Pathology and Pathogenesis of Lassa Fever: Novel Immunohistochemical Findings in Fatal Cases and Clinico-pathologic Correlation

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Abstract

Background.—Lassa fever is a zoonotic, acute viral illness first identified in Nigeria in 1969. An estimate shows that the "at risk" seronegative population (in Sierra Leone, Guinea, and Nigeria) may be as high as 59 million, with an annual incidence of all illnesses of 3 million, and fatalities up to 67 000, demonstrating the serious impact of the disease on the region and global health.

Methods.—Histopathologic evaluation, immunohistochemical assay, and electron microscopic examination were performed on postmortem tissue samples from 12 confirmed Lassa fever cases.

Results.—Lassa fever virus antigens and viral particles were observed in multiple organ systems and cells, including cells in the mononuclear phagocytic system and other specialized cells where it had not been described previously.

Conclusions.—The immunolocalization of Lassa fever virus antigens in fatal cases provides novel insightful information with clinical and pathogenetic implications. The extensive involvement of the mononuclear phagocytic system, including tissue macrophages and endothelial cells, suggests participation of inflammatory mediators from this lineage with the resulting vascular dilatation and increasing permeability. Other findings indicate the pathogenesis of Lassa fever is multifactorial and additional studies are needed.

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Keywords

Lassa fever virus; emerging infections; clinico-pathologic correlation; electron microscopy; immunohistochemistry

Lassa fever (LF) is an acute febrile illness caused by Lassa fever virus (LASV), a member of the arenavirus group known to be endemic in West Africa. It was first isolated from 2 missionary nurses who died from LF in Nigeria in 1969 [1]. It is endemic in most of West Africa, with higher incidence in Sierra Leone, Liberia, Guinea, and Nigeria because the animal reservoir and vector for LASV, *Mastomys natalensis*, is distributed throughout these regions [2]. It is a source of considerable economic hardship in these endemic areas and remains a worldwide concern for public health officials due to potential importation of patients with LF [3, 4]. A significant increase in LF cases occurred in eastern Sierra Leone from January 1996 to April 1997. In May 1996, an LF ward was established in Kenema Hospital, Sierra Leone, by the Medical Emergency Relief International (MERLIN) and the Ministry of Health and Sanitation, Sierra Leone, with technical support from the Centers for Disease Control and Prevention (CDC) [5]. Four postmortem examinations were performed by a CDC pathologist in this Kenema Hospital. Specimens were collected and forwarded to CDC for virus isolation, serology, routine histopathologic examination, and special pathologic testing.

The incubation period of LF ranges from 6 to 21 days. The onset of the disease usually starts with fever, general weakness, and malaise. After a few days, flu-like symptoms, such as headache, sore throat, myalgias, chest pain, nausea, vomiting, diarrhea, cough, and abdominal pain, may follow. In severe cases, facial swelling, subconjunctival effusion, pleural effusion, pericardial effusion, low blood pressure, and bleeding from the mouth, nose, vagina, or gastrointestinal tract may develop [1, 2]. Lassa fever is associated with a high morbidity and mortality, especially in pregnant women [6, 7].

The clinical diagnosis of LF is often presumptive, and laboratory confirmation is essential. Lassa fever is most often diagnosed by using enzyme-linked immunosorbent serologic assays (ELISAs) that detect immunoglobulin (Ig) M and IgG antibodies as well as LASV antigens [8]. Reverse transcription–polymerase chain reaction (RT-PCR) can be used in the early stage of disease with the understanding of potential mismatch due to the existence of several molecular clades of Lassa viruses [9]. The virus itself may be cultured in 7 to 10 days [1], but high containment (BSL-4) is required. Because of the biosafety hazards associated with the handling and testing of LASV, these assays should be performed in specialized laboratories with extreme caution. Transportation of potentially dangerous biological specimens using a cold chain with stringent packaging and shipping procedures from remote sites where the disease occurs to those specialized laboratories may delay timely diagnosis [2]. This report describes the development of a diagnostic immunohistochemical (IHC) assay for LASV infections using formalin-fixed tissue specimens obtained from postmortem examination. In addition to pathologic diagnosis, immunolocalization of LASV antigens in tissues can also help elucidate the pathogenesis of the illness. The pathogenesis of LASV is largely unknown. Previous pathologic studies

performed by Walker and colleagues [10, 11] demonstrated hepatocellular, adrenal, and splenic necrosis and other nonspecific histopathologic changes in heart, lung, and kidney. However, the extent of these histopathologic changes does not sufficiently explain the clinical severity of and cause of death in LASV infection. Previous and recent animal studies by using necropsy tissue samples have helped illustrate the histopathology and antigen distribution of LASV [12, 13] and other arenaviruses, such as lymphocytic choriomeningitis virus (LCMV) [14] and Pichinde virus [15]. This report is the first study demonstrating the tissue and cellular tropism of LASV in fatal human cases and may help explain the pathogenesis with clinico-immunopathologic correlation.

METHODS

Postmortem Tissue Specimens

The specimens in this study include formalin-fixed, paraffin-embedded tissue blocks from 8 cases with previously confirmed LASV infection from the CDC archive and 4 cases where autopsies were performed during the 1997 LF outbreak in Sierra Leone (Table 1). In these 4 autopsy cases, various tissues were also fixed in glutaraldehyde and processed for electron microscopy (EM) examination. Routine hematoxylin and eosin–stained sections were examined in all cases, and clinical and laboratory reports were reviewed when available.

Antibodies

The following antibodies were tested for their suitability in IHC assays on formalin-fixed tissues: (1) an anti-Lassa glycoprotein 2 (GP2) monoclonal antibody, (2) an anti-Lassa glycoprotein 1 (GP1) monoclonal antibody, and (3) an anti-Lassa nucleocapsid protein (NP) monoclonal antibody [16].

Immunohistochemical Assays

Immunohistochemical assays were performed by using a labeled streptavidin-biotin method described earlier [17]. Briefly, 4-μm paraffin-embedded tissue sections were deparaffinized and rehydrated by immersion in graded alcohol solutions. The tissue sections were digested in 0.1 mg/mL Proteinase K (Boehringer Mannheim Corporation, Indianapolis, IN) in 0.6 M Tris (pH 7.5)/0.1% CaCl2. The primary antibody was applied to the tissue sections and incubated at room temperature for 90 minutes. This step was followed with a 15-minute incubation at room temperature with biotinylated swine anti-mouse and anti-rabbit immunoglobulins and a subsequent 15-minute incubation at room temperature with streptavidin alkaline phosphatase conjugate (Dako Corporation, Carpinteria, CA). The alkaline phosphatase activity was detected by using naphthol/fast red substrate (Dako Corporation), and the sections were counterstained in Mayer's hematoxylin (Fisher Scientific, Hampton, NH) and mounted with aqueous mounting medium (Signet Laboratories, Dedham, MA). The specificity of Lassa virus IHC staining was confirmed in all instances by replacing primary antibodies with different antibodies (isotype-identical murine antibodies, hyperimmune mouse ascitic fluid, non-immune sera, and irrelevant immune sera). Control tissues included formalin-fixed and paraffin embedded LASV- and

non-Lassa virus–infected Vero E6 cell lines. Additional controls included noninfected cell lines as well as non–Lassa virus autopsy tissues.

Electron Microscopy

Autopsy tissues were fixed in buffered 2.5% glutaraldehyde and stored in phosphate buffer. Specimens were post-fixed in 1% buffered osmium tetroxide, en bloc stained in 4% uranyl acetate, dehydrated through a graded series of alcohols and propylene oxide, and embedded in a mixture of Epon-substitute and Araldite. Thin sections were stained with 4% uranyl acetate and Reynold's lead citrate [18].

RESULTS

Histopathologic Findings

A characteristic and consistent histopathologic finding, although variable in degree among individual fatal LF cases, is multifocal hepatocellular necrosis with eosinophilic Councilman bodies (Figure 1A). The lesions are often associated with minimal to mild mononuclear inflammatory cell infiltrate scattered throughout the lobules. Necrosis also occurs sporadically in spleen (Figure 1C), abdominal lymph nodes, and adrenal cortex; however, these necrotic foci are not as widespread as those in the liver. Necrosis in the spleen and lymph nodes primarily involves foci within or adjacent to the follicles (Figure 1C). Other major organs show either nonspecific changes, such as intra-alveolar edema in lung (Figure 1E), or no significant histopathologic findings.

Immunolocalization of LASV Antigens by Immunohistochemical Assays

The anti-GP2 monoclonal antibody was selected for this study because of its higher sensitivity for LASV antigen than anti-GP1 or anti-NP antibodies in formalin-fixed tissues. No staining was observed with noninfected cells, cells infected with viruses other than LASV, or in non–LASV-infected tissues used as negative controls.

Immunostaining of LASV is seen in a wide spectrum of cells and tissues. The cellular immunolocalization can be summarized by the cells and tissues involved as the following:

1. Liver: the LASV antigen–positive cells in the liver are abundant in all cases. Immunostaining revealed LASV antigens primarily in those hepatocytes within and around the necrotic foci, including Councilman-like bodies (Figure 1B). LASV antigens are also present in sinusoidal lining cells, mainly endothelial cells and Kupffer cells.

2. Endothelial cells and mononuclear phagocytic cells: LASV antigens are seen in endothelial cells in all tissues examined, including major organs (Figure 1D, 1F), mucosal membranes (Figure 2A–D), reproductive and endocrinologic organs, (Figure 3E, 3F), and fetal tissues (Figure 1H). In addition to endothelial cells, tissue macrophages and dendritic cells in various tissues are also involved in LASV infection with immunostaining (Figure 1D, 1G). LASV antigens in the lymphoid tissues are mainly observed in macrophages and dendritic cells (Figure 1D, 1G); antigen is not detected in lymphocytes or polymorphonuclear cells.

3. Mucosal tissues: immunostaining of LASV antigens is seen in various mucosal tissues, including nasal mucosa (Figure 2A), oral mucosa (Figure 2B), and conjunctiva (Figure 2C, 2D). The immunolocalization is mainly within endothelial cells and scattered macrophages.

4. Serous membranes and mesothelial cells: immunostaining of LASV antigen is seen in mesothelial cells of various serous (serosa) membranes, including pleura (Figure 2E), pericardium (Figure 2F), and visceral peritoneum of liver, spleen (Figure 2G), intestinal tract, and uterus (Figure 2H).

5. Reproductive and endocrinologic tissues: immunostaining of LASV antigen is seen in ductal epithelial cells in breast (Figure 3A); cortical cells of adrenal gland, especially zona fasciculata and zona reticularis (Figure 3B); theca cells (Figure 3C) and stromal cells (Figure 3D) in ovary; endothelial cells of endometrium vasculature (Figure 3E); endothelial cells of fallopian tube vasculature (Figure 3F); and trophoblastic cells in placenta (Figure 3G, 3H).

Electron Microscopy

Lassa fever virus particles were located in a number of organs, including breast, ovary, placenta, and conjunctiva (Figure 4A–D). Mostly spherical virions had a characteristic appearance, composed of a variety of sizes with a dark envelope and containing host cell ribosomes [19].

DISCUSSION

The pathogenesis of LF is largely unknown and is very likely multifactorial [20, 21]. Previous pathologic studies on human autopsy and animal experimental samples showed a paucity of histopathologic lesions that did not sufficiently explain the clinical severity and cause of death of LASV infection. These studies suggested the involvement of the immune system, and receptor affinity may play important roles in systemic infection of LASV. Our study in this report demonstrated the immunolocalization of LASV antigens in a variety of cells and tissues as discussed below and provides insightful information with clinical and pathogenetic implications (Table 2).

Liver

The liver synthesizes all coagulation factors and is involved in both primary and secondary hemostasis [22]. Although a large amount of hepatocellular necrosis may be associated with bleeding tendency, the histopathologic finding is not sufficient to suggest coagulopathy is induced by various degrees of hepatocellular necrosis in LF. From previous studies, coagulopathy may not contribute significantly to the pathogenesis of LASV infection [20, 21].

Endothelial Cells and the Mononuclear Phagocyte System

Lassa fever virus antigens are seen in endothelial cells in all tissues examined. One of the important physiologic roles of the endothelium is maintaining fluid barrier functions of capillaries in tissues. Endothelial cells respond to diverse signals from tissues and control

fluid and cellular efflux without leakage [23]. They elicit responses that regulate platelets and complement and immune cell activation as well as capillary dilation and permeability. Viral hemorrhagic fevers, such as Ebola, could cause the injury of endothelial cells leading to endotheliopathy and endothelial dysfunction [24]. It is known that endotheliopathy triggers many molecular events that promote the activation of independent endothelial pathways, mainly inflammatory and microthrombotic [25].

Similar to Ebola virus, LASV infection may regulate signaling pathways that induce interferon or cellular responses to interferon receptor activation [26]. Given the delicate balance of endothelium-regulated hemostatic responses, a systemic viral infection of endothelium alone has the potential to cause thrombocytopenia, capillary dilation/ contraction, and increased vascular permeability, which are the core pathogenesis of viral hemorrhagic and edematous diseases.

In addition to endothelial cells, tissue macrophages and dendritic cells in various tissues are also involved in LASV infection. Lassa fever virus antigens in the lymphoid tissues are mainly observed in macrophages and dendritic cells. The mononuclear phagocyte system (MPS) is a part of the immune system that consists of the phagocytic cells, mainly monocytes, macrophages, and dendritic cells. These cells share a number of important pathophysiologic functions related to host immune responses against pathogens [27]. The involvement of tissue macrophages and dendritic cells demonstrated by immunolocalization provides unequivocal evidence to show the participation of MPS in the pathogenesis of LF.

Mucosal Tissues

Mucosal tissues or mucous membrane lines various cavities in the body and covers the surface of internal organs. It is continuous with the skin at various body openings, such as the eyes, ears, nasal cavity, oral cavity, lip (labium oris), vagina, urethral opening, and the anus. Immunostaining of LASV antigen is seen in various mucosal tissues, including oral mucosa, nasal mucosa, and conjunctiva. The immunolocalization is mainly in endothelial cells and scattered macrophages. No epithelial staining was observed. Similar immunolocalization was observed in the guinea pig model [12]. Lassa fever virus particles are also observed in conjunctiva by electron microscopy. The implication of such a finding is that LASV can be present in secretions associated with these mucosal membranes, such as tears, nasal discharge, or saliva [28]. Persons with direct contact with these secretions can therefore contract LASV infection. Some patients with LF clinically present with subconjunctival effusion, pharyngeal edema, and sore throat [29]. The involvement of LASV in the mucosal vasculature may explain such manifestations.

Serous Membranes and Mesothelial Cells

Immunostaining of LASV antigens is seen in mesothelial cells of various serous membranes, including pleura, pericardium, and visceral peritoneum of liver, spleen, intestinal tract, and uterus. Serous membrane is a smooth tissue membrane that lines and encloses several body cavities, where mesothelial cells in the membrane secrete a lubricating fluid to reduce friction from muscle movement. Reactive mesothelial cells can be found when there is an infection or an inflammatory response present in a body cavity [30]. The presence of LASV

antigens in the mesothelial cells of various serous membranes may explain why some of the patients with LF clinically develop prominent pleural effusion, ascites, or pericardial effusion [29].

Reproductive and Endocrinologic Tissues

Immunostaining of LASV antigens is observed in multiple reproductive and endocrinologic tissues, including (1) ductal epithelial cells in breast, (2) cortical cells of adrenal gland, (3) theca cells and stromal cells in ovary, and (4) trophoblastic cells in placenta.

Breast—A mature mammary gland is composed of alveoli lined with milk-secreting ductal epithelial cells in the breast. These epithelial cells enlarge during pregnancy and their cytoplasm becomes vacuolated. The presence of immunostaining in these cells implies LASV may be transmitted through breastfeeding, if the pregnant patient survives and practices breastfeeding post-partum. Indeed, previous studies have shown that LASV can be detected in breast milk [31]. Pregnancy has significant effects on the breast, especially by the increased levels of estrogen and progesterone [32]. These hormones play an important role in readying the breasts for lactation. Estrogen stimulates growth of the breast duct cells and generates secretion of prolactin, which stimulates breast enlargement and milk production. Progesterone supports the formation and growth of milk-producing cells within the glands of the breasts. The presence of LASV antigens in breast may imply an interaction between the virus and the hormone-regulated mammary epithelial cells.

Adrenal—The adrenal cortex is divided into 3 zones histologically with an individual function to produce various hormones [33]. The outermost zona glomerulosa is the main site for aldosterone production. The middle zona fasciculata is mainly responsible for producing glucocorticoids, such as cortisol. The innermost zona reticularis produces androgens that are subsequently converted into more potent sex hormones, such as testosterone or estrogens. The presence of immunostaining of LASV antigens in adrenal cortical cells, especially zona fasciculata and zona reticularis, may imply an interplay between LASV infection and cortical hormones, including cortisol and sex hormones.

Ovary—Immunostaining of LASV antigens is seen in theca cells and stromal cells. Theca cells are a group of endocrine cells in the ovary made up of connective tissue surrounding the follicle. The theca cells are responsible for the production of androstenedione, and indirectly the production of 17β-estradiol, which is an estrogen steroid hormone and the major female sex hormone [34]. The stroma of the ovary is a unique type of connective tissue abundantly supplied with blood vessels. Ovarian stromal cells associated with maturing follicles may acquire endocrine function and secrete estrogens [35].

Placenta—The trophoblasts are specialized cells of the placenta that play an important role in embryo implantation and interaction with the decidualized maternal uterus. The syncytiotrophoblasts secrete human chorionic gonadotropin in order to maintain progesterone secretion and sustain a pregnancy [36]. The observation of LASV immunostaining in trophoblasts may suggest an interaction of LASV with these hormone-producing cells during pregnancy. Further studies are needed to evaluate the association

of LASV infection with the above-mentioned hormones. Moreover, the IHC findings in placenta provide evidence to document transplacental transmission of LASV, similar to Ebola virus [37]. Therefore, vertical transmission of LASV is firmly supported by evidence of transplacental, transvaginal (immunostaining in cervix and endometrium), or breastfeeding (immunostaining in breast) routes. In addition, immunostaining in fetal tissues was observed in endothelial cells and mononuclear cells of various tissues. Such findings may be involved in premature abortion or the low birth weight often observed in maternal LF.

The overall finding of immunostaining in a variety of cells in reproductive and endocrinologic tissues may implicate an interplay between LASV infection and hormones. Similar to LF, hepatitis E virus (HEV) infection during pregnancy can have severe consequences on mother and child, such as vertical transmission, fulminant hepatic failure, and fetal or maternal mortality [38]. A recent study by Singh et al [39] demonstrated that levels of estrogen were considerably higher in HEV‐infected pregnant women. They concluded that estrogen plays an important role in preterm delivery, low-birth-weight infants, and fetal mortality in pregnant women with HEV infection through placental dysfunction. Levels of hormones and other serum factors that may modulate lymphocyte or macrophage synthesis, activation, and function shift considerably during pregnancy [40]. It is well documented that LASV infection in pregnant women has been associated with a significantly higher morbidity and mortality. The pathogenesis is not clear; however, it is possible that hormones, especially estrogen and/or progesterone, may play an important role in LASV infection during pregnancy similar to HEV infection.

There is a wide spectrum of disease associated with LF. This study describes the development of an IHC assay to detect LASV antigens in tissues and explores 1 extreme of this polyphenotypic disease. Some of the IHC findings parallel the antigen distribution in callitrichid primates infected with LCMV [14], Hartley guinea pigs infected with Pichinde virus [15], and guinea pigs infected with LASV [12]. The immunolocalization of LASV antigens provides novel insightful information with clinical and pathogenetic implications (Table 2), as well as helps depict the areas needing future studies to better understand the pathogenesis of LASV infections.

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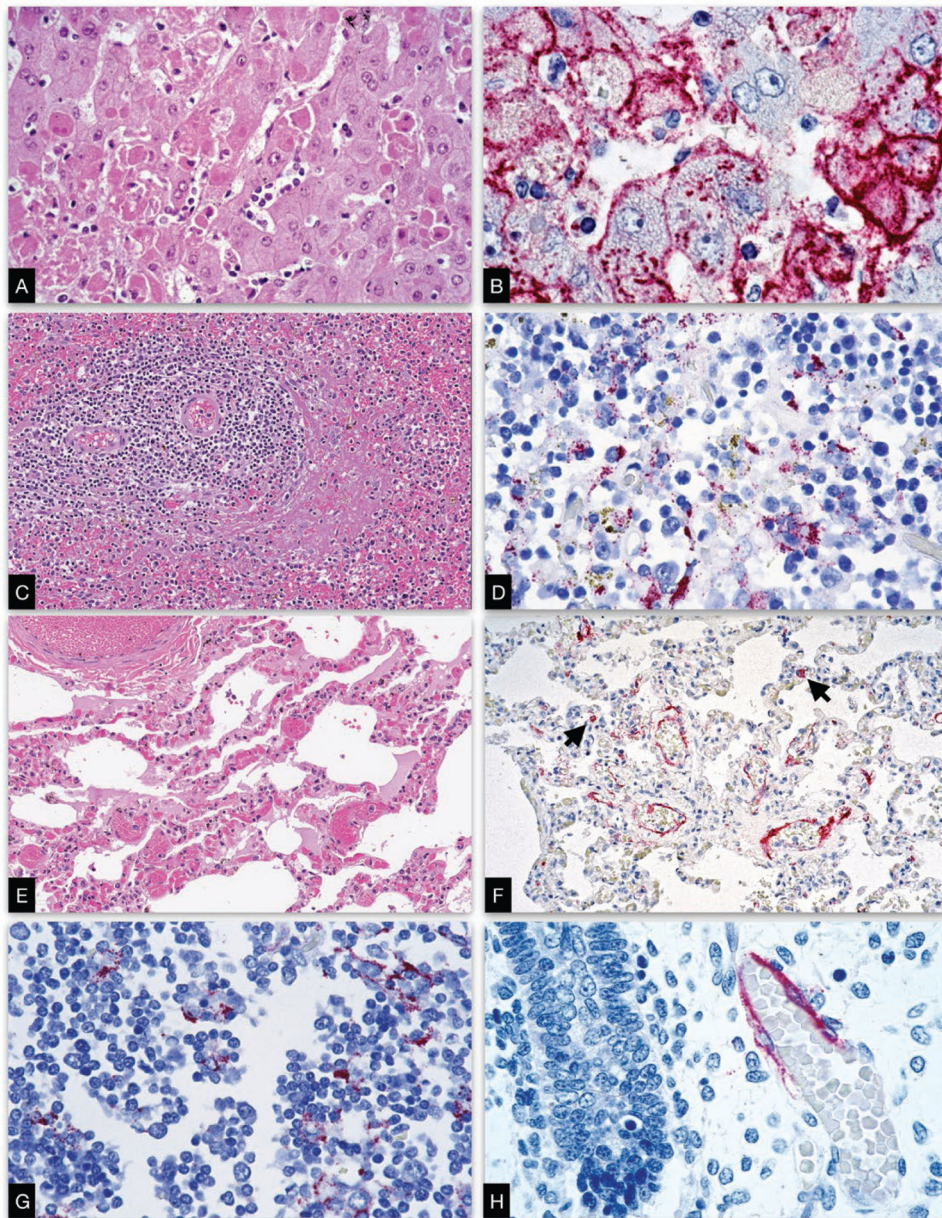
Figure 1.
Histopathologic findings and immunolocalization of LASV antigens in liver, endothelial cells, and the mononuclear phagocyte system. *A*, Patchy necrosis of hepatocytes with scattered round, acidophilic structures resembling apoptotic (Councilman-like) bodies. *B*, Immunostaining of LASV antigens primarily in those hepatocytes within and around the necrotic foci, including Councilman-like bodies. *C*, Necrosis in the spleen usually involves foci within or adjacent to the follicles. *D*, LASV antigens in macrophages and dendritic cells in spleen. *E*, Intra-alveolar edema in lung. *F*, LASV antigens in endothelial cells of pulmonary vasculature. *G*, LASV antigens in macrophages and dendritic cells in tonsil. *H*, LASV antigens in endothelial cells of fetal tissues. H&E stain: *A*, *C*, and E. Original magnifications: ×400 (*A*), ×100 (*C*, *E*). Immunoalkaline phosphatase with napthol fast red

substrate and hematoxylin counterstain: *B*, *D*, *F*, *G*, and *H*. Original magnifications: ×630 (*B*, *D*, *G*, *H*), ×100 (*F*). Abbreviations: H&E, hematoxylin and eosin; LASV, Lassa fever virus.
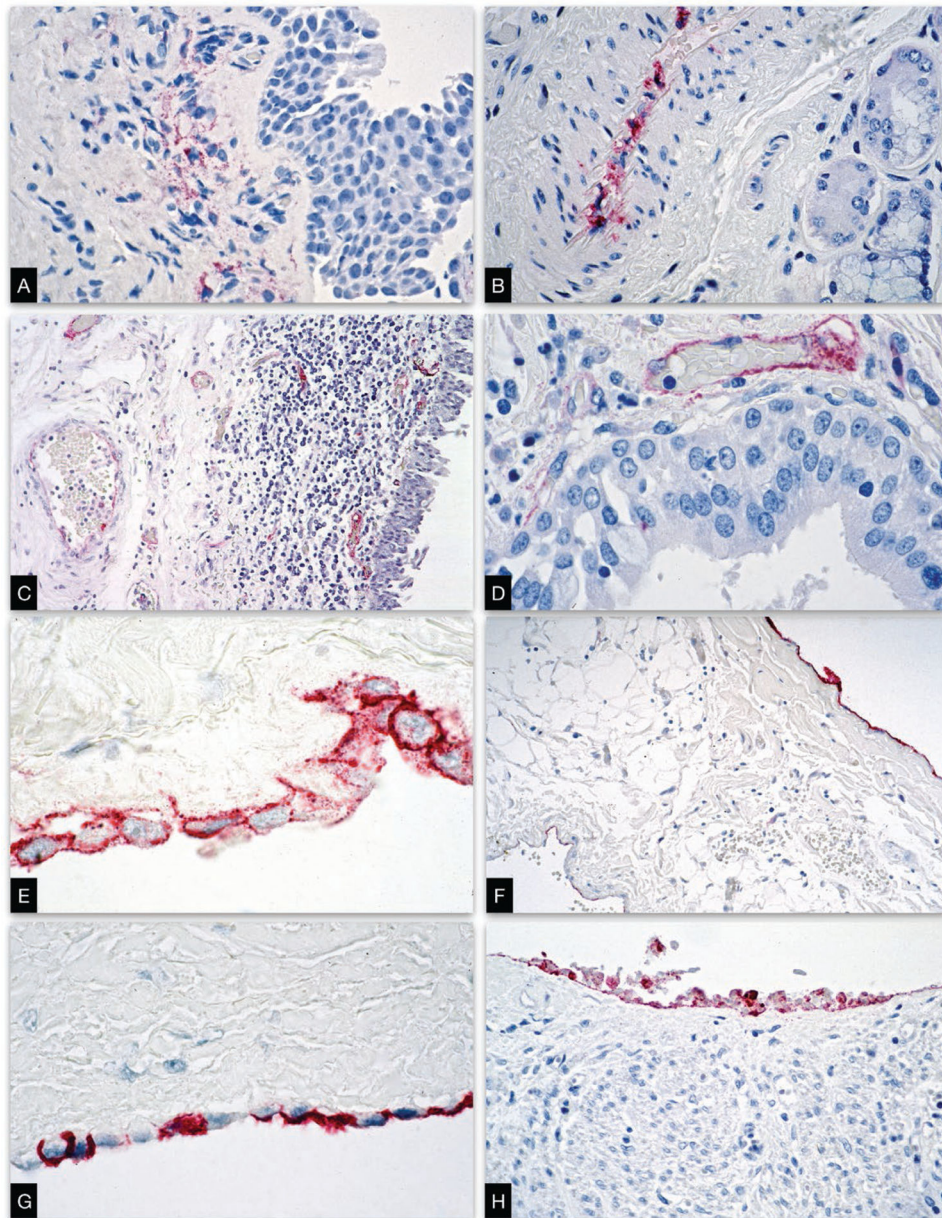
Figure 2.
Immunolocalization of LASV antigens in mucosal tissues (*A–D*) and mesothelial cells of serous membranes (*E–H*). *A*, nasal mucosa; *B*, oral mucosa; *C* and *D*, endothelial cells of conjunctival vasculature; *E*, pleura; *F*, pericardium; *G*, serosa of spleen; *H*, serosa of uterus. Immunoalkaline phosphatase with napthol fast red substrate and hematoxylin counterstain: *A–H*. Original magnifications: ×400 (*A, B, G, H*), ×100 (*C, F*), ×630 (*D, E*). Abbreviation: LASV, Lassa fever virus.
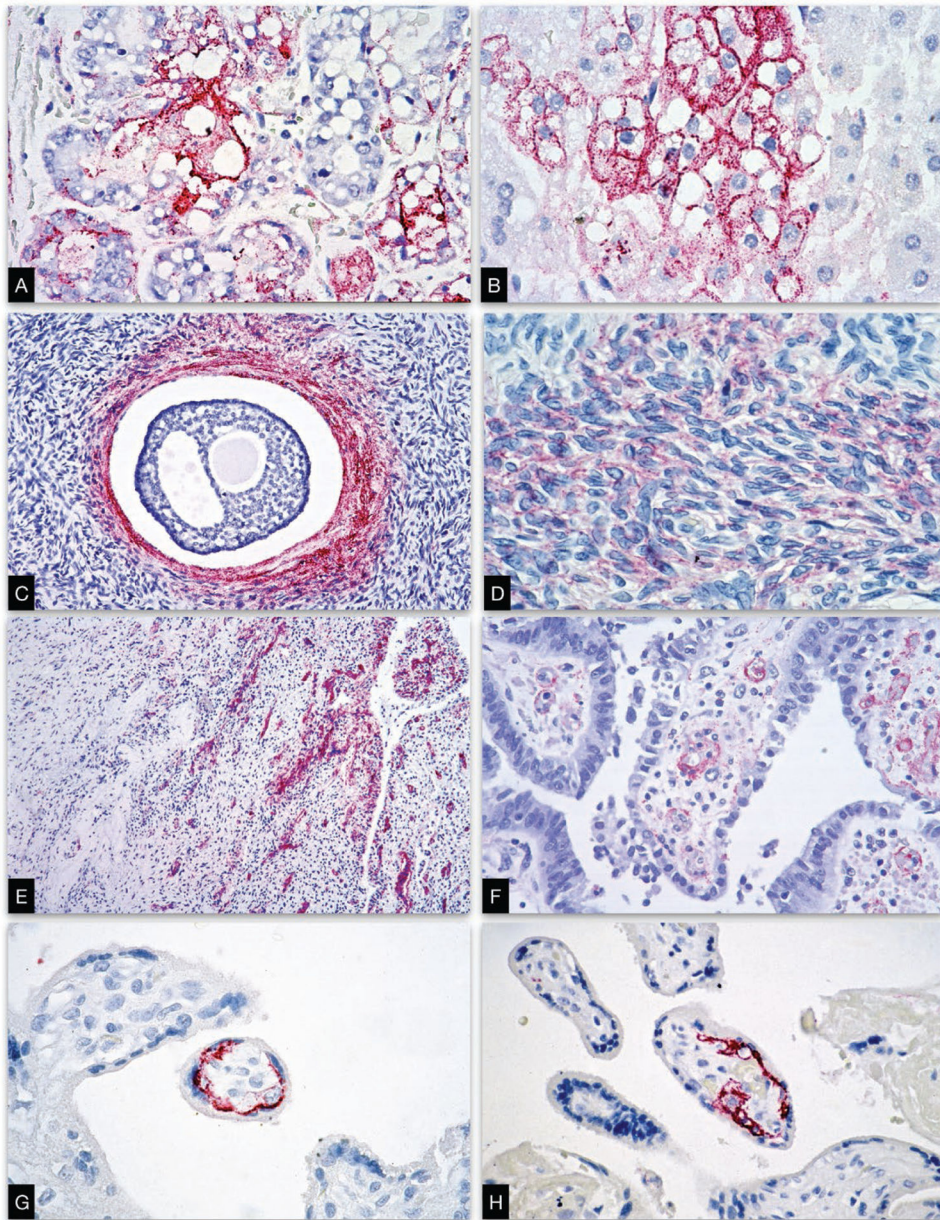
Figure 3.

Immunolocalization of LVF antigens in reproductive and endocrinologic tissues. *A*, LASV antigens in breast, including vacuolated ductal epithelial cells and cuboidal milk-secreting cells (lactocytes). *B*, LASV antigens in cortical cells of adrenal gland, especially zona fasciculata and zona reticularis. *C*, LASV antigens in theca cells, both theca interna and theca externa. *D*, LASV antigens in stromal cells of ovary. *E*, LASV antigens in endothelial cells of endometrium vasculature. *F*, LASV antigens in endothelial cells of fallopian tube vasculature. *G* and *H*, LASV antigens in trophoblastic cells of placenta. Immunoalkaline phosphatase with napthol fast red substrate and hematoxylin counterstain: *A–H*. Original magnifications: ×400 (*A, B, D*), ×630 (*C, G, H*), ×200 (*C, F*), ×50 (*E*). Abbreviations: LASV, Lassa fever virus.
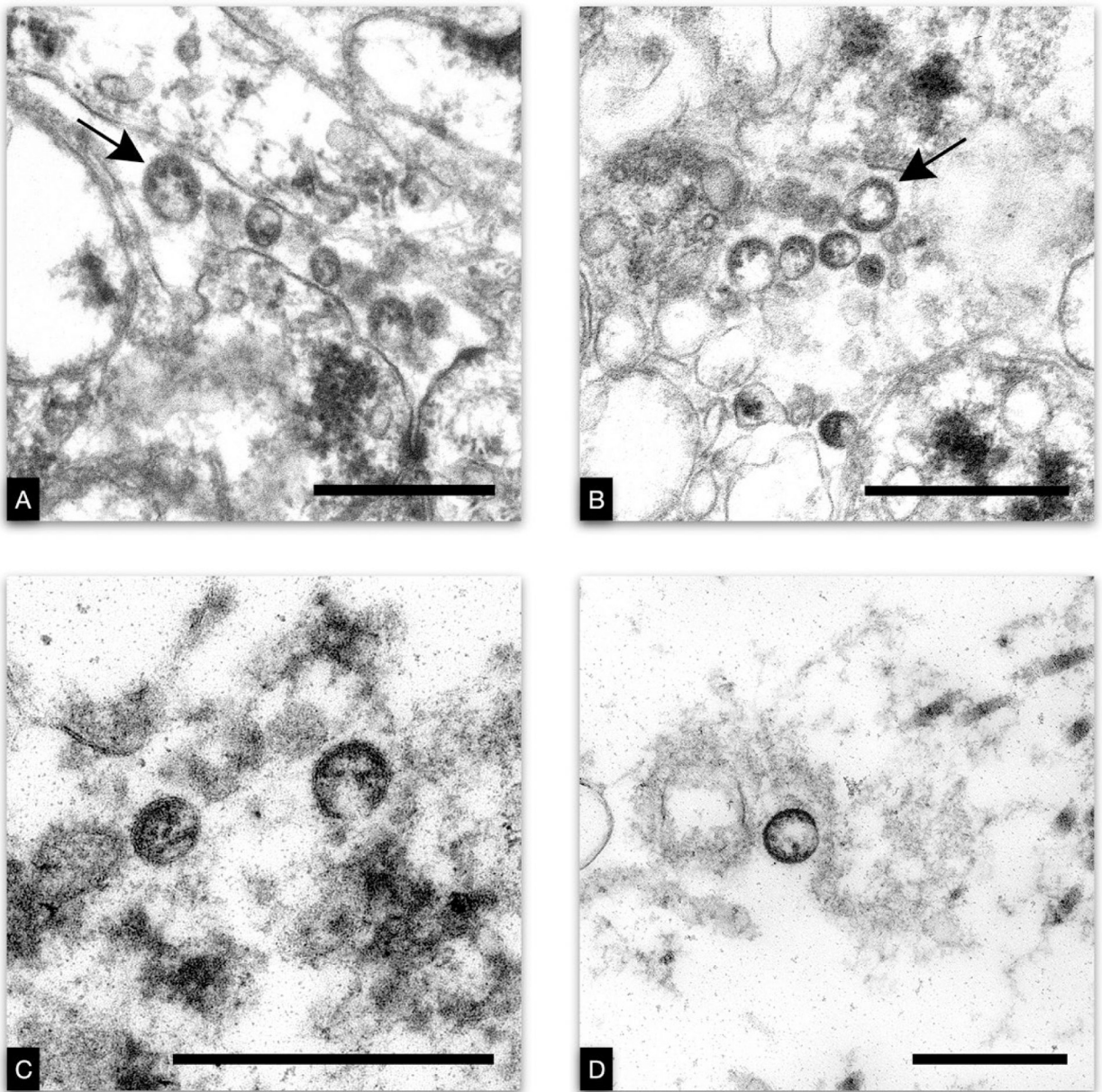
Figure 4.
Electron microscopic images of Lassa virus in fatal cases. Spherical virions (arrows) were found in the (*A*) ovary, (*B*) breast, (*C*) placenta, and (*D*) conjunctiva. Note the ribosomes within the particles, a characteristic feature of arenaviruses. Bars, 500 nm.

Table 1.

Tissue Source and Availability for Studies

Case Number	Source	Tissues Available for Histopathologic Examination and IHC
1	CDC archive	Brain, spleen, pancreas, heart, intestine, lung
2	CDC archive	Brain, liver, pancreas, kidney, heart, lung
3	CDC archive	Heart, lung, liver, spleen, pancreas, kidney, placenta, umbilical cord, ovary, skeletal muscle, adrenal, intestine
4	CDC archive	Lung, liver, spleen, urinary bladder, kidney, adrenal, pancreas, muscle, lymph node
5	CDC archive	Heart, lung, pancreas, intestine, ovary, uterus
6	CDC archive	Heart, lung, liver, spleen, kidney, pancreas, intestine, uterus, muscle, fetal lung
7	CDC archive	Brainstem, pons, cerebellum, heart, lung, liver, spleen, pancreas, kidney, adrenal, lymph node, uterus, ovary, placenta
8	CDC archive	Heart, lung, liver, spleen, pancreas, kidney, adrenal, muscle, ovary, intestine, breast
9	1997 Sierra Leone autopsy, pregnant female	Heart, lung, liver, spleen, pancreas, kidney, adrenal, urinary bladder, tonsil, conjunctiva, parotid gland, oral mucosa, nasal mucosa, skin, muscle, breast, nipple, esophagus, stomach, small intestine, large intestine, mesentery, mesenteric lymph node, ovary, uterus, endocervix, Fallopian tube
10	1997 Sierra Leone autopsy, aborted fetus of case 9	Fetal tissues, placenta, umbilical cord
11	1997 Sierra Leone autopsy, pregnant female	Heart, lung, liver, spleen, pancreas, kidney, adrenal, urinary bladder, tonsil, conjunctiva, parotid gland, oral mucosa, nasal mucosa, skin, muscle, breast, nipple, esophagus, stomach, small intestine, large intestine, mesentery, mesenteric lymph node, ovary, uterus, endocervix, Fallopian tube
12	1997 Sierra Leone autopsy, aborted fetus of case 11	Fetal tissues, placenta, umbilical cord

Abbreviations: CDC, Centers for Disease Control and Prevention; IHC, immunohistochemical; LN, lymphnode.

Table 2.

Summary of LASV Immunolocalization and Associated Implications

Tissue Tropism	Cellular Tropism	Clinical Implications	Pathogenetic Implications
Liver	Hepatocytes and sinusoidal lining cells, mainly Kupffer cells and endothelial cells	Mainly transaminitis; may increase bleeding tendency with large amount of hepatic necrosis	Direct cytopathic damage of hepatocytes in large amount may decrease production of multiple coagulation factors
Endothelial cells and mononuclear phagocyte system	Endothelial cells, tissue macrophages, and dendritic cells	Systemic shock, facial edema, lung edema, dyspnea, thrombocytopenia; vertical transmission of LASV, evidenced by immunostaining of endothelial cells in fetus	Systemic immunologic responses, including release of cytokines, interferon, and other inflammatory mediators; increased capillary permeability; secretion or lack of secretion of particular products
Mucosal tissues	Endothelial cells and macrophages in nasal mucosa, oral, mucosa, and conjunctiva	Subconjunctival effusion, pharyngeal edema, and sore throat; transmission of LASV via contact with patient's nasal discharge, saliva, or tear	Mucosal congestion and edema due to endothelial and vasculature involvement
Serosal membranes	Mesothelial cells in pleura, pericardium, and visceral peritoneum	Pleural effusion, pericardial effusion, ascites	Aggravate cardiac or respiratory failure if large amount of effusion is present
Reproductive and endocrinologic tissues	Ductal epithelial cells in breast, cortical cells of adrenal gland, theca cells and stromal cells in ovary, and trophoblastic cells in placenta	Vertical transmission of LASV via breastfeeding or trans-placental route; higher mortality in pregnant females may be attributed to effects of hormones, especially estrogen	An interplay between LASV infection and hormones, such as cortisol, estrogen or progesterone, which may induce receptors to facilitate virus entry or affect immune responses

Abbreviation: LASV, Lassa fever virus.