Pathology of Experimental Ebola Virus Infection in African Green Monkeys

Involvement of Fibroblastic Reticular Cells

Kelly J. Davis, DVM; Arthur O. Anderson, MD; Thomas W. Geisbert; Keith E. Steele, DVM; Joan B. Geisbert; Peter Vogel, DVM; Brett M. Connolly, PhD; John W. Huggins, PhD; Peter B. Jahrling, PhD; Nancy K. Jaax, DVM

Background.—Ebola virus has been responsible for explosive lethal outbreaks of hemorrhagic fever in both humans and nonhuman primates. Previous studies showed a predilection of Ebola virus for cells of the mononuclear phagocyte system and endothelial cells.

Objective.—To examine the distribution of lesions and Ebola virus antigen in the tissues of six adult male African green monkeys (Cercopithecus aethiops) that died 6 to 7 days after intraperitoneal inoculation of Ebola-Zaire (Mayinga) virus.

Methods.—Tissues were examined histologically, immunohistochemically, and ultrastructurally.

Ebola and Marburg viruses cause severe and often fatal hemorrhagic fevers in both humans and nonhuman primates.\textsuperscript{1}–\textsuperscript{16} Ebola and Marburg viruses form one genus, Filovirus, within the family Filoviridae. The Filovirus genus is represented by four species: Ebola virus Zaire (EBOV-Z), Ebola virus Sudan, Ebola virus Reston, and Marburg virus.\textsuperscript{17} A new Ebola virus, recently isolated from a single, nonfatal human case in Côte d’Ivoire,\textsuperscript{12} is genetically distinct from previous Ebola isolates.\textsuperscript{11} The taxonomic classification of this new Ebola virus within the Filoviridae family has not been definitively established. Of the three recognized species of Ebola virus, EBOV-Z and Ebola virus Sudan are highly lethal for humans and nonhuman primates.\textsuperscript{1}–\textsuperscript{12} In contrast, although Ebola virus Reston infects humans,\textsuperscript{13}–\textsuperscript{20} it appears to be a lethal pathogen only for nonhuman primates.\textsuperscript{21} The 1995 outbreak of Ebola hemorrhagic fever in and around Kikwit, Zaire,\textsuperscript{13,14} and a more recent outbreak of Ebola hemorrhagic fever in Makokou, Gabon,\textsuperscript{22} have emphasized the public health threat of these pathogens, primarily because proven prophylactic or therapeutic measures to combat filoviral infections do not yet exist.

The origins of the pathophysiologic changes that make filovirus infections of humans so devastating are still not understood,\textsuperscript{22} although studies have suggested that several mechanisms, including viral infection of mononuclear phagocyte system cells, may be operative.\textsuperscript{22–24} Previous studies showed that the disease course of EBOV-Z in Asian monkeys and African green monkeys (Cercopithecus aethiops) is similar to that seen in humans.\textsuperscript{22,23,27,29–31} The distribution of lesions and EBOV-Z antigen in experimentally infected Asian monkeys has been documented.\textsuperscript{27} Complete details of the distribution of lesions and EBOV-Z antigen in tissues of African monkeys have not been reported.

The objective of this study was to examine the distribution of lesions and virus antigen by immunohistochemistry and electron microscopy in tissues of African green monkeys experimentally infected with EBOV-Z. Characterization of the distribution of EBOV-Z antigen, virus particles, and virus inclusions in various tissues and cell types should lend additional insight into the pathogenesis of Ebola hemorrhagic fever. Additionally, this information will be useful for comparing Asian and African nonhuman filoviruses.

Results.—A major novel finding of this study was that fibroblastic reticular cells were immunohistochemically and ultrastructurally identified as targets of Ebola virus infection.

Conclusions.—The role of Ebola virus–infected fibroblastic reticular cells in the pathogenesis of Ebola hemorrhagic fever warrants further investigation. This is especially important because of recent observations indicating that fibroblastic reticular cells, along with the reticular fibers they produce, maximize the efficiency of the immune response.

(Arch Pathol Lab Med. 1997;121:805–819)
man primate species as models of the human disease. A better understanding of the pathogenesis of the disease in nonhuman primates may facilitate the development of effective prophylactic and treatment strategies for Ebola hemorrhagic fever.

MATERIALS AND METHODS

Monkeys

Tissues from six 4.4- to 7.5-kg adult male African green monkeys (C. aethiops) that died 6 to 7 days after intraperitoneal inoculation of EBOV-Z were examined in this study. The monkeys were placebo controls in a drug efficacy trial. Before exposure, the monkeys had normal hematologic and serum biochemical values, and no demonstrable antibody titers or history of exposure to any filovirus. The monkeys were maintained in individual stainless steel cages in a BL-4 facility fully accredited by the American Association for Accreditation of Laboratory Animal Care. Monkeys were fed commercial monkey chow and provided fresh fruit twice daily and filtered tap water ad libitum. Monkeys were sedated with tiletamine-zolazepam combination (3 mg/kg intramuscularly, Telazol, A.H. Robbins, Richmond, Va) on days −7, −2, 0, 2, 4, and 6 (relative to virus inoculation) to obtain femoral vein blood samples.

Inoculum

After sedation with tiletamine-zolazepam combination (3 mg/kg intramuscularly), each monkey was inoculated intraperitoneally with 1000 plaque-forming units of EBOV-Z (Mayinga) suspended in 1 mL of phosphate-buffered saline.

Necropsy

The following tissues were collected at necropsy and immersion-fixed for 30 days in 10% neutral-buffered formalin for histology and immunohistochemistry: haired skin, mammary gland, submandibular salivary gland, lymph nodes (mandibular, mesenteric, and mediastinal), mediastinum, brachial plexus, tonsils, tongue, trachea, thyroid glands, parathyroid glands, lungs, esophagus, heart, liver, spleen, adrenal glands, kidneys, urinary bladder, seminal vesicles, testes, stomach, duodenum, pancreas, jejunum, ileum, ileocecal junction, proximal and distal colon, sciatic nerve with skeletal muscle, femoral bone marrow, nares, lips, eyes, brain, spinal cord, and pituitary gland. The following tissues were collected and immersion-fixed in 4% paraformaldehyde plus 1% glutaraldehyde in 0.1 mol/L Millonig’s phosphate buffer (pH 7.4) for transmission electron microscopy examination: lung, liver, spleen, adrenal gland, kidney, urinary bladder, mesenteric lymph node, aorta, submandibular salivary gland, tongue, nasal vestibule, and ileum.

Histochemistry, Immunohistochemistry, and Ultrastructure

Tissues were processed and embedded in paraffin. Histology sections were cut at 5 to 6 μm on a rotary microtome, mounted on glass slides, and stained with hematoxylin-eosin. Replicate sections from kidney, liver, lung, spleen, and duodenum were stained with phosphotungstic acid-hematoxylin to demonstrate polymerized fibrin. Replicate tissue sections were immunohistochemically stained for Ebola virus antigen by using mouse anti-Ebola ascitic fluid and an alkaline phosphatase-labeled streptavidin method as de
scribed previously. Negative controls included substitution of isotype-identical mouse anti-Marburg ascitic fluid for mouse anti-Ebola ascitic fluid and application of mouse anti-Ebola ascitic fluid to tissues from Marburg virus–infected monkeys. Replicate sections from lymph nodes were stained for reticular fibers by silver impregnation followed by immunohistochemical staining for Ebola virus antigen.

Tissue samples that had been fixed in 4% paraformaldehyde plus 1% glutaraldehyde in 0.1 mol/L Millonig’s phosphate buffer (pH 7.4) were postfixed in 1% osmium tetroxide in 0.1 mol/L Millonig’s buffer, rinsed, stained with 0.5% uranyl acetate in ethanol, dehydrated in ethanol and propylene oxide, and embedded in POLY/BED 812 resin. Areas to be evaluated by transmission electron microscopy were selected from 1-μm sections stained with toluidine blue. Ultrathin sections were cut, placed on 200-mesh copper grids, stained with uranyl acetate and lead citrate, and examined with a JOEL 1200 EX transmission electron microscope (JOEL Ltd, Peabody, Mass) at 80 kV. In addition, areas of tongue and lip (hair bulb papillae, sweat glands, and labial salivary gland) were selected by positive immunohistochemistry findings and recovered from paraffin blocks. These specimens were deparaffinized, treated with 1% osmium tetroxide, and processed for electron microscopy as described above.

Virus Isolation From Blood and Tissues

For infectivity assays, blood was collected on days −7, −2, 0, 2, 4, and 6 (relative to virus inoculation), and blood and portions of spleen, liver, adrenal glands, lungs, kidneys, aorta, mesenteric lymph nodes, heart, and brain were aseptically collected during the necropsy. Blood and tissues were processed and assayed as described previously.

RESULTS

Necropsy Findings

All monkeys in this study died 6 to 7 days postexposure (mean time to death, 6.5 days). In five of six monkeys, the liver was friable with a prominent reticulated pattern. There were bilateral petechiae in the periorbital dermis and petechiae in the mucosa/submucosa of the glandular stomach in these monkeys. Other findings included petechiae in the mesentery (two of six); ecchymoses in the mucosa/submucosa of the distal ileum (two of six); gastric erosions in the glandular stomach (two of six); and luminal blood in the stomach, duodenum, jejunum, and ileum (two of six).

Histology, Immunohistochemistry, and Ultrastructure

Lymphoid Tissues.—Histologically, there was depletion, apoptosis, and/or necrosis of lymphoid cells in B-cell follicles and germinal centers in the mandibular, mediastinal, and mesenteric lymph nodes, spleen, tonsils, and the gut-associated lymphoid tissue (GALT) present in sections of the intestinal tract. The subcapsular, cortical, and medullary sinuses of the lymph nodes in each monkey contained increased macrophages, necrotic cellular debris, small numbers of neutrophils, and extravasated red blood cells. Elongate, often

*Ebola Virus Infection of African Green Monkeys—Davis et al.*

Arch Pathol Lab Med—Vol 121, August 1997
branching, fibroblast-like cells (Fig 1, A through C) lining and traversing the subcapsular, cortical, and medullary sinuses were immunoreactive for Ebola virus antigen. At low magnification, the immunoreactive cells lining and traversing the medullary sinuses were arranged in a trabecular-like pattern (Fig 1, D). The immunoreactive cells were closely associated with reticular fibers in replicate lymph node sections stained for reticular fibers by silver impregnation followed by immunohistochemical staining for Ebola virus antigen (Fig 2, A). Infrequently, fibroblast-like cells were immunoreactive in the paracortex and medullary cords of the lymph nodes, as well as in the tonsils and GALT of each monkey. In the lymph nodes, tonsils, and GALT, there was strong Ebola virus antigen immunoreactivity surrounding venules, including the high endothelial venules (HEVs) (Fig 2, B). Endothelial cells, including HEV endothelium, were also multifocally immunoreactive (Fig 2, C). Cells, interpreted as follicular dendritic cells, were occasionally immunoreactive (Fig 2, D) in the follicular centers of the lymph nodes, tonsils, and GALT. In the paracortex of the lymph nodes and in the interfollicular areas of the tonsils and GALT, scattered macrophage-like cells, interpreted as interdigitating dendritic cells or macrophages, were also immunoreactive. Cell-free and macrophage/monocyte-associated immunoreactivity for Ebola virus antigen was present in the lymph sinuses and within the vasculature of the lymph nodes. Ebola virus antigen was not associated with lym-

![Image](https://example.com/image1.png)

**Fig 3.**—Electron micrograph of mesenteric lymph node from an Ebola virus–infected African green monkey. Ebola virus inclusion (arrow) in the cytoplasm of a fibroblastic reticular cell lining the subcapsular sinus (Bar = 4.0 μm).

![Image](https://example.com/image2.png)

**Fig 4.**—Electron micrograph of mesenteric lymph node from an Ebola virus–infected African green monkey. Enlargement of fibroblastic reticular cell from Fig 3 shows details of intracytoplasmic Ebola virus inclusion (arrow) and close association of the cell with reticular fibers (asterisk) (Bar = 1.7 μm).

![Image](https://example.com/image3.png)

**Fig 5.**—Electron micrograph of mesenteric lymph node from an Ebola virus–infected African green monkey. Enlargement of fibroblastic reticular cell from Fig 4 shows details of intracytoplasmic Ebola virus inclusion (Bar = 0.6 μm).
phocytes. In all examined tissues, negative controls, consisting of replacement of the mouse anti-Ebola ascitic fluid with mouse anti-Marburg ascitic fluid and application of mouse anti-Ebola ascitic fluid to Marburg virus-infected monkey tissues, did not immunoreact.

Ultrastructural examination of the lymph nodes confirmed light microscopy findings. The architecture of the lymph nodes was disrupted by necrosis and depletion of lymphoid follicles. Necrotic cellular debris, macrophages, fibrin, Ebola virions, and small numbers of neutrophils...
and red blood cells were in the lymph sinuses. The fibroblast-like immunoreactive cells lining the lymph sinuses observed by light microscopy were identified as fibroblast-like reticular cells (FRCs) by ultrastructure. Briefly, the FRCs were characterized by elongated branching cytoplasmic processes, irregular nuclei with small deposits of heterochromatin, abundant rough endoplasmic reticulum, and fine filaments most prevalent at the periphery of the cells. The FRCs were in close association with, often surrounding, reticular fibers composed of narrow bundles of collagen fibrils with an amorphous granular material. Many FRCs in the lymph nodes contained characteristic Ebola virus inclusion material, (Figs 3 through 8) budding virions at the cell membrane, or both. Ebola virus-infected FRCs were seen at all stages of degeneration (Figs 6 and 7), ranging from disruption of cellular organelles and increased autophagosomes to complete dissolution of plasma and nuclear membranes. Fibroblastic reticular cells surrounded the basal lamina of venules, including the HEVs, and often contained Ebola virus inclusions (Fig 5) and/or budding virions. Extracellular Ebola virions were present in the adjacent lymph sinuses, between the fibrillar components of the reticular fibers (Fig 8), surrounding the basal lamina of venules, including the HEV, and in the bloodstream. Multilocally, Ebola virus inclusions and budding virions were observed in endothelial cells, including the endothelium of the HEVs (Fig 6), intravascular monocytes, and macrophages in the lymph sinuses.

In the spleen, there was marked congestion of the marginal zone sinuses. Fibrin thrombi were present in the red and white pulp. Abundant karyorrhectic cellular debris was present throughout the red pulp. Macrophages/monocytes and neutrophils were also present in the red pulp and marginal zone. Scattered macrophages/monocytes and fibroblast-like cells were immunoreactive for Ebola virus antigen. Ebola virus antigen was not associated with lymphocytes or neutrophils. Widespread deposition of fibrin, numerous necrotic cells, and large aggregates of free virions in the red pulp of the spleen hindered ultrastructural identification of infected cells. However, Ebola virus inclusions and budding virions were seen in splenic macrophages.

Mononuclear Phagocyte System.—In each monkey, the cytoplasm of most circulating monocytes was immunoreactive for Ebola virus antigen. Macrophages in the lymph nodes, spleen, liver (Kupffer cells), lungs (alveolar and interstitial macrophages), lamina propria of the intestine, bone marrow, skin, and other organs were also immunoreactive. Ultrastructural examination confirmed the immunohistochemical findings. Virus replication, demonstrated by intracytoplasmic Ebola virus inclusions and budding virions, was present in circulating monocytes and in macrophages within the lymph nodes, spleen, liver, lungs, skin, and lamina propria of the tongue and ileum.

Circulatory System.—Endothelial cells, circulating monocytes, and the cell-free portion of the blood were multifocally immunoreactive for Ebola virus antigen in all examined tissues. Multilorgan congestion was evident. Fibrin thrombi and fibrinocellular thrombi, composed of monocytes enmeshed in fibrin, were identified in capillary and small venules of numerous tissues, but were particularly prevalent in renal glomeruli, spleen, liver, lungs, and capillaries and venules adjacent to Brunner’s glands of the duodenum in each monkey. The fibrin and fibrinocellular thrombi were immunoreactive for Ebola virus antigen. Light microscopy findings were confirmed by ultrastructural examination, which showed large amounts of free virions in the blood, virions enmeshed in fibrin and fibrinocellular thrombi, and Ebola virus inclusions and budding virions in endothelial cells (Fig 9, A). The sinusoids contained necrotic cellular debris and small numbers of neutrophils and monocytes. Hepatocytes and Kupffer cells contained large (5 to 25 μm), pleomorphic, acidophilic, intracytoplasmic inclusions (Fig 9, A). Immunohistochemically, numerous hepatocytes within and adjacent to areas of hepatocellular degeneration and necrosis contained Ebola virus antigen (Fig 9, B). Kupffer cells, sinusoidal endothelial cells, and circulating monocytes, were also immunoreactive for Ebola virus antigen. Cell-free and cellular debris–associated immunoreactive material was present in the sinusoids.

Electron microscopy of the liver revealed numerous free virions in the sinusoids and in the space of Disse (Fig 10). In addition to virus particles, sinusoids also contained fibrin and necrotic cellular debris. Ebola virus inclusions and budding virions were present in hepatocytes (Fig 10), Kupffer cells, sinusoidal endothelial cells, and circulating monocytes.

Epithelial cells of the tongue (Fig 9, C), esophagus, and ducts of the submandibular salivary gland (six of six monkeys); tonsils, crypts of the ileum, and ducts of the labial and lingual salivary glands (four of six monkeys); and gastric glands and duodental Brunner’s glands (two of six monkeys) multifocally contained Ebola virus antigen. Macrophages, fibroblast-like cells, and endothelial cells in the lamina propria of the tongue, esophagus, and small and large intestines were multifocally immunoreactive in each monkey. Ebola virus antigen was not present in the exocrine pancreatic cells in any of the six monkeys.

Ultrastructural examination showed Ebola virus inclusions in epithelial cells of the tongue, in macrophages, in fibroblast-like cells, and in endothelial cells of the lamina propria of the tongue and ileum. Viral inclusions were not observed in the epithelium of submandibular, lingual, or labial salivary glands, or in enterocytes of the ileum by electron microscopy.

Endocrine System.—The adrenal glands of each monkey had foci of degeneration and necrosis of cortical cells in the zona glomerulosa and zona fasciculata accompanied by small numbers of neutrophils. The sinusoids were congested. Intracytoplasmic inclusions were occasionally present in adrenal cortical cells. Cortical cells of the zona glomerulosa, zona fasciculata, and less frequently the zona reticularis were immunoreactive for Ebola virus antigen (Fig 9, D).

Ultrastructural examination supported immunohistochemical findings. Electron microscopy showed numerous free virions in the sinusoids and subendothelial spaces. Ebola virus replication in adrenal cortical cells was striking, as virions frequently appeared to be budding from all available plasma membranes (Fig 11). In addition, Ebola virus inclusions and budding virions were present in endothelial cells lining the sinusoids and in fibroblast-like cells positioned between adrenal cortical cells of the zona glomerulosa and fibroelastic tissue of the capsule (Fig 12).

Ebola virus antigen was multifocally present in testicular interstitial cells of Leydig (six of six monkeys), thyroid interstitial cells and thyroid follicular epithelial cells
(three of six monkeys), and parathyroid chief cells (two of six monkeys). Ebola virus antigen was not present in endocrine cells of the pituitary gland or pancreatic islets in any of the six monkeys.

**Respiratory System.**—In the alveolar capillaries of the lungs, each monkey had diffuse neutrophilic leukocytosis. Minimal intra-alveolar foci of hemorrhage occurred in two monkeys. Alveolar and interstitial macrophages, bronchial and bronchiolar epithelial cells, pneumocytes, and intravascular monocytes were multifocally immunoreactive for Ebola virus antigen in each monkey. Electron microscopy of the lungs corroborated immunohistochemical findings. While Ebola virus–infected cells and extracellular virions were most prevalent in the interstitium, intracytoplasmic Ebola virus inclusions and budding virions were occasionally seen in alveolar macrophages (Fig 13) and infrequently observed in bronchial and bronchiolar epithelial cells and Type II pneumocytes.

In the trachea and larynx of each monkey, Ebola virus antigen was occasionally present in the respiratory epithelial cells. The immunoreactive epithelial cells were overlying areas of the lamina propria containing immunoreactive macrophages, fibroblast-like cells, and endothelial cells.

---

**Fig 8.**—Electron micrograph of mesenteric lymph node from an Ebola virus–infected African green monkey. Ebola virions (arrowheads) are between fibrillar components (spearheads) of reticular fibers. Note close association of the fibers with a fibroblastic reticular cell containing an Ebola virus inclusion (asterisk) (Bar = 1.3 μm).

**Fig 7.**—Electron micrograph of mesenteric lymph node from an Ebola virus–infected African green monkey. Degenerative Ebola virus–infected fibroblastic reticular cells (F). Note intracytoplasmic Ebola virus inclusions (arrowheads), close association of the cells with reticular fibers (asterisk), and numerous extracellular virions (spearheads) (Bar = 2.3 μm).
Skin and Adnexa.—Sections of haired skin from the chest and near the mucocutaneous junction of the nose and lip were examined. Blood vessels were congested and occasionally contained thrombi. In each monkey, immunoreactive endothelial cells, fibroblast-like cells, and macrophages were present in the superficial dermis and in the connective tissue adjacent to hair follicles, sebaceous glands, sweat glands, and mammary gland ducts. In four monkeys, mammary gland ductal epithelium, acinar and ductal epithelium of sweat glands, and fibroblast-like cells in the dermal papillae of the hair follicles were occasionally immunoreactive for Ebola virus antigen. Electron microscopy of haired lip failed to show Ebola virus inclusions in the epithelial cells of sweat glands or in the fibroblast-like cells of the dermal papillae of the hair follicles.

Urinary System.—Although proximal tubular epithelial cells in four of the six monkeys immunohistochemically contained Ebola virus antigen, we could not confirm the presence of Ebola virus in these cells by electron microscopy. In the urinary bladder of each monkey, immunoreactive endothelial cells, fibroblast-like cells, and macrophages were multifocally present in the lamina propria. In one monkey, the urothelium was immunoreactive above several foci of subepithelial immunoreactivity. Ultrastructural examination of the urinary bladder confirmed the immunohistochemical findings.

Reproductive System.—In three of the six monkeys, the epididymides and testes (predominately in the tunica albuginea) were congested and contained thrombi. Interstitial cells of Leydig and endothelial cells contained Ebola virus antigen in the testes of each monkey. Ebola virus antigen was not present in the seminiferous tubules. In three monkeys, infrequent epithelial cells of the seminal vesicles were immunoreactive for virus antigen.

Central and Peripheral Nervous System.—Central nervous system tissues in all six monkeys were congested. Ebola virus antigen in the central and peripheral nervous system was limited to endothelial cells and circulating monocytes.

Virology

Onset of viremia occurred between days 2 and 4 postexposure (monkeys were not bled on day 3 postexposure). By day 4 postexposure, all six monkeys were viremic. The mean EBOV-Z virus titer in sera collected at necropsy was 6.2 log_{10} per milliliter. In the spleen, liver, adrenal glands, lungs, kidneys, aorta, mesenteric lymph nodes, heart, and brain, mean organ EBOV-Z titers ranged from 6.85 to 8.95 log_{10} per gram.

COMMENT

In most respects, the pathology of EBOV-Z infection that we observed in African green monkeys resembles previously reported findings in humans and rhesus mon-
A major novel finding of this study was that FRCs in lymph nodes of African green monkeys were identified as targets of Ebola virus infection.

Fibroblastic reticular cells are also referred to as fibroblastic reticulum cells, reticular cells, reticulum cells, or stromal cells. These terms denote a fibroblastic cell subpopulation identified morphologically as elongate cells closely associated with reticular fibers. Fibroblastic reticular cells are present in all lymphoid tissues and produce the networks of reticular fibers found within the connective tissue of all organs. The networks of reticular fibers interlace hepatic cells, cells of many endocrine organs, and cells of the lymphoid tissues and thereby help hold the parenchymal elements together. In the lymph nodes, FRCs line the lymph sinuses, are present in the interstitium or parenchyma, and surround the HEVs.

The FRCs, along with their networks of reticular fibers, have been referred to as the reticular network, FRC conduit system, or reticulum. Recent observations indicate that the reticular network serves as a multifunctional system that maximizes the efficiency of the immune response. In addition to its structural function in lymph nodes, the reticular network provides a meshwork for the adhesion and migration of lymphocytes and the attachment of antigen-presenting cells. The reticular network also transports antigen from lymphatics to antigen-pre-
senting cells and provides a conduit system for the rapid transport of soluble molecules to the HEV.

In this study, the FRCs lining the lymph sinuses and surrounding the venules, including the HEVs, of the lymph nodes were immunohistochemically and ultrastructurally identified as targets of Ebola virus infection. In contrast, only infrequent immunoreactive FRCs were present in the parenchyma of the paracortex and medullary cords of the lymph nodes and in the interfollicular areas of the tonsils and GALT of each monkey. This apparent preferential infection of a subpopulation of FRCs may reflect viral recognition of cellular receptors present on FRCs lining the lymph sinuses and surrounding the HEVs but not present on the majority of FRCs in the parenchyma. Or, it may reflect a difference in endocytotic activity of FRCs lining the lymph sinuses versus FRCs that ensheath the reticular fibers that transport materials to the HEVs. This hypothesis is consistent with the reported heterogeneity of FRC antigens and warrants further investigation.

Ebola virus Zaire infection of the FRCs lining the lymph sinuses and surrounding the HEVs may play a role in the pathogenesis of Ebola hemorrhagic fever by amplifying the viral infection. The location of the FRCs in the lymph nodes increases the probability of their exposure to Ebola virus-infected afferent macrophages and lymph. The FRCs provide a large cell population in the lymph nodes for viral replication, which would amplify the number of virions exiting the lymph nodes via the efferent lymph and eventually entering the bloodstream via the lymphatic ducts.

Ebola virus Zaire infection of the FRCs, the mononuclear phagocyte system, and the endothelium of the HEVs may also play a role in the pathogenesis of Ebola hemorrhagic fever by disrupting antigen trafficking, cytokine transport, and/or immune cell trafficking. By infecting the FRCs and the mononuclear phagocyte system, Ebola virus may disrupt normal antigen trafficking. Additionally, Ebola virus infection of the FRCs might also disrupt the transport of soluble molecules, such as cytokines, to the HEVs. Disruption of cytokine transport to the HEVs and Ebola virus infection of the HEVs could interfere with the adhesion cascade for entry of immune system cells into the lymphoid tissues.

In addition to demonstrating Ebola virus–infected FRCs in the lymph nodes, we observed Ebola virus inclusions, budding virions, and Ebola virus antigen in fibroblast-like cells in multiple tissues. Based on the ultrastructural cellular morphology and virus infection of these fibroblast-like cells, we postulate that they are either FRCs or share a common lineage with FRCs.
In most respects, our findings substantiate our previously reported histologic, immunohistochemical, and ultrastructural findings in rhesus monkeys. In concurrence with earlier reports, we showed EBOV-Z replicates to very high titers, has an affinity for a wide variety of cell types, and that thrombi are present in multiple organs. Also consistent with other reports, we determined that the mononuclear phagocytic system and endothelial cells are important targets of EBOV-Z. Monocytes, macrophages, and endothelial cells were infected in every tissue examined. Similar to rhesus monkeys, Ebola virus antigen was immunohistochemically demonstrated in various epithelial cells and multiple endocrine cells of the African green monkeys. The immunoreactive epithelial cells we observed in the seminal vesicles of the male reproductive tract, mammary gland ducts, sweat glands, and in the respiratory, digestive, and urinary systems suggest that many body secretions or excretions are potentially infectious.

Electron microscopy showed Ebola virus inclusions in epithelial cells in the lungs, nasal vestibule, tongue, and urinary bladder. However, we were unable to corroborate our immunohistochemical findings of reactive epithelial cells in the salivary glands, sweat glands, intestine, and proximal tubules of the kidneys (epithelial cells of the seminal vesicles in the male reproductive tract, esophagus, and mammary gland ducts were not examined ultrastructurally). Ebola virus inclusions were previously seen in ductal epithelial cells of the salivary gland in cynomolgous monkeys (Macaca fascicularis) experimentally infected with Ebola virus Reston. Our inability to detect Ebola virus inclusions or budding virions in the immunoreactive epithelium of the salivary glands, sweat glands, intestine, and proximal tubules of the kidneys of EBOV-Z-infected African green monkeys may be due to either a low frequency of virus-infected cells in these tissues, an absence of morphologically identifiable filoviral structures in cells at early stages of infection, or cellular uptake by these tissues of dissociated Ebola virus antigen. In situ hybridization techniques currently being developed for Ebola virus–infected tissues, employed in conjunction with immunoelectron microscopy assays, should be useful for resolving these inconsistencies.

Although the liver and adrenal gland lesions were consistent with those previously reported in EBOV-Z infections in Asian monkeys, we found differences in the major immunoreactive cells in these tissues. In the African green monkeys, the hepatocytes and cortical cells were the major immunoreactive cells in the liver and adrenal glands, respectively. In contrast, in rhesus monkeys infected with EBOV-Z orally and conjunctivally, we found monocytes and macrophages to be the major immunoreactive cells in the liver and adrenal glands. These differences in immunoreactivity for Ebola virus antigen in the liver and adrenal glands may be due to differences in the nonhuman primate species, the route of virus exposure, or both.

In agreement with previous studies, we noted the absence of a maculopapular skin rash in our EBOV-Z–infected African green monkeys. In contrast, maculopapular skin rashes have been reported in Ebola virus–infected rhesus monkeys and humans.
In summary, the pathology of EBOV-Z infection in African green monkeys was generally consistent with observations previously reported in humans and rhesus monkeys. A major novel finding of this study was that FRCs lining the lymph sinuses and FRCs surrounding the HEV in lymph nodes of African green monkeys were identified as targets of Ebola virus infection. Ebola virus infection of fibroblast/fibroblast-like cells has been reported in Asian monkeys infected with Ebola virus Reston and EBOV-Z.37,38 Also, the FRC network has been shown to play an important role in the pathogenesis of several arenaviruses,39-41 including Junin virus,37 which frequently causes fatal hemorrhagic fever in humans. Whether infection of the FRCs is unique to Ebola virus and some arenaviruses or is a general mechanism used by other hemorrhagic fever viruses is not known. Based on our findings, the role of the FRCs in the pathogenesis of Ebola hemorrhagic fever and other viral hemorrhagic fever warrants further investigation. This is especially important because of recent observations indicating that the fibroblastic reticulum, composed of FRCs along with the reticular fibers they produce, maximizes the efficiency of the immune response.42-46 The fibroblastic reticulum forms networks that link the lymphatic spaces with the parenchyma and vasculature of lymphoid tissues and provides anchorage for antigen-presenting cells of the mononuclear phagocyte system.47,48 We postulate that immune responses may be adversely affected by Ebola virus infection of this interconnected system of FRCs, mononuclear phagocyte system cells, and HEV endothelial cells.

We thank Eric Kaldjian, MD, for sharing with us his recent immunohistochemical data on the fibroblastic reticular cell conduit system in human lymph nodes. We thank Phil Fogle, Jeff Brubaker, Lynda Miller, Denise Braun, Linda Madero, Al Denn, and Steve Ferendo for their expert technical assistance.

References

Ebola Virus Infection of African Green Monkeys—Davis et al


47. Anderson AO, Shaw S. T cell adherence to endothelium: the FRC conduit system and other anatomic and molecular features which facilitate the adhesion cascade in lymph node. Immunology. 1993;95:271-282.


