection is retinitis. Retinal complications occur in up to 10% of those affected and can cause lasting loss of vision.[6,10–12] Other severe complications can include encephalitis (8%), hepato-renal failure,[8] hepatitis,[10] and hemorrhagic fever (1%).[11,13] Case-fatality rates have been reported as high as 31%, but the actual value is likely between 0.5% and 2% because a small proportion of infected persons are diagnosed,[11,13] especially those with milder disease.

The onset of RVFV retinitis in humans occurs 5–14 days (mean = 8.8 days) after initial symptoms,[4] which coincides with the activation of the adaptive immune response.[14] For the present study, we hypothesized that ocular complications of humans could be triggered by an antibody-mediated autoimmune reaction.

To explore part of this hypothetical pathway, we used immunohistochemical (IHC) analysis of normal eye tissue to compare serum levels of IgG against retinal tissue among persons with and without RVFV retinitis. Serum samples were collected during household-based cluster surveys of residents of Masalani (2008)[5] and Sangailu (2011), North East Province, Kenya, where a RVF epidemic had occurred in 2006–2007. The persons participated in a formal interview regarding demographics, potential viral exposures, their recent symptoms, and their vision. Complete physical examinations with vision testing and indirect ophthalmoscopic examination were performed to identify previous or current retinal inflammation.

Exposure to RVFV was determined by screening serum for IgG against RVFV by enzyme-linked immunosorbent assay and confirmation with 80% plaque reduction neutralization testing as previously detailed.[5,6] Four groups were available for comparison: 1) persons with retinitis after exposure to RVFV (n = 8); 2) persons without retinitis after exposure to RVFV (n = 9); 3) persons with retinitis and no exposure to RVFV (n = 9); and 4) persons without retinitis and exposure to RVFV (n = 17). This study was approved by the institutional review boards at Case Western Reserve University and the Kenya Medical Research Institute.

For IHC analysis, healthy human eyes from a deceased Caucasian donor were preserved in paraformaldehyde, fixed in paraffin, and 4-μm sections were placed on glass slides. The slides were deparaffinzied in xylene, serially rehydrated in 100%, 95%, and 80% ethanol, and then set in Diva decloaking solution (Biocare Medical, Concord, CA). Potentially hidden epitopes were identified in a decloaking chamber heated to 125°C for 10 seconds, followed by 90°C for 30 seconds. Slides were rinsed and blocked for 30 minutes with 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) before incubation with human sera diluted 1:50 in BSA-PBS for 30 minutes at room temperature. The antibody dilution was optimized to that which gave the best consistently reproducible discrimination among the specimen panel, with mouse anti-human retinal S antigen (S-arrestin) (Abcam, Cambridge, UK) diluted 1:100 used as a positive control. The slides were rinsed three times with PBS to remove unbound human serum antibodies. Secondary horseradish peroxidase–conjugated chicken anti-human IgG, diluted 1:500 in BSA-PBS, was added for 30 minutes. Excess secondary antibody was removed with PBS rinses, and horseradish peroxidase substrate added for approximately 10 minutes. The slides were washed and stained with CAT hematoxylin, then preserved with crystal mount and a coverslip. All samples were tested in duplicate.

Slides were evaluated for retinal staining by two masked observers, who scored the slides from 0 (no staining) to 4 (dark). Each serum sample was tested at least twice, and the four scores were averaged to produce a final score for each study participant. If scores for a given serum sample from different slides or observers diverged by more than one point, the test was repeated. Samples with an average score of two or more were considered positive for antibodies against retinal tissue. The proportion of positive samples in each group was compared in a pairwise fashion by using a two-tail Fisher's exact test. Serum samples from 9 of 43 persons tested were positive Table 1.

The greatest proportion of positive scores was among persons with retinitis and RVFV exposure; four of eight had

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TABLE 1
Sample and final score for autoimmune pathogenesis of Rift Valley Fever virus retinitis, Kenya*

Study group	Village	No.	No. tested	No. positive	Immunohistochemical scores	Group average
RVFV retinitis	Masalani	5	8	4	0.6, 0.7, **2, 2.16, 2.33**	1.45
	Sangailu	3			0.83, 1, **2**	
RVFV unexposed with retinitis	Masalani	6	9	1	0, 0.5, 1.25, 1.33, 1.75, 1.75	1.24
	Sangailu	3			0.14, 1.6, **3**	
RVFV exposed without retinitis	Masalani	6	9	1	0, 0, 0.17, 0.5, 1, 1.25	0.67
	Sangailu	3			0, 0.75, **2.33**	
RVFV unexposed without retinitis	Masalani	9	17	3	0, 0, 0.25, 0.25, 0.75, 0.75, 1, 1.5, **2.33**	1.09
	Sangailu	8			0, 1.2, 1.25, 1.25, 1.33, 1.5, **2.33, 3**	

*RVFV = Rift Valley Fever virus. The nine positive scores (≥ 2) are indicted in **boldface**.

averaged scores ≥ 2 (2.00, 2.00, 2.16 and 2.33), and the remaining four had negative scores. Of the five persons with antibodies against retinal tissue who were not diagnosed with RVFV retinitis, one had serologic evidence of RVFV exposure but no retinitis, one had no RVFV exposure but had retinitis (presumably caused by another etiology), and three did not have RVFV exposure or retinitis. There was a trend toward differences in the prevalence of antibodies against retinal tissue for the RVFV-exposed retinitis groups compared with each of the other three groups, but these differences did not reach statistical significance, defined as $P < 0.05$ (Table 1).

Our small study examined levels of antibodies against retinal tissue in RVFV-seropositive humans. When the groups were compared individually to cases of retinitis with RVFV exposure, we had insufficient power to detect significant differences. Four of eight of the RVFV-exposed retinitis-positive serum samples had increased levels of antibodies against retinal tissue compared with 1 of 9 in the RVFV-exposed retinitis negative group and RVFV-unexposed retinitis-positive group and 3 of 16 of the RVFV-unexposed, retinitis negative control group. It is clear that larger numbers of persons in each of the groups are needed to assess the implications of our findings. Our attempts to address this were limited by the logistical impediments to performing eye examinations in this and other remote areas of sub-Saharan Africa where RVFV epidemics occur.

The ocular examinations showed a variety of pathologies. Persons who were RVFV-exposed and had retinitis had the following findings: retinal hemorrhage, maculopathy, peripapillitis, and retinitis (positive IHC results) and ischemia, maculopathy, retinitis, and retinopathy (negative IHC results). Those who were RVFV-unexposed with retinitis showed optic atrophy (positive IHC results), retinitis, maculopathies, retinal exudation, ischemic hemorrhage and vasculitis (negative IHC results).

Animal studies suggest that direct viral effect and autoimmunity have a role in the pathogenesis of RVFV retinitis.[15] Ruminants tend to become more severely ill than humans after RVFV infection, but can provide valuable information on severe features of human RVF. The study of Galindo-Cardiel and others of RVFV infection in sheep found ocular complications in 4 of 16 experimentally infected lambs, and viral RNA was detected by reverse transcription–polymerase chain reaction in 2 of 4 of the lambs with ocular involvement.[15] Kinetics of human and ovine infections are similar, and ocular complications occur 5–14 days after infection.[15,16] However, the lack of any histologic descriptions from human cases in the published literature makes direct comparisons to ovine models problematic.[15]

If a true association exists between RVFV retinitis and antibodies against retinal tissue, it may have been obscured in the present study by several factors. These factors include misclassification of participants resulting in the inclusion of RVFV seropositive persons with non–RVFV-related retinitis in the RVFV-exposed retinitis positive group, and persons in the RVFV–non-exposed groups who may have also been exposed to RVFV but had since lost their levels of antibodies against RVFV (i.e., seroreversion to negative). Furthermore, the serum from Sangailu was frozen at −80°C for one year and the serum from Masalani[5] was frozen for four years,[6] which may have affected the antibody content. Differences between the black African population studied and the Caucasian donor of the ocular tissue could also affect the validity of our study.

Alternatively, the association we observed could be caused by chance, with levels of antibody against retinal tissue randomly associated with retinitis in the RVFV-positive patients in our study. We observed that healthy persons have low levels of antibodies against retinal tissue, and this finding has been reported elsewhere among healthy persons.[17] Rift Valley Fever virus could also directly infect ocular tissues, as it does in calves,[18] causing greater permeability of the circulation in the eye with exposure of normally-hidden ocular epitopes to pre-formed antibodies against retinal tissue.

Further studies are needed to determine the pathogenesis of this common complication of RVFV infection and provide possibilities for therapeutic interventions. Postmortem examination of eye tissue from persons who die of RVFV infection during outbreaks could provide important data on pathogenesis. More affected persons need to be studied, and the change in profiles of antibodies against retinal tissue before, during and after the onset of retinitis should be delineated.

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