11
Viral Infections of the Lung

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The most widespread and fatal of all acute diseases, pneumonia, is now Captain of the Men of Death.

—Sir William Osler, 1901[1]

The lungs are among the most vulnerable to microbial assault of all organs in the body. From a contemporary vantage, lower respiratory tract infections are the greatest cause of infection-related mortality in the United States, and rank seventh among all causes of deaths in the United States.[2,3] From a global and historic perspective, the scope and scale of lower respiratory tract infection is greater than any other infectious syndrome, and viral pneumonias have proven to be some of the most lethal and dramatic of human diseases. The 1918–1919 influenza pandemic, perhaps the most devastating infectious disease pandemic in recorded history, resulted in an estimated 40 million deaths worldwide, including 700,000 deaths in the U.S.[4] The global outbreak of severe acute respiratory syndrome (SARS) during 2003, although considerably smaller in scale, resulted in 8098 cases and 774 deaths[5] and is a dramatic contemporary example of the ability of viral pneumonias to rapidly disseminate and cause severe disease in human populations.

Although viruses are commonly identified causes of pneumonia of infants and young children, they are relatively infrequently recognized as agents of community-acquired pneumonia in adults.[6] In several large series that investigated a microbiologic cause of community-acquired pneumonia, viral etiologies were identified in only 9% to 18% of cases (Table 11.1).[7–12] However, it is likely that viral pneumonias are underrecognized and underdiagnosed for various reasons. Although some agents may cause distinct cytopathology or inclusions (e.g., adenoviruses, herpesviruses, and paramyxoviruses), many important pathogens (e.g., influenza viruses) do not, and none of these agents are resolved specifically in tissue by routine histologic stains. Viruses require live cells for cultivation, and are generally more difficult than bacteria to isolate from clinical samples. For some viral pneumonias,

the pathogen appears to initiate a cascade of destructive host responses that continue or progress even in the absence of the specific infectious agent, and in these patients the etiologic agent may be absent from host tissues at the time of autopsy.[13] Thirty to sixty percent of community-acquired pneumonias are etiologically undetermined,[14] and it is entirely possible that viruses directly cause more episodes of pneumonia than currently appreciated.

Because viral infections of the lower respiratory tract often precede bacterial pneumonias, viruses may indirectly exert considerable influence on the cumulative morbidity and mortality of infectious pneumonias.[15,16] The mechanisms by which viruses may facilitate bacterial invasion of the respiratory tract are complex and varied. Certain viruses cause the death of ciliated respiratory epithelium and thereby disrupt normal ciliary activity. Viruses may also inhibit the phagocytic or bactericidal activities of neutrophils, T lymphocytes, and alveolar macrophages, and predispose the host to secondary infections. Certain gram-positive and gram-negative bacteria adhere to and colonize virus-infected epithelium more readily than to noninfected cells by various hypothetical mechanisms, including alteration and induction of receptors at the host–cell surface and changes in the extracellular environment.[17–19] Finally, viral infections of the lung may exacerbate noninfectious pulmonary conditions (e.g., asthma and chronic obstructive pulmonary disease) and indirectly contribute to aggregate morbidity and mortality associated with these conditions.[20]

Although influenza viruses remain the most frequently identified cause of viral pneumonia in adults (Table 11.1), the diversity of agents identified as causes of viral pneumonias has expanded considerably. Several newly recognized viral pneumonias have been identified since 1992 that are among the most feared and lethal of all emerging infections, including those caused by hantaviruses, Nipah virus, and SARS coronavirus (CoV).[13,21–23] Certain causes

TABLE 11.1. Viral etiologies identified by culture isolation or serologic assays in adult patients with community-acquired pneumonias

Reference	Study interval	No. of patients studied (% with viral etiology)	Influenza A or B	No. of viral etiologies identified					
				RSV	HPIV	Adeno	CMV	Varicella	Other[a]
Fekety et al.[11]	1965–66	100 (17)	10	0	3	1	0	0	3
Sullivan et al.[9]	1967–68	292 (12)	27	1	4	3	0	0	0
Macfarlane et al.[8]	1980–81	127 (9)	7	2	0	1	0	1	0
Woodhead et al.[10]	1984–85	236 (13)	19	5	1	5	0	0	1
Ortqvist et al.[7]	1987	277 (16)	7	8	13	15	0	0	0
de Roux et al.[12]	1996–2001	338 (18)	37	5	11	5	0	0	3
Total (% of viral etiologies identified)		107 (54)		21 (11)	32 (16)	30 (15)	1 (0.5)	1 (0.5)	6 (3)

[a]Rubella (2), rhinovirus (1), mixed viral infections (3).
Source: Adapted from Greenberg.[6]

of viral pneumonia, particularly those that occur in vulnerable patient cohorts, have diminished during this same interval. By example, the U.S. incidence of varicella pneumonia has dropped by two thirds since universal childhood vaccination for varicella was implemented in 1995,[24,25] and advances in the clinical management of transplant recipients have reduced the incidence of cytomegalovirus (CMV) pneumonia.[26] Also occurring during the last decade has been the development and use of powerful molecular techniques that have unveiled the identity of historic pathogens (e.g., the H1N1 "Spanish" influenza A virus),[27,28] and have facilitated the rapid characterization of emerging agents (e.g., SARS-CoV).[13]

It should be noted that the disease manifestations of several of these agents (e.g., human parainfluenza virus [HPIV], respiratory syncytial virus [RSV], and influenza) are often confined to the upper airway and are not invari-ably associated with pneumonia. With some of these pathogens (e.g., influenza viruses, RSV, hantaviruses, and HPIV viruses) respiratory disease is the primary mani-festation. For other agents, such as measles, Nipah virus, and herpesviruses, typically the lungs are involved as part of a multisystem syndrome.

The diagnosis of viral pneumonia, suspected by patient history and clinical manifestations, also can be supported histopathologically, and the general pattern of histopath-ologic lesions may suggest a specific diagnosis. Many viruses can be identified in lung by examining the tissue response and cytopathic changes. Some of these viruses cause recognizable tissue reaction patterns including nec-rotizing tracheobronchitis, bronchiolitis, and interstitial pneumonia. A summary of the key diagnostic histopatho-logic and ultrastructural features for the most common viral pathogens that cause a majority of pulmonary infec-tions is provided in Tables 11.2 and 11.3.

TABLE 11.2. Diagnostic histopathologic features of viral pneumonias

Family	Virus	Inclusions (location)	Pulmonary tissue reaction
Adenoviridae	Adenovirus	Yes (nuclear)	Necrotizing bronchiolitis; smudge cells; DAD
Bunyaviridae	Hantavirus	No	Severe edema, early DAD
Coronaviridae	SARS Coronavirus	No	Interstitial pneumonia; DAD; occasional multinucleation
Herpesviridae	Cytomegalovirus	Yes (nuclear and cytoplasmic)	Interstitial pneumonia; DAD; cytomegaly
	Herpes simplex	Yes (nuclear)	DAD; necrosis and rare multinucleation
	Varicella-zoster	Yes (nuclear)	DAD; necrosis and rare multinucleation
Orthomyxoviridae	Influenza	No	DAD; necrotizing bronchiolitis
Paramyxoviridae	Measles	Yes (nuclear and cytoplasmic)	Interstitial pneumonia with multinucleation DAD
	Parainfluenza	Yes (cytoplasmic; ill-defined)	DAD; interstitial pneumonia with occasional multinucleation
	Respiratory syncytial virus	Yes (cytoplasmic; ill-defined)	Necrotizing bronchiolitis, interstitial pneumonia with occasional multinucleation
	Human metapneumovirus	Yes (cytoplasmic; ill-defined)	Recently recognized; human pathology not well described
	Nipah	Yes (nuclear and cytoplasmic)	Interstitial pneumonia with multinucleation; DAD

DAD, diffuse alveolar damage.

TABLE 11.3. Diagnostic ultrastructural features of viral pneumonias

| Family	Diagnostic ultrastructural features
Adenoviridae	70–90 nm nucleocapsids (NCs) with a dense core are found in cell nuclei, sometimes in paracrystalline arrays
Bunyaviridae	Virions difficult to detect; characteristic NC granulofilamentous inclusions in endothelial cells are seen occasionally in human tissues
Coronaviridae	Spherical, enveloped virions, approx. 75 but up to 160 nm in diameter, accumulate in cytoplasmic vesicles and are often found adherent to the plasma membrane
Herpesviridae	100 nm viral NCs are found in cell nuclei; in cytoplasm, tegument surrounds the NCs; enveloped virions, 120–200 nm, are found in Golgi cisternae and extracellularly
Orthomyxoviridae	Pleomorphic and filamentous virions, 80–100 nm in diameter, are composed of enveloped, filamentous NCs found budding at the plasma membrane of infectious cells; cytoplasmic and nuclear inclusions are sometimes seen
Paramyxoviridae	*Paramyxovirinae* (measles, Nipah, HPIV) are enveloped, pleomorphic virions, 125–250 nm in diameter, consisting of an enveloped aggregation of filamentous NCs; the 18-nm-wide NCs align under plasma membranes as virions bud into extracellular space; *Pneumovirinae* (RSV, HMPV) are smaller than *Paramyxovirinae*; the roughly spherical, enveloped particles average 90–130 nm, and contain 14 nm wide NCs

Only certain viruses can cause cytopathic changes that are sufficiently distinct to enable the pathologist to recognize a specific diagnosis on routine histologic examination of lung specimens. With the availability of special diagnostic techniques, such as immunohistochemistry (IHC) and in-situ hybridization (ISH), many viruses can be detected in formalin-fixed, paraffin-embedded tissue samples even if specific viral inclusions cannot be found in histologic examination of tissue sections. Among the techniques, IHC utilizing specific antibodies can be routinely performed on formalin-fixed tissue and can enhance the pathologist's accuracy in identifying organisms in tissue specimens.

In addition to histologic pattern recognition, IHC, and ISH in tissue, several other diagnostic tests are available to aid the pathologist. Cell culture techniques, serology, polymerase chain reaction (PCR), and electron microscopy (EM) all play vital roles in the diagnosis of these infections. While histologic techniques can be an excellent means of demonstrating organisms, tissue culture isolation remains essential for definitive identification of the virus. When a viral pneumonia is suspected, samples of lung tissues should be evaluated by cell culture, which has the advantage of being a nonbiased method for screening purposes that does not rely on the availability of specific antibodies or probes. Electron microscopy offers the same utility as a broad scope diagnostic tool and has been especially critical in outbreaks of unknown etiology. It played a critical role during the Hendra and Nipah virus outbreaks in 1994 and 1999, respectively, and more recently, in the early recognition of a novel coronavirus associated with SARS in 2003 and in the diagnosis of emerging transplant-associated infections.[13,22,29–33] The advantage of this approach is that viral particles may be demonstrated by negative stain or thin section EM, either directly in clinical material or after amplification in cell culture. Like culture, EM is not limited by narrow specificity of reagents or by prior clinical bias (Fig. 11.1).

FIGURE 11.1. Negative stain electron microscopic images of different viruses that can cause pulmonary infections. **A.** Adenovirus. Adenoviruses are protein-shelled icosahedral-shaped nonenveloped viruses that measure approximately 70 to 90 nm in diameter. Two of the viruses are stain penetrated revealing the DNA-containing nucleoprotein. **B.** Sin Nombre virus. Sin Nombre virus, the causative agent of hantavirus pulmonary syndrome, belongs to the genus hantavirus in the family *Bunyaviridae*. The envelopes of hantaviruses are checkerboard in appearance, and particles measure 90 to 150 nm in diameter. **C.** Herpes simplex virus. The stain has penetrated the envelope of several of these herpesvirus particles, delineating the icosahedral-shaped nucleocapsids, which measure 90 to 100 nm in diameter. **D.** Cytomegalovirus. Another herpesvirus; one virus particle (left) is intact while two nearby particles are stain-penetrated and show the viral nucleocapsids. The nucleocapsid of the upper right particle shows a central core that harbors the DNA of the virus. **E.** Influenza virus. Influenzaviruses belong to the family Orthomyxoviridae; viral particles are pleomorphic and can be filamentous or spherical in shape. The evenly spaced spikes cover the entire virus surface and contain both the hemagglutinin and neuraminidase surface glycoproteins. **F.** Human metapneumovirus. These paramyxoviruses are heterogeneous in size and shape, and range in size from 150 nm to 1 μm in diameter. **G.** Parainfluenza virus. Another paramyxovirus, the viral nucleocapsid, with its typical herringbone appearance, can be seen both within the stain-penetrated particle as well as partially extruded from the virion. An important feature that can help distinguish between Paramyxovirinae (parainfluenza and measles viruses) and Pneumovirinae (human metapneumovirus and respiratory syncytial virus) is the diameter of the nucleocapsids, which measure 18 nm and 14 nm, respectively. **H.** Severe acute respiratory syndrome (SARS) coronavirus. These 80- to 100-nm particles are named for the characteristic crown-like fringe on the surface. Scale bars, 100 nm. (**A,B,D,F,H:** courtesy of C. Humphrey; **C,G:** courtesy of E. Palmer; **E:** courtesy of F.A. Murphy, all at Centers for Disease Control and Prevention, Atlanta, GA.)
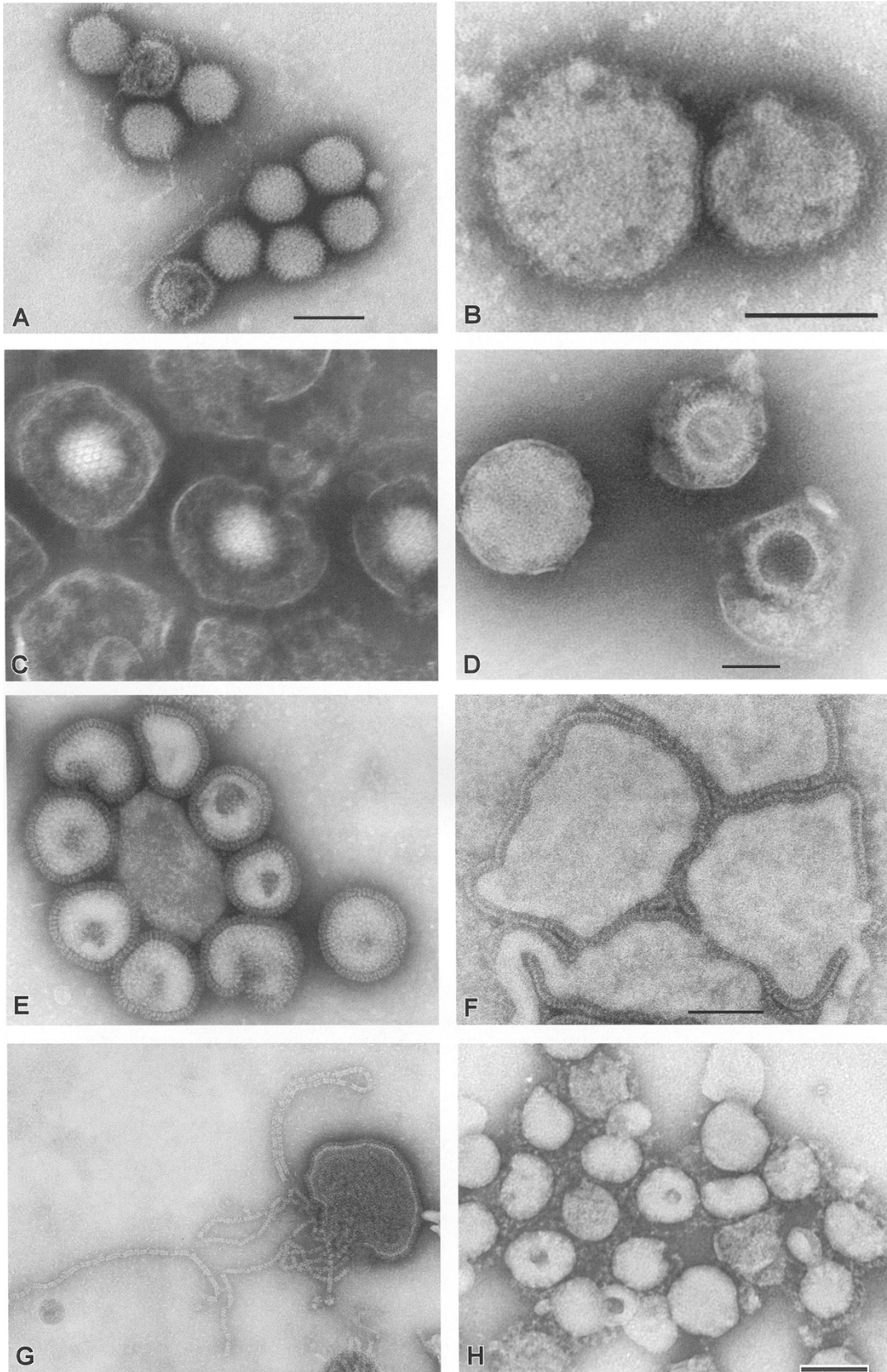
Adenoviruses

Adenoviruses were first cultured and identified during the early 1950s by investigators searching for etiologic agents of acute respiratory infections. The initial adenovirus isolate was made serendipitously from adenoid tissues obtained from children during efforts to establish a primary human adenoid cell line.[34] A related virus was identified the following year by investigators studying respiratory disease in military recruits.[35] These agents were subsequently named *adenoviruses* after the original source of tissue from which the prototype strain was identified.[36] Adenoviruses are nonenveloped viruses with a single, linear, double-stranded DNA genome that is contained within an icosahedral capsid that measures 70 to 90 nm in diameter (Fig. 11.1A). The capsid is comprised of seven known polypeptides, including the hexon capsomere, which contains group-specific antigenic determinants.[37]

Adenoviruses are a ubiquitous and diverse group of viruses found naturally in the upper respiratory tracts and gastrointestinal systems of humans, other mammals, and birds. Most adenoviruses infect mucosal epithelium, although some pathogens of animals are trophic for endothelial cells, and endothelial infection has been identified in some immunocompromised humans.[38] Adenoviruses are represented by at least 51 serotypes on the basis of resistance to neutralization by antisera to other known adenovirus serotypes, and comprise six subgroups or subgenera (A through F) that are distinguished by differential hemagglutination with erythrocytes from various animal species.[37,39,40] More than 50% of the known adenovirus serotypes are associated with human diseases of the upper and lower respiratory tract, conjunctiva, urinary tract, intestine, and occasionally heart, liver, and central nervous system. The others are rarely encountered and may or may not act as pathogens in recognizable disease.[37]

It is estimated that approximately 5% to 10% of all pneumonias in infants and young children are caused by adenoviruses.[41,42] Most pediatric cases of adenovirus pneumonia occur between 6 months and 5 years of age, and serotypes 3, 7, and 21 (all members of the B subgenus), are the most common causes of pneumonia in this patient cohort.[43–45] Serotypes 3 and 7 are particularly pathogenic adenoviruses that can cause disseminated and often fatal disease in previously healthy children.[46] In adults, pneumonia is generally associated with serotypes 3, 4, and 7.[47] Periodic epidemics of adenovirus pneumonia in young adults have been identified, particularly among military recruits.[48,49]

In a manner similar to other pathogens, adenoviruses take advantage of impaired or destroyed immune systems to establish persistent and disseminated infections in immunocompromised hosts. In this patient cohort, the case fatality rate of adenoviral pneumonia approaches 60%, compared with an approximately 15% mortality in immunocompetent patients.[37] Immunocompromised patients are also susceptible to a broader range of different adenovirus serotypes. By example, the commonly recognized serotypes in normal children account for only about 50% of the adenovirus serotypes reported for children with congenital immune deficiencies.[37,46] Because some adenoviruses establish latency in lymphoid tissues and the kidneys of their host, it is believed that many, possibly most, cases of clinical disease caused by adenoviruses in immunocompromised patients are reactivated infections.[37]

The lungs of patients with adenovirus pneumonia are typically heavy and edematous, and the bronchi are generally filled with mucoid, fibrinous, or purulent exudates. The histopathologic findings (Fig. 11.2) include necrotizing bronchitis and bronchiolitis with extensive denudation of the surface epithelium, particularly in medium-sized (1 to 2 mm in diameter) intrapulmonary bronchi (Fig. 11.2A). Affected airways may be occluded by homogeneous eosinophilic material, mixed inflammatory cells, detached epithelium, and cellular debris. The lamina propria of bronchi and bronchioles is typically congested and infiltrated by predominantly mononuclear inflammatory cell infiltrates. Bronchial serous and mucous glands are also often involved and show necrosis and mixed infiltrates.[50] As the infection progresses, there is involvement of the pulmonary parenchyma, forming bronchocentric necrosis with hemorrhage, neutrophilic and mononuclear cell infiltrates, and karyorrhexis. These findings generally occur against a background of exudative diffuse alveolar damage, with filling of the air space by macrophages, fibrin, and detached pneumocytes, and hyaline membrane formation.[51] Patients with fatal pneumonia may develop disseminated intravascular coagulopathy and demonstrate fibrin thrombi in vessels of the lungs, kidney, heart, adrenals, and central nervous system (see Fig. 4.20 in Chapter 4).[48]

Adenoviruses form intranuclear inclusions in respiratory epithelial cells of the trachea, bronchi, and bronchioles, in the acinar cells of bronchial glands, and in alveolar pneumocytes, and are generally most abundant at the viable edges of necrotic foci. By using the hematoxylin and eosin (H&E) stain, early inclusions appear as small, dense, amphophilic structures surrounded by a cleared zone and peripherally marginated chromatin, similar to herpetic inclusions. As the cellular infection progresses, the inclusion becomes larger (as large as 14 μm in some cells) and more basophilic, and the margins of the nuclear membrane become blurred to form the characteristic "smudge cell" (Fig. 11.2B,C).[50,51] Tracheal aspirates of patients with adenovirus pneumonia may show distinctive features on cytologic preparations that include cells with fine strands of chromatin that radiate from a central
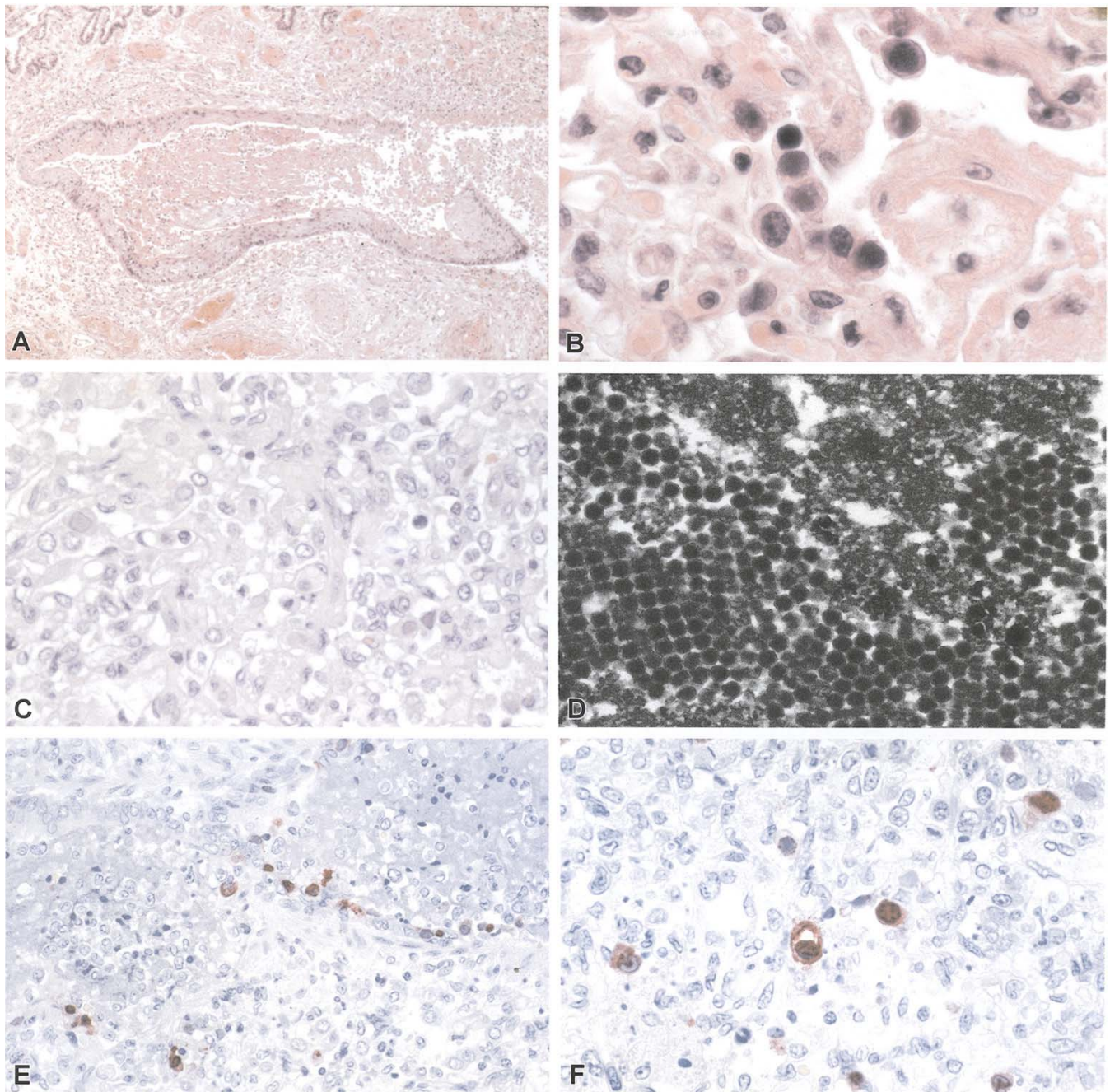
FIGURE 11.2. Adenovirus pneumonia. **A.** Bronchiole containing necrotic debris composed of surface epithelium, fibrin, and mixed inflammatory cells. **B,C.** Large, basophilic, intranuclear inclusions in alveolar pneumocytes, forming characteristic "smudge cells" that can be observed in advanced adenovirus infections. **D.** Paracrystalline arrays of 70 to 90 nm of adenovirus particles in the nucleus of an infected pneumocyte. (Courtesy of C. Goldsmith, Centers for Disease Control and Prevention, Atlanta, GA.) **E,F.** Immunohistochemical localization of adenovirus-infected cells in the pulmonary parenchyma of patient with fatal adenovirus pneumonia. Scale bar, 100 nm. **A–C,** hematoxylin and eosin (H&E); **E,F,** immunoalkaline phosphatase staining, naphthol fast-red, and hematoxylin counterstain; **D,** uranyl acetate and lead citrate stain.

inclusion to the marginated chromatin at the nuclear membrane ("rosette cells"), and cells with foamy, "honeycomb" nuclei, as well as typical smudge cells (see Fig. 7.45 in Chapter 7).[52]

Various methods can be used to diagnose adenovirus infections that include antigen detection, cell culture, electron microscopy, molecular assays, and serology. Direct detection techniques that identify the common group-reactive hexon antigen in tissues or body fluids include fluorescence antibody assays and enzyme immunoassays.[53] Immunohistochemistry staining methods have been used successfully to detect adenovirus-infected cells in formalin-fixed, paraffin-embedded tissues using various commercially available, adenovirus group-specific antibodies (Fig. 11.2E,F).[38,51] Electron microscopy of adenovirus-infected tissues reveals a paracrystalline array of virions (Fig. 11.2D).[54,55]

Most adenoviruses can be isolated in cell culture from bronchial washings, tracheal aspirates, or lung biopsy specimens during the early stage of the illness and grow well in various cell lines, including human embryonic kidney, HeLa, and HEp-2 cells.[47] Cell cultures infected with adenoviruses exhibit a relatively characteristic cytopathologic effect, described as a "cluster of grapes," within 3 to 5 days after inoculation. Serotyping of the isolate is accomplished by using hemagglutination inhibition and neutralization tests with hyperimmune type-specific animal antisera.[56] Molecular assays, particularly gene amplification using PCR, and ISH methods, have been developed to detect adenovirus nucleic acid in respiratory secretions and in formalin-fixed, paraffin-embedded tissues.[51,57,58] Broad-range, sensitive assays that can detect any adenovirus amplify common genomic sequences (e.g., the hexon gene region). Other more specific assays detect specific adenovirus types with unique genomic sequences.[59] Serologic assays include tests for group-specific antibodies (e.g., complement fixation and enzyme immunoassays), or type-specific antibodies (e.g., neutralization and hemagglutination-inhibition assays). Pitfalls associated with serologic testing for adenoviruses include occasional rises in heterotypic antibodies when type-specific assays are used, and relatively low sensitivity demonstrated by complement fixation assays.[59]

Hantaviruses

Human hantaviral diseases are caused by a group of closely related, trisegmented, negative-sense RNA viruses of the genus *Hantavirus*, of the family Bunyaviridae.[60–62] Members of the genus *Hantavirus* have similar morphologic features.[63,64] Virus particles are 70 to 130 nm in diameter and generally appear spherical to ovoid, although pleomorphic forms may be seen. A lipid envelope containing glycoprotein spikes surrounds a core consisting of the genome and its associated proteins (nucleocapsids)

arranged in delicate tangles of filaments showing occasional granulation. The presence of characteristic inclusion bodies in thin section electron microscopy and a unique grid-like pattern on negative-stain electron microscopy differentiate these viruses from other members of the family Bunyaviridae (Fig. 11.1B).[65,66]

The severity and disease type largely depends on the viral serotype. Two categories of hantavirus-associated illnesses are described: hemorrhagic fever with renal syndrome (HFRS) for disease in which the kidneys are primarily involved, and hantavirus pulmonary syndrome (HPS) for disease in which the lungs are primarily involved.[67–69] The isolation of the first recognized hantavirus (Hantaan virus, named for the river in South Korea), and its subsequent identification as the causative agent of HFRS was reported in 1978.[70] In 1993, the deaths of several previously healthy individuals due to a rapidly progressive respiratory disease in the southwestern United States were etiologically linked to a previously unrecognized hantavirus. Clinically, the disease differs from HFRS in its pronounced pulmonary involvement and higher mortality rates and is known as HPS.[23,69,71,72]

Hantavirus-associated diseases primarily affect blood vessels and result in different degrees of generalized capillary dilatation and edema.[73] In contrast to severe HFRS where abundant protein-rich, gelatinous retroperitoneal edema fluid is found, all HPS patients have large bilateral pleural effusions and heavy edematous lungs.[74–77] In fatal Far Eastern HFRS, a distinctive triad of hemorrhagic necrosis can be seen in the renal medullary junctional zone, cardiac right atrium, and anterior pituitary.[75,78] However, in patients with HPS, hemorrhages are rare, and ischemic necrotic lesions, except those attributed to shock, are not seen.[72,74]

Histologically, morphologic changes of the endothelium are uncommon but, when seen, consist of prominent and swollen endothelial cells. Vascular thrombi and endothelial cell necrosis are rare. In HFRS, the most severe and characteristic microscopic lesions involve the kidney; however, an interstitial pneumonitis can also be seen in some fatal cases. In contrast, the microscopic changes of North and South American HPS are principally seen in the lung and spleen.[72,74] The lungs show a mild to moderate interstitial pneumonitis characterized by variable degrees of edema and an interstitial mononuclear cell infiltrate comprised of a mixture of small and enlarged mononuclear cells with the appearance of immunoblasts (Fig. 11.3A). Focal hyaline membranes composed of condensed proteinaceous intraalveolar edema fluid, fibrin, and variable numbers of inflammatory cells are observed (Fig. 11.3B). Typically, neutrophils are scanty and the alveolar pneumocytes are intact with no evidence of cellular debris, nuclear fragmentation, or hyperplasia. In fatal cases, with a prolonged survival interval, tissues show features more characteristic of the exudative and
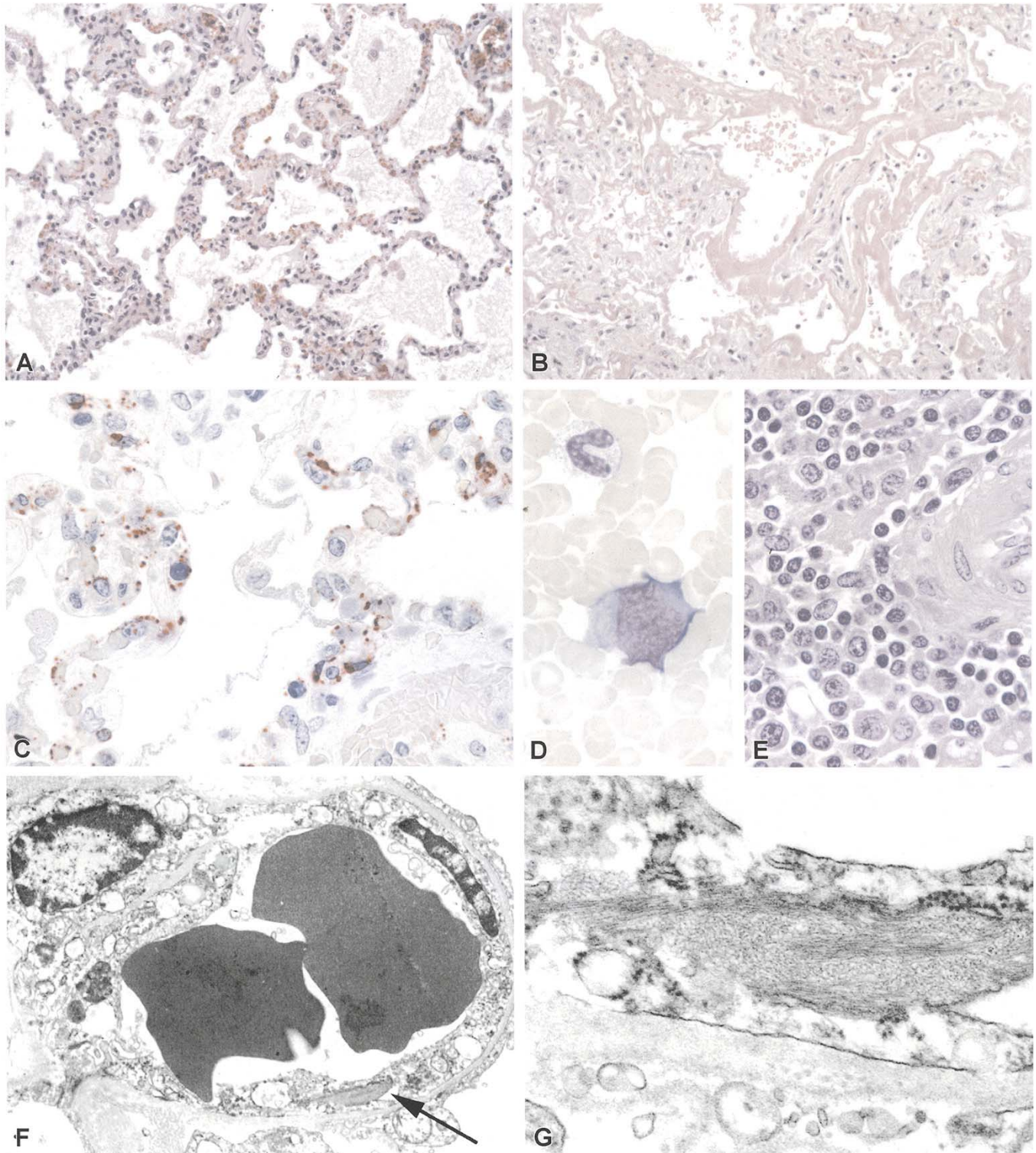
FIGURE 11.3. Hantavirus pulmonary syndrome (HPS). **A.** Lung showing a mononuclear interstitial pneumonitis and intraalveolar edema in a typical case of HPS. **B.** Patchy areas of alveolar septal thickening and prominent hyaline membranes in an HPS patient who died on the 19th day of illness. **C.** Widespread immunostaining of hantaviral antigens in pulmonary microvasculature of an HPS patient. **D.** Peripheral blood smear showing a circulating immunoblast with deeply basophilic cytoplasm, high nuclear-to-cytoplasmic ratio, and prominent nucleolus. **E.** Spleen from a fatal case in which immunoblasts are seen in the periarteriolar sheath. Note prominent nucleoli and high nuclear-to-cytoplasmic ratio. **F.** Ultrastructural appearance of typical hantavirus inclusion within a pulmonary capillary (arrow). **G.** High magnification of inclusion in **F** showing a granulofilamentous viral inclusion within capillary endothelium. **A,B,D,E,** H&E; **C,** immunoalkaline phosphatase staining with naphthol fast-red and hematoxylin counterstain; **F,G,** uranyl acetate and lead citrate stain.

proliferative stages of diffuse alveolar damage. Lung biopsies taken from patients who survive their illness appear similar with proliferated reparative type II pneumocytes, severe edematous and fibroblastic thickening of the alveolar septa, and severe air-space disorganization with distorted lung architecture (see Chapter 4). Other characteristic microscopic findings in HPS cases include variable numbers of immunoblasts within the splenic red pulp and periarteriolar white pulp, lymph nodal paracortical zones, hepatic portal triads, and peripheral blood (Fig. 11.3D,E). Similarly, in severe HFRS cases, large mononuclear cells can be present in the spleen, lymph nodes, blood, and hepatic portal triads.[75,76]

Electron microscopic studies of HPS lung tissue demonstrate infection of endothelial cells and macrophages.[69,72] The virus or virus-like particles observed are infrequent and extremely difficult to identify in autopsy tissues because of the considerable degree of viral pleomorphism and the postmortem deterioration of tissues. However, typical hantaviral inclusions are seen more frequently and their identity can be confirmed by immunolabeling (Fig. 11.3F,G). Similar inclusions are observed in epithelial cells in HFRS and are considered to be ultrastructural markers of hantavirus-infected cells.[63,79,80]

Using immunohistochemistry, viral antigens are found primarily within capillary endothelium throughout various tissues in both HPS and HFRS. In HPS, marked accumulations of hantaviral antigens are in the pulmonary microvasculature and in splenic and lymph nodal follicular dendritic cells (Fig. 11.3C).[72] Despite the extensive endothelial cell accumulations of hantaviral antigens, there is little ultrastructural evidence of cytopathic effect.

Hantavirus pulmonary syndrome should be suspected in cases of adult respiratory distress syndrome (ARDS) without a known precipitating cause among previously healthy individuals. The level of suspicion should be particularly high when patients have a known exposure to rodents in areas where *Peromyscus maniculatus* or other reservoirs of hantavirus are found. Physicians need to differentiate HPS from other common acute respiratory diseases, such as pneumococcal pneumonia, influenza virus, and unexplained ARDS. The diagnosis of HPS, suspected by patient history and clinical manifestations, can also be supported histopathologically. Although there is no single pathognomonic lesion that would permit certain histopathologic diagnosis of HPS, the overall constellation of histopathologic hematologic findings suggests the diagnosis.[72,74] Diseases that need to be distinguished pathologically from HPS include a relatively large number of different viral, rickettsial, and bacterial infections, as well as various noninfectious disease processes.

Virus-specific diagnosis and confirmation can be achieved through serology, PCR for hantavirus RNA, or IHC for hantaviral antigens.[21,72] Serologic testing can detect hantavirus-specific immunoglobulin M or rising titers of immunoglobulin G in patient sera and is considered the method of choice for laboratory confirmation of HPS. Immunofluorescent assays and enzyme-linked immunosorbent assays (ELISAs), which demonstrate the presence of specific antihantaviral antibodies, are currently used as rapid diagnostic tests and provide results within a few hours. Recently, synthetic hantaviral nucleocapsid proteins have been used to improve the sensitivity and specificity of serologic assays. These proteins are more available than inactivated hantaviral antigens.[81,82] Polymerase chain reaction detects viral RNA in blood and tissues and is extremely useful for diagnostic and epidemiologic purposes. Hantaviral RNA can also be detected in formalin-fixed, paraffin-embedded archival tissue by reverse-transcriptase (RT)-PCR.[83] Immunohistochemistry testing of formalin-fixed tissues can be used to detect hantavirus antigens, and is a sensitive method to confirm hantaviral infections.[72] It has a unique role in the diagnosis of fatal HPS cases when serum samples and frozen tissues are unavailable but formalin-fixed autopsy tissues are obtainable.[84,85]

Severe Acute Respiratory Syndrome

The causative agent of SARS is an enveloped, positive-stranded RNA virus that is a member of the genus *Coronavirus*, of the family Coronaviridae. Coronaviruses have the largest genomes of all RNA viruses and replicate by a unique mechanism that results in a high frequency of recombination. Maturation of SARS coronavirus (SARS-CoV) is similar to features previously described for other coronaviruses.[86-90] Virions form by alignment of the helical nucleocapsids along the membranes of the endoplasmic reticulum or Golgi complex and acquire an envelope by budding into the cisternae. The cellular vesicles become filled with virions and progress to the cell surface for release of the virus particles; large numbers of particles remain adherent to the plasma membrane at the cell surface.

Severe acute respiratory syndrome was recognized during a global outbreak of severe pneumonia that began in late 2002 in Guangdong Province, China, and gained prominence in early 2003 as cases were identified in more than two dozen countries in Asia, Europe, North America, and South America. The disease causes an influenza-like illness with fever, cough, dyspnea, and headache, and in severe cases it can cause death in humans. Person-to-person transmission, combined with international travel of infected persons, accelerated the worldwide spread of the illness.[5,13,91]

Several reports have described diffuse alveolar damage with various levels of progression and severity as the main histopathologic findings in SARS patients (Fig. 11.4A,B).[13,92-98] Lungs typically show changes
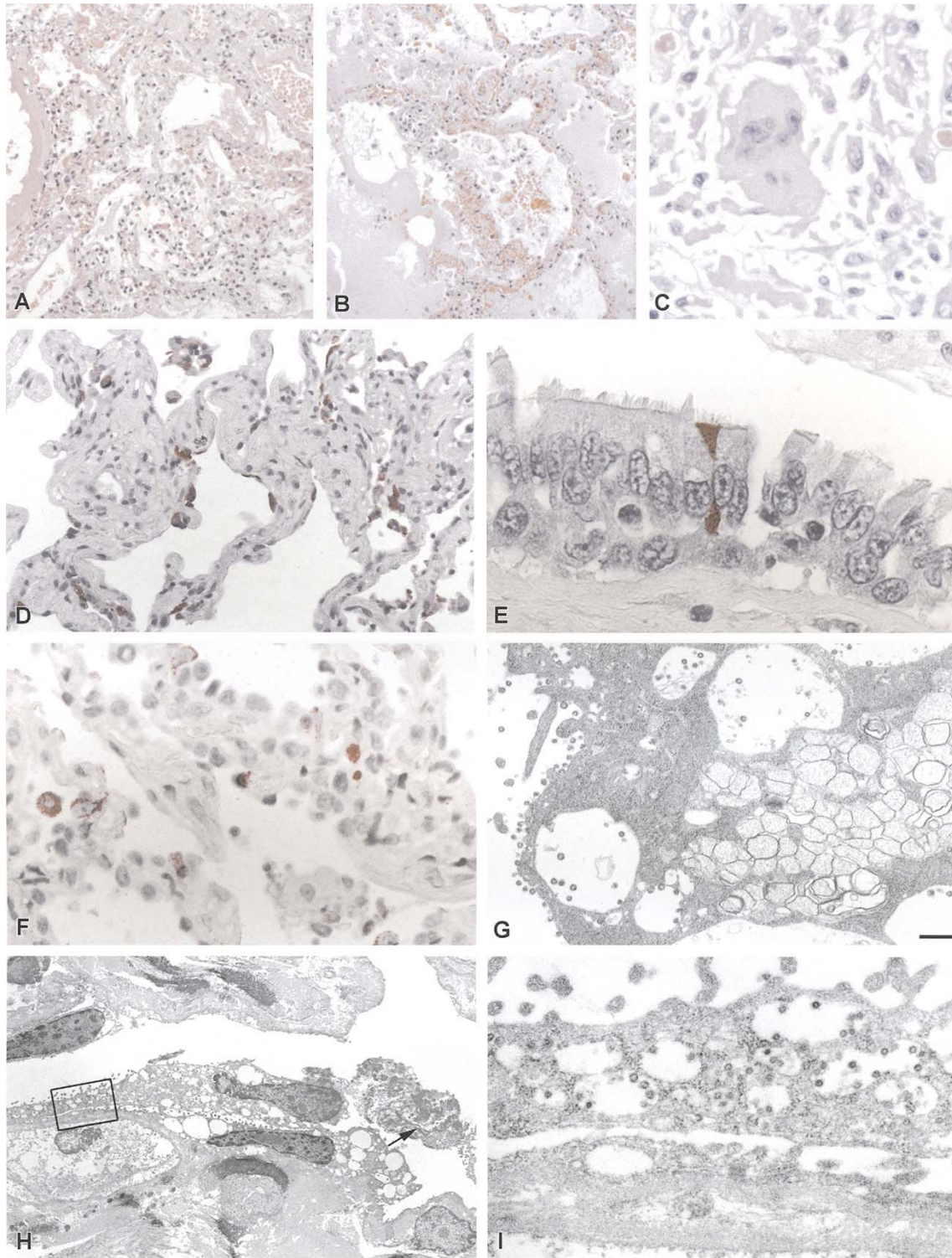
FIGURE 11.4. Severe acute respiratory distress syndrome (SARS). **A,B.** Lung showing interstitial pneumonia, patchy hyaline membranes and prominent intraalveolar edema. **C.** Multinucleated syncytial giant cells can be seen in some cases of fatal SARS. Note absence of discernible viral inclusions. **D.** Abundant immunostaining of coronavirus antigens in alveolar-lining pneumocytes. **E.** Ciliated epithelial cell in upper airway epithelium containing viral antigens. **F.** In-situ hybridization (ISH) showing abundant infected pneumocytes and intraalveolar macrophages containing viral nucleic acids. **G.** Cytoplasmic and extracellular virions are apparent in this electron micrograph of a sloughed pneumocyte from a bronchioalveolar lavage obtained from a patient with SARS. Note the region of double-membrane vesicles, a common feature of coronavirus-infected cells. **H.** Electron micrograph showing an infected pneumocyte, attached at one edge to the basement membrane. **I.** Higher magnification of boxed area in **H,** showing numerous coronavirus particles within the cytoplasmic vesicles of this pneumocyte. **A–C,** H&E; **D,E,** immunoalkaline phosphatase staining, naphthol fast-red, and hematoxylin counterstain; **F,** digoxigenin-labeled probe followed by immunoalkaline phosphatase staining, naphthol fast-red, and hematoxylin counterstain; **G–I,** uranyl acetate and lead citrate stain.

described for the proliferative phase of diffuse alveolar damage, with hyaline-membrane formation, desquamation of epithelial cells, fibrin deposit in the alveolar space, and hyperplasia of type 2 pneumocytes. Increased mononuclear infiltrate in the interstitium can be seen in some cases. Other findings identified in some patients included focal intraalveolar hemorrhage, necrotic inflammatory debris in small airways, and organizing pneumonia. In addition, multinucleated syncytial cells may be seen in the intraalveolar spaces of some patients who died 14 days or more after onset of illness (Fig. 11.4C). Infection with some coronaviruses, including SARS-CoV, is known to induce cell fusion in culture producing syncytial cells similar to those sometimes observed in lungs of patients who die from SARS. These cells contain abundant vacuolated cytoplasm with cleaved and convoluted nuclei, but without obvious intranuclear or intracytoplasmic viral inclusions. The ISH and IHC studies of tissues from SARS patients have identified coronavirus infection of upper airway bronchiolar epithelium (Fig. 11.4D–F).[92,99–101] Infected ciliated columnar epithelial cells can be seen focally in lining epithelium of trachea and larger bronchi (Fig. 11.4E). Many of these infected cells slough from the epithelium and can be observed by using ISH within the bronchial lumen. Abundant viral antigens can also be found distributed focally in parenchyma of lungs of some patients and are seen predominantly in the cytoplasm of pneumocytes, in occasional macrophages, and in association with intraalveolar necrotic debris and fibrin (Fig. 11.4D). Double-stain studies indicate that most SARS-CoV–infected cells are type 2 pneumocytes. Double-stain studies also detected viral nucleic acids with a distribution similar to that seen in IHC studies, mainly in pneumocytes and in some macrophages.[100] Electron microscopic examination of lung tissues selected from areas with abundant IHC staining shows numerous coronavirus particles and nucleocapsid inclusions. Virions are seen in cytoplasmic vesicles and along the cell membranes of pneumocytes, in phagosomes of macrophages, and associated with fibrin in alveolar spaces (Fig. 11.4G–I). Because coronavirus particles may be confused morphologically with other nonviral cellular components, definitive ultrastructural identification can be achieved by using immunogold labeling electron microscopy.

The primary histopathologic lesions seen in the lungs of patients who die from SARS are somewhat nonspecific and can also be seen in acute lung injury cases caused by infectious agents, trauma, drugs, or toxic chemicals.[102] Multinucleated syncytial cells similar to those seen in some SARS patients can also be found in a number of virus infections, including measles, parainfluenza viruses, RSV, and Nipah virus infections.[102–104] In an early study of four human SARS patients,[13] viral antigens were not detected in the lung by IHC. The most likely explanation is that all patients in the study had a clinical course aver-

aging more than 2 weeks. For many virus infections, viral antigens and nucleic acids are cleared within 2 weeks of disease onset by the host immune response. It is also possible that the pulmonary damage associated with SARS is not caused directly by the virus, but represents a secondary effect of cytokines or other factors induced by the virus infection. Similarly, in influenza virus infections, viral antigens are seen predominantly in respiratory epithelial cells of large airways and are only rarely identified in pulmonary parenchyma despite concomitant and occasionally severe interstitial pneumonitis.[105] In recent reports by Shieh et al.[100] and Chong et al.,[92] the temporal relationship between the duration of illness and clearance of SARS-CoV in human lung tissue was examined. Viral antigens and nucleic acids were detected only in pulmonary tissues of patients who died early in the disease.

The development of specific IHC, ISH, and immunoelectron microscopy (IEM) assays to identify SARS-CoV in formalin-fixed, paraffin-embedded samples has facilitated the assessment of the cellular tropism of SARS-CoV infection in human lung tissues. Localization of SARS-CoV in the lung occurs mainly in the cytoplasm of pneumocytes, primarily type 2, and occasionally in alveolar macrophages (Fig. 11.4F). Type 2 pneumocytes are known to secrete pulmonary surfactant, resulting in reduced surface tension and preservation of the integrity of the alveolar space. These cells also play an important role in tissue restitution following lung damage. Moreover, there is mounting evidence to support their contribution to the development of acute inflammatory lung injury following exposure to biological or chemical agents. Additional studies are needed to further define the role of type 2 pneumocytes and alveolar macrophages in SARS-CoV infection.

Cynomolgus macaques inoculated with SARS-CoV develop pathologic findings of pneumonia and have been proposed as an animal model.[106] Haagmans et al.[107] showed extensive SARS-CoV antigen expression in experimentally infected macaques 4 days after infection. The antigens were mainly in alveolar lining epithelial cells with morphologic characteristics of type 1 pneumocytes, indicating type 1 pneumocytes are the primary target for SARS-CoV infection early in the disease. Type 1 pneumocytes normally represent 90% of the alveolar epithelial cell volume and are easily damaged during pulmonary infections or other types of injury. In a more recent study on nonhuman primates,[108] evidence of infection of type 1 pneumocytes in addition to some type 2 pneumocytes and macrophages was found.

Small animal models, such as rodents, would be very useful for evaluating vaccines, immunotherapies, and antiviral drugs, and we have recently identified the mouse as an animal model for this purpose.[109] In those studies, microscopic examination of trachea, bronchus, lung,

thymus, and heart on day 2 after infection revealed mild and focal peribronchiolar mononuclear inflammatory infiltrates with no significant histopathologic change in other organs. Viral antigens and nucleic acids were focally distributed in bronchiolar epithelial cells, and virions were found in these same areas by ultrastructural analysis. Data suggest that SARS-CoV replicates in mice to a titer sufficient to evaluate vaccines and antiviral agents. The mouse and other small animal models[110] might also be used to test the ability of the virus to replicate and cause disease and facilitate identification of host-immune mechanisms that contribute to the resolution of SARS-CoV infection.

Cytomegalovirus

Cytomegaloviruses (CMV) comprise a distinct and ancient group of herpesviruses that are widely distributed in nature, share similar growth characteristics in cell culture, and cause cellular enlargement and form distinctive inclusions in infected cells. These cytopathic changes, identified by early pathologists in the salivary glands of children dying from various unrelated diseases,[111-113] led to the early designation of cytomegalic inclusion disease many years before the causative agent was isolated in the mid-1950s. The name cytomegalovirus was proposed in 1960 to reflect the cytopathic changes caused by these viruses.[114]

Cytomegaloviruses are highly host-specific, and various mammalian hosts, including nonhuman primates, rodents, and domesticated animals, are infected with their own distinct CMV. In this context, human CMV is stringently species-specific and, with rare exception, only infects cells of human origin.[115] Several cell types are permissive for CMV replication, including alveolar pneumocytes, vascular endothelium, fibroblasts, monocytes, dendritic cells, and exocrine and endocrine glandular epithelial cells.[116] Cytomegalovirus is a β-herpesvirus with the largest genome (230 kilobase pair [kbp]) of all the herpesviruses known to infect humans. The double-stranded linear DNA genome is contained within a 90 to 100 nm icosahedral capsid and is surrounded by an amorphous material known as the tegument. These components are enclosed in a lipid bilayer envelope that is derived from the host cell nuclear or Golgi membranes and contains several virally encoded glycoproteins necessary for infection of other cells. Mature enveloped virions range from 150 to 200 nm, making CMV one of the largest viruses that infect humans (Fig. 11.1D).[117] The structure of CMV is typical of other human herpesviruses, but demonstrates some subtle ultrastructural differences from other viruses in this group including greater pleomorphism of the lipid envelope and dense body inclusions in the cytoplasm of infected cells.[118]

Cytomegalovirus is a ubiquitous human pathogen, and in North America infects approximately 50% to 90% of the population.[117] Most of these infections are inapparent, although some cases of primary infection in otherwise healthy individuals result in a self-limited mononucleosis syndrome similar to that caused by Epstein-Barr virus; it is estimated that 20% to 50% of cases of heterophile-negative mononucleosis, and 8% of all cases of mononucleosis, are caused by CMV.[119] Pulmonary involvement in CMV mononucleosis is infrequent and occurs in approximately 6% of these cases.[120] Congenitally acquired CMV infection has various deleterious effects on the fetus, including mental retardation, neurologic abnormalities, sensorineural hearing loss, and retinitis, and in one series pulmonary involvement occurred in 64% of symptomatic infants.[121]

Like all herpesviruses, CMV remains with its host for life after primary infection and establishes latency in various cell types, including vascular endothelial cells, monocytes and macrophages, neutrophils, and renal and pulmonary epithelial cells.[122] Activation of viral replication occurs in persons with severely compromised immunity. Patients with advanced HIV disease and recipients of hematopoietic stem cell or lung transplants are particularly at risk of developing CMV pneumonia. Before the use of CMV screening and effective antiviral prophylaxis regimens, 10% to 30% of all patients undergoing allogeneic bone marrow transplantation for leukemia, and 15% to 55% of solid organ transplants, developed CMV pneumonia with case fatality rates of greater than 80% in some series.[26,123,124] The relatively high frequency of CMV pneumonia in lung transplant recipients may be a correlate of animal model data that indicate the lungs are a major site of latent CMV infection.[125] Before the use of ganciclovir as therapy for CMV disease in AIDS patients, the case fatality rate for CMV pneumonia in this patient cohort was 75% when CMV was the only pathogen identified. Mixed infections with CMV and another pathogen had an even worse prognosis, and the case fatality rate for patients with pulmonary disease caused by CMV and Pneumocystis jiroveci (formerly carinii) was 92%.[126]

Cytomegalovirus pneumonia can show various histopathologic patterns (Fig. 11.5). Extensive intraalveolar hemorrhage with scattered cytomegalic cells and relatively few inflammatory cell infiltrates may occur (Fig. 11.5A).[127] In a similar manner, extensive involvement of the alveolar epithelium with minimal inflammation or overt evidence of parenchymal injury has also been described.[128] Other patterns include multifocal lesions with mixed inflammatory cell infiltrates, hemorrhage, necrosis, and cytomegalic cells, or a diffuse, predominantly mononuclear cell infiltrate, interstitial pneumonitis with intraalveolar edema and fibrin deposition, and diffusely distributed cytomegalic cells (Fig. 11.5E).[129-131]
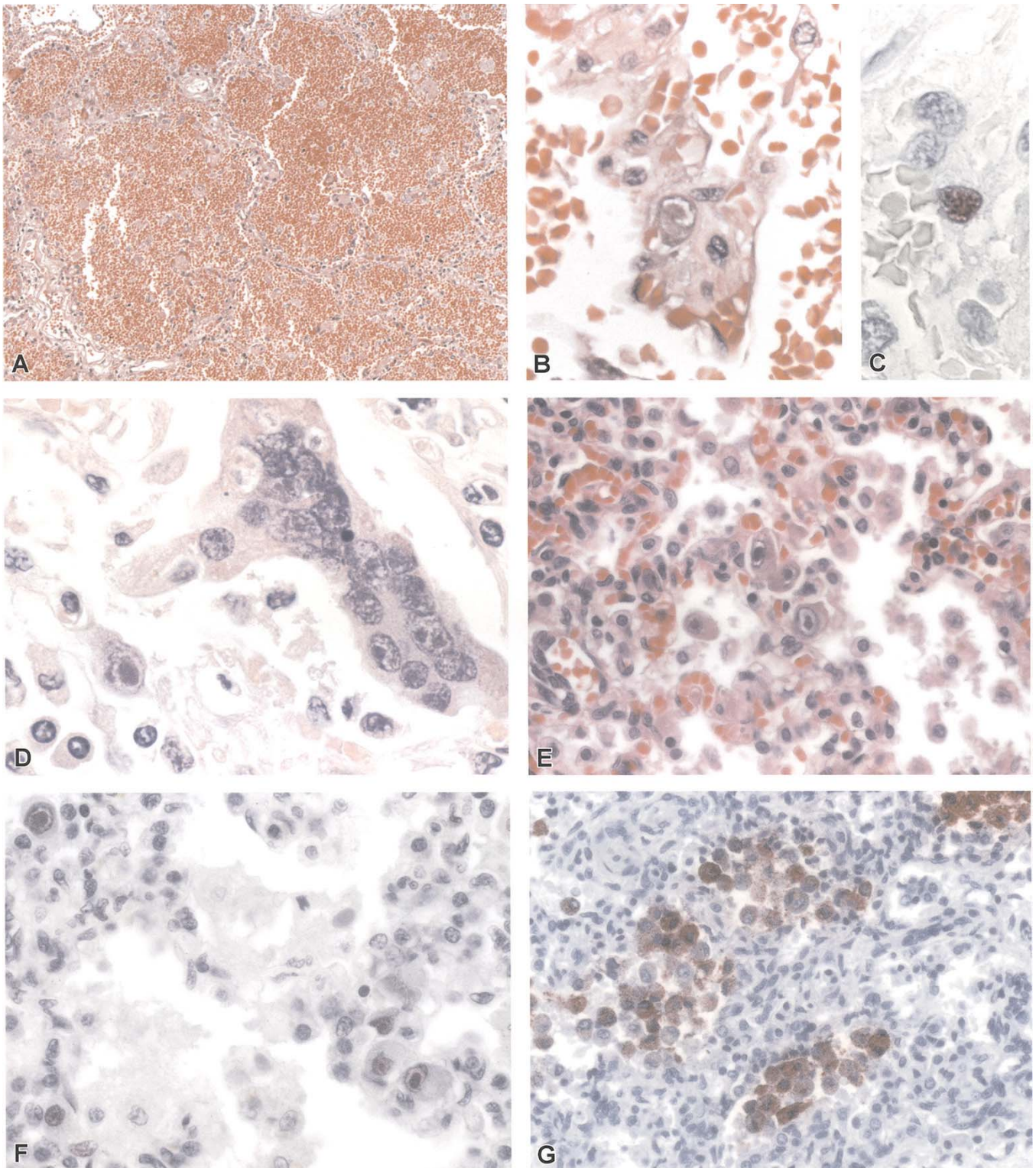
FIGURE 11.5. Cytomegalovirus (CMV) pneumonia. **A,E.** Diffuse intraalveolar hemorrhage with relatively little inflammation; interstitial pneumonitis, with diffusely distributed CMV-infected cells. **B.** CMV-infected cell showing large, amphophilic, owl's-eye intranuclear inclusion and smaller cytoplasmic inclusions. **C,F.** Immunohistochemical localization of CMV-infected cells in the pulmonary parenchyma. **D,G.** CMV as a pulmonary coinfection in patients with fatal measles pneumonia **(D)**, and in a patient with fatal pertussis **(G)**. **A–D,** H&E; **C,F,G,** immunoalkaline phosphatase staining, naphthol fast-red, and hematoxylin counterstain.

The cytomegalic changes of CMV-infected cells are evident by standard H&E staining and are virtually pathognomonic of active CMV infection. The cells are enlarged (25 to 40 μm) and contain amphophilic to deeply basophilic intranuclear and intracytoplasmic inclusions (Fig. 11.5B,E). The single intranuclear inclusion is composed of viral nucleoprotein and assembled capsids, and is a large (up to 20 μm), round to ovoid body with a smoothly contoured border that is generally surrounded by a clear halo that gives the inclusion a distinctive owl's-eye appearance. The host cell nucleolus is often retained in the inclusion.[131] Cytoplasmic inclusions are small (1 to 3 μm), granular bodies that appear after the intranuclear inclusion is well developed and are not uniformly present in all CMV-infected cells (Fig. 11.5B). These inclusions represent a mixture of virions and various cellular organelles, and increase in size and number as the infection progresses.[132] Unlike the intranuclear inclusion, the cytoplasmic inclusions stain with periodic acid-Schiff stain and are deeply argyrophilic with Gomori's methenamine silver stain.[133]

Cytomegalovirus pneumonia is defined by the presence of signs or symptoms of pulmonary disease combined with the detection of CMV in bronchoalveolar lavage (BAL) fluid or lung tissue samples. In this context, detection methods that support this definition include virus isolation, histopathologic observation of cytomegalic cells, ISH, or IHC stains (Fig. 11.5F). Detection by PCR alone is considered too sensitive for the diagnosis of CMV pneumonia and is insufficient for this purpose.[134] Cytomegalovirus is most often cultured in human diploid fibroblasts such as human embryonic lung and human foreskin fibroblasts. It grows slowly in conventional cell culture, and the cytopathic effect is generally not detected until the second week or longer after inoculation. For this reason, a shell vial method using centrifugation to enhance infectivity has become the standard isolation technique and can usually yield diagnostic results within 48 hours.[135]

Herpes Simplex Viruses

For centuries, the term *herpes,* derived from the Greek *erpein* (to crawl), was used in medicine to describe any spreading cutaneous lesion. By the end of the 19th century, investigators surmised that the herpetic lesions of the lips and genitalia were manifestations of a single infectious agent, and recognized that it was a disease distinct from herpes zoster.[136,137] As with all human herpesviruses, HSV is a large, enveloped, double-stranded DNA-containing virion with an icoshedral nucleocapsid approximately 100 to 110 nm in diameter, composed of 162 capsomers. The nucleocapsid is surrounded by an amorphous, sometimes asymmetric material (the tegument) that is surrounded by a thin, trilaminar envelope that contains numerous glycoprotein spikes (Fig. 11.1C). The assembly of HSV begins in the nucleus of its host cell and the virus acquires its envelope as the capsid buds through the inner lamella of the nuclear membrane.[120,138] Two serologic types are recognized and each is most frequently associated with particular disease syndromes; however, either serotype may cause any of the aggregate clinical syndromes. Herpes simplex virus-1 causes gingivostomatitis, pharyngitis, esophagitis, keratoconjunctivitis, and encephalitis, and is the serotype most commonly associated with adult HSV pneumonia. Herpes simplex virus-2 typically infects genital sites such as the penis, urethra, vulva, vagina, and cervix, and is the serotype associated with approximately 80% of disseminated disease and pulmonary infections in newborn infants.[136]

All herpesviruses have the ability to persist in an inactive state for varying periods of time and then recur spontaneously following undefined stimuli associated with physical or emotional stress, trauma to nerve roots or ganglia, fever, immunosuppression, or exposure to ultraviolet radiation.[138] During the primary infection, HSV replicates at the portal of entry (typically oral or genital mucosae), and infects sensory nerve endings. The virus is transported centripetally along peripheral sensory nerves to central axons and finally to nerve cell bodies in the trigeminal, sacral, and vagal ganglia, where it replicates briefly before becoming latent.[139–141] Antiviral drugs have no effect on latent infection with HSV. Following cues that initiate viral reactivation, HSV replicates in sensory ganglia and is transported centrifugally along sensory nerves to epithelial cells on mucosal surfaces.[138] Reactivation of HSV from the trigeminal ganglion is associated with asymptomatic excretion of virus in saliva, and with the development of herpetic ulcers on the vermillion border of the lip, oral mucosa, or external facial skin.[142]

Newborn infants, severely immunosuppressed or burned patients, and patients with severe trauma are at greatest risk of developing HSV pneumonia.[143–146] Lower respiratory tract disease in neonates is most commonly associated with disseminated herpetic infections. Disseminated HSV infection in the newborn was first described in 1935 as "hepatoadrenal necrosis"[147] because of the prominent and frequent necroses that occur in the livers and adrenal glands of affected neonates.[148] Most cases of neonatal disease represent primary HSV infections and are acquired during parturition from HSV-infected mothers. The incidence of neonatal HSV infection is approximately 1 in 3200 deliveries, and disseminated disease develops in approximately 25% of infected neonates.[149] In disseminated infections, signs and symptoms

appear a mean of 5 days after birth (range, 0 to 12 days), and approximately 40% to 50% of these patients develop pneumonia. In the preantiviral era, 85% of neonates with disseminated disease died from the infection. With early diagnosis and high-dose acyclovir therapy, mortality has been reduced to approximately 30%.[136,138,149] The disease can be exceedingly difficult to diagnose in a timely manner as only 10% of mothers of affected infants have clinically apparent HSV infection at the time of delivery.[150] Neonates that survive severe disseminated disease may develop hepatic and adrenal calcifications evident on abdominal radiographs.[151]

In adults, infection of the respiratory tract with HSV may be associated with disseminated herpetic infection, but is more commonly identified as an isolated disease manifestation resulting from reactivation of latent herpetic infections in the oropharynx. Herpetic tracheobronchitis is an ulcerative process characterized by large areas of denuded mucosal epithelium and fibrinopurulent exudate containing necrotic cells with densely eosinophilic cytoplasm. Despite extensive tissue damage, cells with intranuclear inclusions may be sparse, and, when identified, are found most often at the margins of the ulcerated epithelium or occasionally in the mucous glands subjacent to ulcerated surfaces.[152] Aspiration of virus-containing secretions into the lower respiratory tract is believed to be the most frequent cause of pulmonary infection with HSV; however, oral lesions may be absent in patients with herpetic laryngotracheobronchitis and bronchopneumonia.[153] Disease can be also associated with airway trauma caused by tracheal intubation or from hematogenous dissemination of HSV.[144,152,154] Chest radiographs of HSV pneumonia generally show ill-defined nodular or reticular densities of various sizes scattered in both lung fields. During the early stages of disease, these nodules measure 2 to 5 mm and are best seen in the periphery of the lungs. As the disease progresses, these lesions coalesce and enlarge to form more extensive infiltrates.[137]

Herpetic infections of the airways and lung are characteristically difficult to diagnose clinically and HSV pneumonia was not described as a distinct clinical entity until 1949.[155] Several studies attest to the relative infrequency with which this diagnosis is considered in patients with respiratory disease. For example, none of the 15 cases of HSV disease of the middle and lower respiratory tract identified in a review of autopsies at Brooke Army Medical Center during 1965 to 1968 were suspected prior to autopsy.[143] In a 1982 review of 20 culture-confirmed cases of HSV pneumonia, none of the patients had been diagnosed prior to death and all 16 had oral herpetic lesions at the time of death.[144] Recent investigations also suggest that lower respiratory tract disease caused by HSV may be more common than currently appreciated. In a study from Sweden, HSV was cultured from pro-

tected brush and BAL specimens of 34 (26%) of 132 consecutive patients who required assisted ventilation for a suspected acute lower respiratory infection.[156] A similar study from Australia identified an herpetic infection of the lower respiratory tract cytologically in tracheobronchial secretions of 14 (30%) of 46 consecutive patients with ARDS.[157] Concomitant bacterial or fungal pulmonary infections are identified in approximately 20% to 30% of patients with HSV pneumonia.[144,156]

A diagnosis of tracheobronchitis and pneumonia is best established histologically (Fig. 11.6). Because lower respiratory tract HSV infections are often focused in the tracheobronchial tree, open lung biopsy may be less sensitive than bronchoscopy.[154] Herpetic lesions show extensive necrosis and karyorrhectic debris and are associated with hemorrhage and a sparse-to-moderate neutrophilic infiltrate (Fig. 11.6A,B). Intranuclear inclusions are best appreciated in cells at the leading edge of necrotic foci (Fig. 11.6B,C). Inclusions appear either as homogeneous, amphophilic, and glassy (Cowdry type B inclusions), or as eosinophilic with a halo separating the inclusion from the nuclear membrane (i.e., Cowdry type A inclusions).[158] Cowdry type B inclusions contain actively replicating virus. Type A inclusions, considered noninfectious and devoid of viral nucleic acid or protein, represent the nuclear "scar" of HSV infection.[136,141] Other changes associated with HSV, including multinucleation and nuclear molding, and ballooning degeneration of the cytoplasm, are more frequently associated with squamous epithelium and are seldom encountered in the lung. Because of the high frequency of hepatic and adrenal involvement with disseminated HSV infection in young children (Fig. 11.6F,G), liver biopsy has been suggested as a diagnostic technique in this patient cohort.[148] Commercially available antibodies exist for IHC detection of HSV in tissues (Fig. 11.6D).[159]

Virus isolation remains an important diagnostic method; however, because HSV can be isolated from oropharyngeal secretions and occasionally from the lower respiratory tract of patients who lack overt pulmonary disease, virologic cultures must be interpreted in the context of complementary clinical, radiographic, and histopathologic findings as much as possible. Cell culture systems susceptible to HSV include Vero cells and foreskin fibroblasts. Cytopathic effects generally develop within 24 to 48 hours after cultures are inoculated with infectious specimens. Suitable specimens include scrapings made from mucocutaneous lesions, tracheobronchial aspirates, or BAL specimens. In infants with evidence of hepatitis, it may also be useful to obtain duodenal aspirates for HSV isolation.[138] Polymerase chain reaction methods that amplify HSV DNA from clinical specimens, including tissue and blood, can be particularly useful by specifically distinguishing between HSV-1 or HSV-2 infections.[138,149]
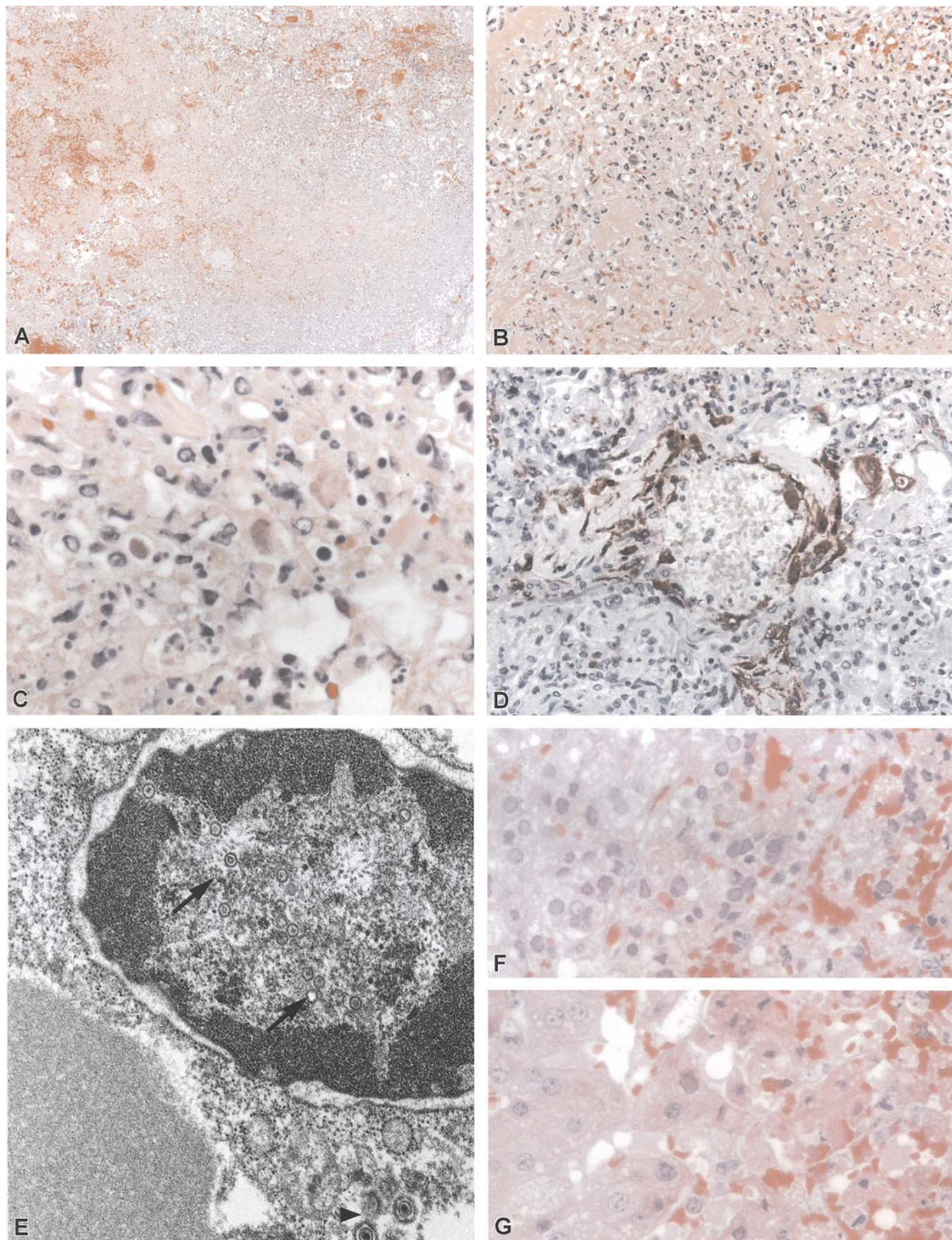
FIGURE 11.6. Herpes simplex virus (HSV) pneumonia. **A,B.** Extensive necrosis and hemorrhage and mild to moderate neutrophilic infiltrate associated with herpes simplex pneumonia. **C.** Glassy, amphophilic, intranuclear inclusions in HSV-infected cells at the margin of a necrotic focus in the lung. **D.** Immunohistochemical localization of HSV in multiple cell types in the lung of a patient with fatal HSV pneumonia. **E.** HSV-infected cell showing viral nucleocapsids (arrows) within the nucleus and complete, enveloped virions (arrowhead) in the cytoplasm. (Courtesy of C. Goldsmith.) **F,G.** Herpetic inclusions in cells of the adrenal cortex **(F)** and in hepatocytes **(G)** of an infant with fatal disseminated HSV-2 infection. Scale bar, 100 nm. **A–C,F,G,** H&E; **D,** immunoalkaline phosphatase staining, naphthol fast-red, and hematoxylin counterstain; **E,** uranyl acetate and lead citrate stain).

Varicella-Zoster Virus

Varicella-zoster virus (VZV), also known as human herpesvirus 3 (HHV-3), is a human α-herpesvirus most closely related to HSV. It has a linear, double-stranded DNA genome with approximately 125 kbp that encodes more than 70 proteins. The icosahedral nucleocapsid is indistinguishable in appearance from other herpesviruses. The nucleocapsid and the tegument are surrounded by a lipoprotein envelope derived from the host cytoplasmic membranes. The enveloped viral particle is pleomorphic to spherical in shape and 180 to 200 nm in diameter.[160] The primary infection is initiated by inoculation of respiratory mucosa by the virus through infectious aerosols or by direct contact of skin lesions of patients with varicella or herpes zoster. After a primary viremia in the reticuloendothelial system, and secondary viremia in circulating mononuclear cells, the virus is disseminated to the skin, where it initiates a vesicular rash, and back to mucosal sites in the lungs. The release of infectious virus into respiratory droplets is a pathogenic characteristic that distinguishes VZV from other human herpesviruses. The attack rate for previously uninfected household contacts exposed to varicella is approximately 90%. For less sustained exposure, it is estimated to be approximately 10% to 30%.[160]

During primary infection with VZV, viral replication in keratinocytes (Fig. 11.7A) and vascular and lymphatic endothelial cells of the superficial dermis produces the generalized, pruritic, vesicular rash of varicella commonly known as chickenpox. Varicella-zoster virus also establishes latent infection within satellite cells and neurons of the trigeminal and dorsal root ganglia and can reactivate under various conditions to cause herpes zoster, a painful vesicular rash commonly referred to as shingles.[161] The origin of the term *chickenpox* is speculative, but believed to derive from *gican*, an Old English term for itching. The term *shingles* originates from the medieval Latin *cinguls*, or girdle, and alludes to the partial encircling of the trunk by the rash of herpes zoster.[137,160]

Varicella-zoster virus is ubiquitous in human populations around the world, and humans are the only known host. During the prevaccine era in the U.S., approximately 4 million cases, 4000 to 9000 hospitalizations, and 50 to 140 deaths were reported annually.[25,162] The risk of severe illness during primary or recurrent VZV infection appears to depend more on host factors rather than a particular viral strain. Chickenpox is considered a relatively benign infection in children, but adult patients are approximately 25 times more likely than children to develop pneumonia. The greatest risk of severe disease and pneumonia occurs in those patients with chronic lung disease, immune-suppressing conditions, neonates, and pregnant women.[163] Varicella-zoster virus–related deaths have declined sharply in the U.S. since universal childhood vaccination was implemented in 1995.[24,25]

Varicella pneumonia was first described in the medical literature in 1942[164] and is the most frequently reported complication of chickenpox in adult patients.[24,165] Pneumonia occurs in approximately 10% to 15% of adults infected with VZV.[166,167] The occurrence of pneumonia during herpes zoster is rare, and limited primarily to profoundly immunosuppressed patients, particularly bone marrow transplant recipients.[137] Varicella-zoster virus pneumonia generally develops within 2 to 7 days following the onset of rash and may be characterized by fever, cough, tachypnea, chest pain, and hemoptysis.[26,168] Hypoxemia is common and may be severe. Radiographically, the lungs show multiple, scattered, defined, nodular densities. Untreated adult varicella pneumonia is fatal in approximately 10% of cases, but mortality is as high as 25% to 40% in certain high-risk cohorts, including pregnant women, transplant recipients, and neonates.[137,169–171] Massive pulmonary hemorrhage is a frequent terminal event.

Gross examination reveals lungs that are generally two to three times heavier than normal, firm, and plum-colored. There are often multiple necrotic and hemorrhagic lesions on the visceral pleura that resemble the pox lesions of skin.[169,172,173] Pox may also be seen on the parietal pleura, although pleural effusions are uncommon and rarely prominent.[163,173] The trachea and bronchi are generally edematous and erythematous with occasional vesicles on the mucosal surfaces, and there may be lobular consolidation of the lungs. Microscopically, pulmonary involvement consists primarily of interstitial pneumonitis and diffuse miliary foci of necrosis and hemorrhage in the pulmonary parenchyma that involve alveolar walls, blood vessels, and bronchioles (Fig. 11.7B,D).[172] Other findings may include intraalveolar collections of edema, fibrin, or hemorrhage, diffuse alveolar damage, and septal edema.[164,168,173] Virally infected cells with intranuclear inclusions may be identified in respiratory epithelial cells, pneumocytes, interstitial fibroblasts, or capillary endothelium (Fig. 11.7E,F).[167] Eosinophilic intranuclear inclusions and multinucleated syncytial cells may be difficult to locate but are best identified at the edges of necrotic foci (Fig. 11.7C). In cases of disseminated disease, similar necrotizing hemorrhagic lesions and occasional viral cytopathic changes in epithelial cells or fibroblasts may be observed in other tissues and organs, including esophagus, pancreas, liver, renal pelves, ureters, urinary bladder, spleen, bone marrow, thymus, lymph nodes, adrenal glands, and brain.[172,174] In those patients who recover from severe VZV pneumonia, some necrotic parenchymal foci may mineralize, and can be identified by chest radiograph years later as miliary, 1 to 5 mm, nodular opacities. Microscopically, the lesions are characterized as discrete collections of dense fibrous connective tissue that surround multiple, small, calcified bodies. The periphery may include a cellular zone of fibroblasts and occasional giant
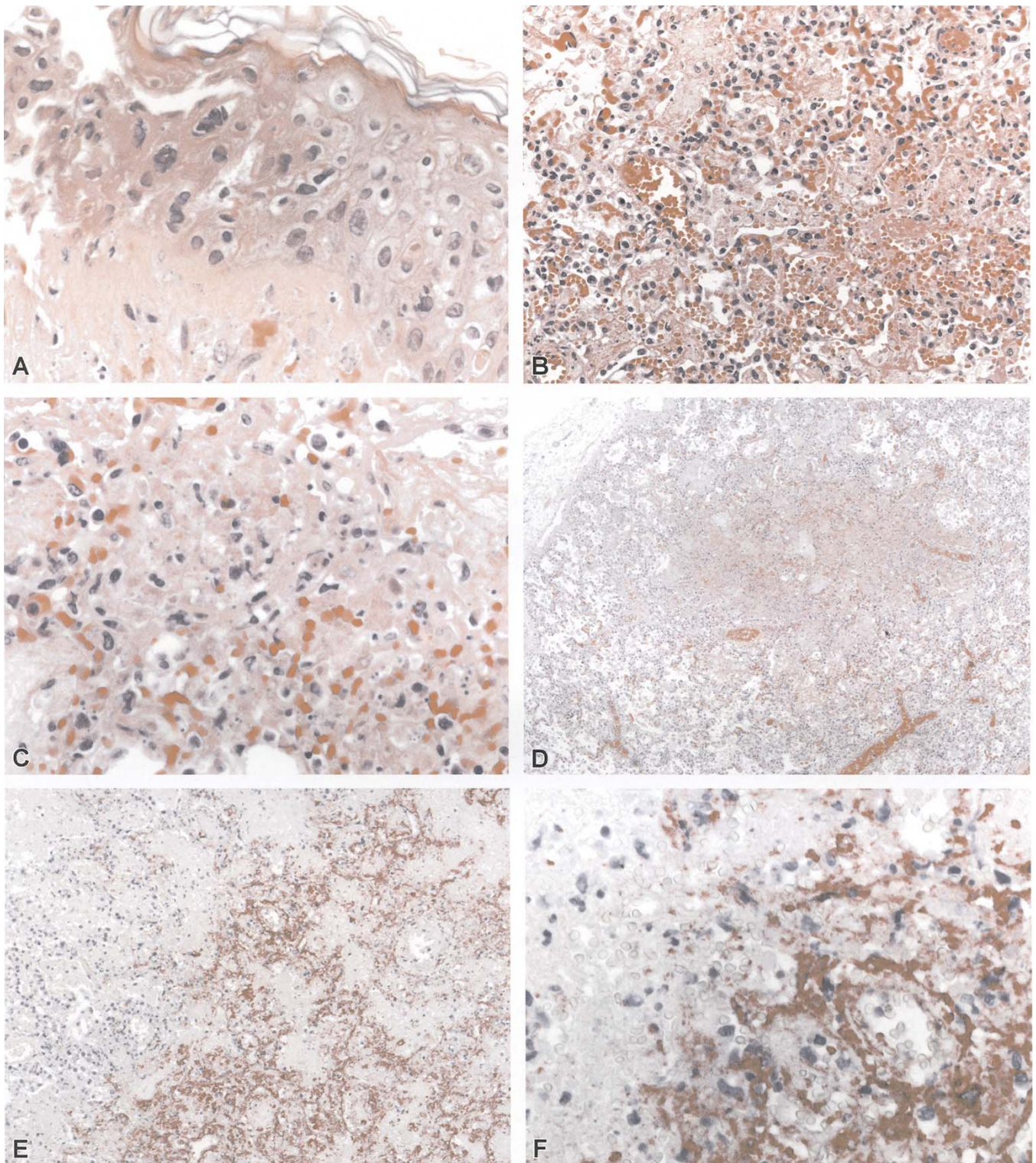
Figure 11.7. Varicella zoster virus (VZV) pneumonia. **A.** Viral cytopathic changes involving the keratinocytes in a cutaneous lesion of a patient infected with VZV. The histopathology includes multinucleate cells with nuclear molding and epidermal necrosis. **B.** Interstitial pneumonitis and intraalveolar collections of fibrin and hemorrhage in a patient with fatal VZV pneumonia. **C.** Eosinophilic intranuclear inclusions in VZV-infected cells at the edge of a necrotic pulmonary focus. **D.** A miliary focus of necrosis and hemorrhage involving air spaces that is characteristic of VZV pneumonia. **E,F.** Immunohistochemical staining of VZV antigens in the pulmonary parenchyma of patient with fatal varicella pneumonia. In the lungs, VZV infects various cell types, including respiratory epithelium, pneumocytes, endothelial cells, and fibroblasts. **A–D,** H&E; **E,F,** immunoalkaline phosphatase staining, naphthol fast-red, and hematoxylin counterstain.

cells.[175] A radiographic survey of 16,894 persons identified pulmonary calcifications in eight (1.7%) of 463 patients who had chickenpox during adulthood compared with only eight (0.05%) of the remaining 16,431 who did not report having varicella as an adult.[176]

A positive history of varicella predicts immunity in >95% of persons.[160] Because pulmonary symptoms most often occur several days following the onset of the characteristic rash of varicella, a pathologic diagnosis is seldom required for a real-time diagnosis of VZV pneumonia. However, hematopoietic cell transplant recipients may present with signs of visceral dissemination and pneumonia 1 to 4 days before the localized cutaneous eruption of herpes zoster appears, and lower respiratory tract disease has been described in the absence of skin lesions, particularly in neonates and bone marrow transplant recipients.[26,137,177] Commercially available antigen detection kits can be used for rapid diagnosis of cutaneous VZV infection. Epithelial cells are scraped from the base of a newly formed vesicle, applied to a slide, and stained by using fluorescein-conjugated VZV monoclonal antibodies to detect specific viral proteins in the specimen. In a similar manner, the Tzanck test uses Wright-Giemsa stain to demonstrate multinucleated giant cells in these specimens; however, this test does not differentiate between HSV and VZV, and false-negative results are common. Commercially available antibodies are also available for IHC detection of VZV in tissue specimens (Fig. 11.7E,F); however, relatively few laboratories are able to provide well-validated assays. Some commercial laboratories offer PCR amplification to detect viral nucleic acid in clinical specimens.

Isolation of the virus in cell culture remains the reference standard for the diagnosis of VZV. In human melanoma cells, an excellent substrate for VZV isolation, the average time for visible cytopathic effect from the virus is 3 to 5 days.[178] Infectious VZV is usually recoverable from the clear fluid of cutaneous vesicles of varicella for approximately 3 days after the appearance of these lesions and for approximately 1 week from herpes zoster lesions. The lungs are the most common organ from which VZV is isolated at autopsy, but isolates have also been obtained from heart, liver, pancreas, gastrointestinal tract, brain, and eyes.[160]

Influenza Viruses

Influenza is derived from the term *influentia*, meaning epidemic in the Italian form of Latin, originally used because epidemics were thought to result from astrologic or other occult influences. Influenza is a highly contagious, acute respiratory illness with a spectrum of clinical illness ranging from asymptomatic or mild disease with rhinitis or pharyngitis to primary viral pneumonia with

fatal outcome. Influenza may also be associated with a broad range of other disorders affecting the heart, brain, kidneys, and muscle.

Influenzaviruses belong to the Orthomyxoviridae family, which consists of four genera that include the two important influenza viruses types A and B associated with significant human disease.[179,180] Influenza A viruses are further classified into subtypes based on the antigenicity of their hemagglutinin (HA) and neuraminidase (NA) surface glycoproteins. Only one type of HA and one type of NA are recognized for influenza B. Influenza A occurs in both pandemic and interpandemic forms. Fortunately, pandemics, defined as worldwide outbreaks of severe disease, occur infrequently. Interpandemic influenza, although less extensive in its impact, occurs virtually every year. The epidemiologic pattern of influenza in humans is related to two types of antigenic variation of its envelope glycoproteins, namely antigenic drift and antigenic shift. During antigenic drift, new strains related to those circulating in previous epidemics evolve by accumulation of point mutations in the surface glycoproteins. This enables the virus to evade the immune system leading to repeated outbreaks during interpandemic years. Antigenic shift occurs with the emergence of a "new" potentially pandemic, influenza A virus that possesses a novel HA alone or in combination with a novel NA.

There are 16 recognized HA subtypes and nine NA subtypes of influenza A virus. Viruses from all HA and NA subtypes have been recovered from aquatic birds, but only three HA subtypes (H1, H2, and H3) and two NA subtypes (N1 and N2) have established stable lineages in the human population since 1918. Since 1997, widespread avian infection with influenza A (H5N1) and associated clusters of human disease have aroused concern about the threat of a pandemic, and attention has been appropriately focused on control measures to deal with such an event.

All Influenzaviruses have a segmented, negative-sense RNA core surrounded by a lipid envelope. Influenzavirus particles are pleomorphic. Among isolates that have undergone a limited number of passages in cell culture or eggs, more filamentous than spherical particles are seen. Spherical morphology becomes dominant when the virus is extensively passaged in the laboratory. A 10- to 12-nm layer of HA (rod-shaped) and NA (mushroom-shaped) spikes project radially on the surface of the influenza A and B viruses. Hemagglutinin facilitates entry of virus into host cells through its attachment to sialic-acid receptors. Because neutralizing antibodies are directed against this antigen, it is a critical component of current influenza vaccines. Neuraminidase, the second major antigenic determinant, catalyzes the cleavage of glycosidic linkages to sialic acid and the release of progeny virions from infected cells. Accordingly, it has become an important target for drug inhibitors such as oseltamivir

and zanamivir. The M2 surface component and channel of influenza A (not present in influenza B virus) regulates the internal pH of the virus and is blocked by the antiviral drug amantadine.

Influenzaviruses are spread person-to-person primarily through the coughing and sneezing of infected persons. The typical incubation period for influenza is 1 to 4 days, with an average of 2 days. Adults can be infectious from the day before symptoms begin through approximately 5 days after illness onset. Children can be infectious for ≥10 days, and young children can shed virus for several days before their illness onset. Severely immunocompromised persons can shed virus for weeks or months.

Uncomplicated influenza illness is characterized by the abrupt onset of constitutional and respiratory signs and symptoms (e.g., fever, myalgia, headache, malaise, non-productive cough, sore throat, and rhinitis). Among children, otitis media, nausea, and vomiting are also commonly reported with influenza illness. Respiratory illness caused by influenza is difficult to distinguish from illnesses caused by other respiratory pathogens on the basis of symptoms alone. Influenza typically resolves after 3 to 7 days in most patients, although cough and malaise can persist for >2 weeks. Among certain persons, influenza can exacerbate underlying medical conditions (e.g., pulmonary or cardiac disease), lead to secondary bacterial pneumonia or primary influenza viral pneumonia, or occur as part of a co-infection with other viral or bacterial pathogens. Young children with influenza infection can have initial symptoms that mimic bacterial sepsis. More than 20% of children hospitalized with influenza can have febrile seizures. Influenza has also been associated with encephalopathy, transverse myelitis, Reye syndrome, myositis, myocarditis, and pericarditis. The risks for complications, hospitalizations, and deaths from influenza are higher among persons aged ≥65 years, young children, and persons of any age with certain underlying health conditions than among healthy older children and younger adults. Influenza-related deaths can result from pneumonia or from exacerbations of cardiopulmonary conditions and other chronic diseases.

The histopathologic features of nonfatal and fatal influenza have been well described and include necrotizing bronchitis, thrombosis, interstitial inflammation, hemorrhage, hyaline membrane formation, and intraalveolar edema (Figs. 11.8A,B, 11.9A,C, and 11.10A).[105,181–191] The pathology is more prominent in larger bronchi, and inflammation may vary in intensity in individual patients. Viral inclusions cannot be identified by light microscopy (Fig. 11.8D). Secondary bacterial infections with organisms such as *Streptococcus pneumoniae* (group A streptococcus [GAS]), *Staphylococcus aureus*, and *Haemophilus influenzae* may occur as a complication in about 50% to 75% of fatal cases and make it difficult to recognize the pathologic changes associated with the primary viral infec-

tion.[190,192,193] The histopathologic features in other organs may include myocarditis, cerebral edema, rhabdomyolysis, and hemophagocytosis (Figs. 11.8H and 11.9E,F). Immunohistochemistry and ISH assays demonstrate that viral antigens and nucleic acids are usually sparse and are primarily seen in the bronchioepithelial cells of larger bronchioles (Figs. 11.8C,E,F and 11.9D).[105,189,190] Antigens are more readily identified in patients who die within 3 to 4 days of onset of illness. Recent studies suggest that unlike human influenza viruses, avian virus H5N1 preferentially infects cells in the lower respiratory tract of humans, resulting in extensive damage of the lungs with minimal pathology in the upper respiratory tract (Fig. 11.10A,C). This may help explain why the H5N1 avian influenza virus is so lethal to humans but so difficult to spread from person to person. These studies show that the avian virus preferentially binds to the α-2,3-galactose receptors, which are found only in and around the alveoli. This is in contrast to the human Influenzaviruses that preferentially bind to the α-2,6-receptors, which are found throughout the respiratory tract from the nose to the lungs.[194,195] In birds and other animals, viral antigens can be detected in the lung as well as a variety of extrapulmonary tissues (Fig. 11.10B).

The diagnosis of influenza, suspected by history and clinical manifestations, can also be supported histopathologically. However, because of the absence of any characteristic viral inclusions and because the overall pathologic features of influenza may resemble other viral, rickettsial, and certain bacterial infections, an unequivocal diagnosis can be made only by laboratory tests such as viral culture, direct fluorescent antibody and rapid antigen assays, serology, and IHC.[190,196,197]

Measles

Measles (rubeola) is an infectious, acute febrile viral illness characterized by upper respiratory tract symptoms, fever, and a maculopapular rash. The causative agent, a member of the genus *Morbillivirus*, of the family Paramyxoviridae,[198,199] is an enveloped virus that contains a negative sense, single-stranded RNA genome of 16,000 nucleotides. Other human pathogens in this family include parainfluenza, mumps, and respiratory syncytial viruses. Measles virions are pleomorphic, generally spherical, enveloped particles from 120 to 250nm in diameter. The virus is morphologically indistinguishable from other members of the Paramyxoviridae family when viewed by negative contrast electron microscopy. A lipid envelope surrounds a helical nucleocapsid composed of RNA and protein. Two transmembrane glycoproteins, hemagglutinin (H) and fusion (F), are present in the envelope and appear as surface projections. These proteins mediate viral attachment and
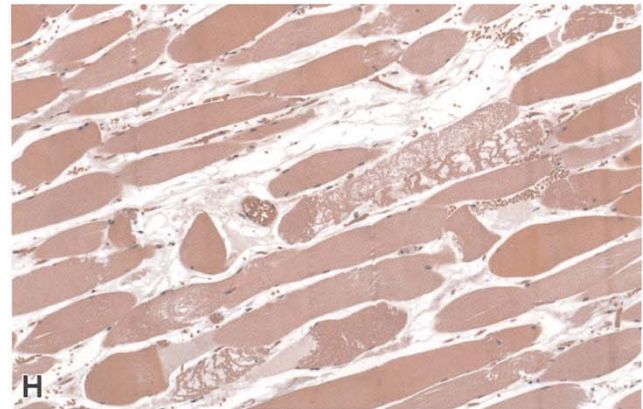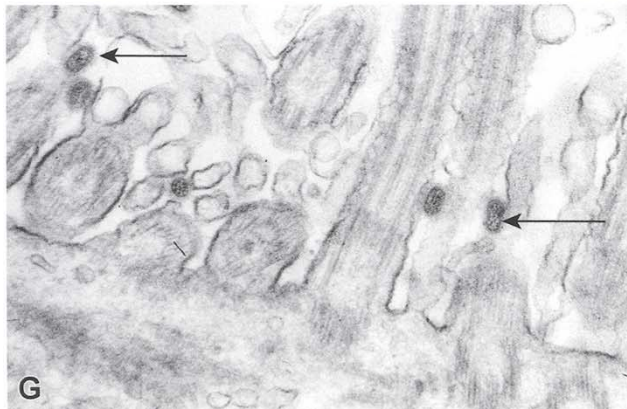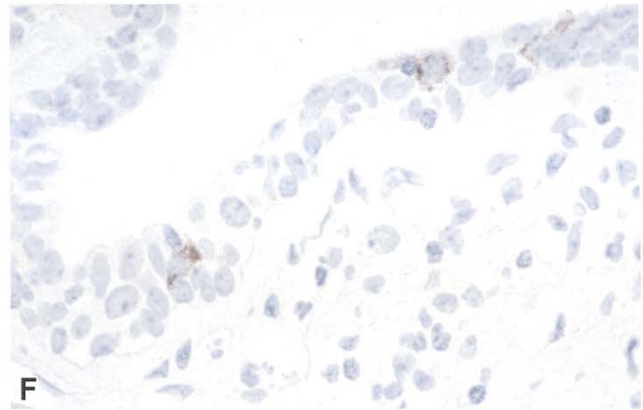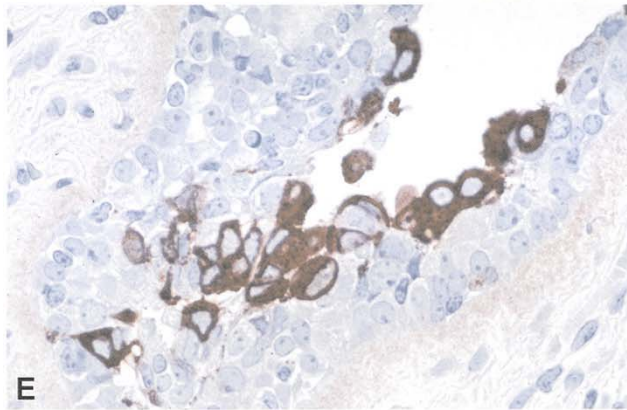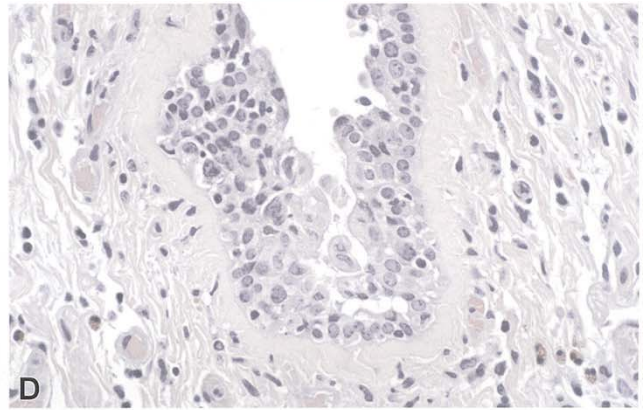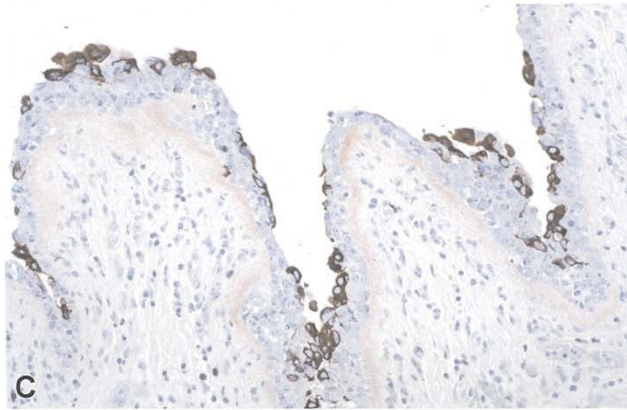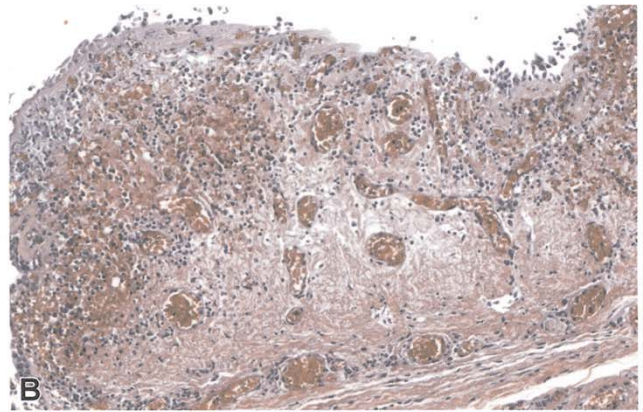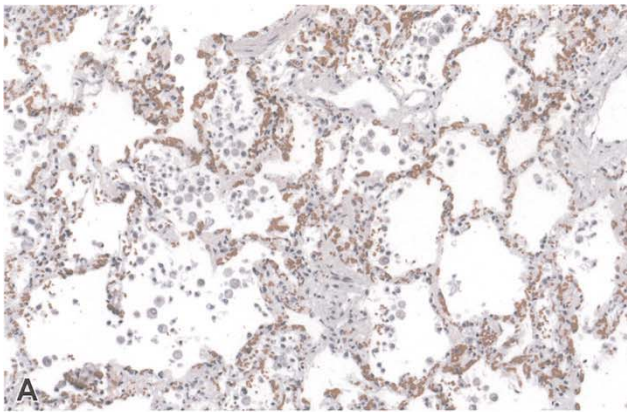
FIGURE 11.8. Influenza A. **A.** Alveolar damage in a patient with fatal influenza A showing prominent congestion with intraalveolar macrophages and fibrin deposits. **B.** Extensive ulceration of respiratory epithelium in a large airway of a patient with fatal influenza A. The lamina propria shows florid vascular congestion and focal hemorrhage, and predominantly mononuclear inflammatory cell infiltrates. **C–E.** Immunohistochemical staining **(C,E)** of influenza virus A hemagglutinin antigens in the cytoplasm of residual respiratory epithelial cells of a large airway. This same focus of extensively infected respiratory epithelium **(D)** demonstrates that, unlike many other viral respiratory pathogens, influenza viruses do not elicit specific cytopathic effects in infected cells. **F.** In-situ hybridization assay demonstrating active replication of influenza A in respiratory epithelium in a large airway. **G.** Electron micrograph of influenza A particles (arrows) attached to the cilia of a rodent tracheal epithelial cell. Viral ribonucleoproteins are evident in the central aspect of the particles. The hemagglutinin and neuraminidase surface glycoproteins make up the peripheral spike layer. (Courtesy of F.A. Murphy.) **H.** Rhabdomyolysis in a patient with fatal influenza A. Bar, 100 nm. **A,B,D,H,** H&E; **C,E,** immunoalkaline phosphatase stain, naphthol fast-red, and hematoxylin counterstain; **F,** digoxigenin-labeled probe followed by immunoalkaline phosphatase staining, naphthol fast-red, and hematoxylin counterstain; **G,** uranyl acetate, lead citrate stain.

fusion with respiratory epithelium. They are also believed to play a role in virus maturation through their interaction with the matrix (M) protein, which, in turn, is thought to interact with the nucleocapsid structure.[200]

Measles is a highly communicable disease of worldwide distribution. Before the introduction of measles vaccines, epidemics occurred about every 2 to 5 years when the percentage of nonimmune members of a population reached critical levels. Recently, epidemics have occurred in cycles of about 10 years. In small and isolated communities, measles circulation may cease altogether unless it is reintroduced. If introduced in nonimmune populations, the disease tends to be more severe and may involve more than 90% of the population because of the highly infectious nature of the virus.

Although still a significant problem in underdeveloped countries, measles infection became uncommon in the U.S. after the development and widespread use of an effective measles vaccine. However, a recrudescence of measles infection occurred in several large U.S. urban centers in recent years, associated with reduced use of the vaccine among children and young adults. During the peak of this activity (i.e., between 1989 and 1991), greater than 50,000 measles cases and approximately 150 measles-associated deaths were reported.[201]

Measles virus is highly contagious, spread by aerosols and droplets from respiratory secretions of acute cases.[202–204] Less frequently, contaminated fomites are involved in transmission. A person with acute measles is infective from just before onset of symptoms to defervescence of fever. In developed countries, likely settings for exposure to measles virus are infectious disease clinics, pediatric emergency rooms, and physicians' offices.[205] Children are usually infected by 6 years of age, resulting in lifelong immunity, and almost all adults are immune. Clinical infection in children younger than 9 months of age is generally uncommon because of passive protection afforded the infant by the transfer of maternal antibodies. However, with the resurgence of measles in the U.S. came the realization that most women of childbearing age acquired immunity to measles through vaccination and not through natural infection. Lower levels of maternal antibodies were found to be transferred from an immunized mother to her infant; thus, a substantial number of measles cases occurred in children younger than 1 year of age in the U.S. in recent years.

After an incubation period of about 1 to 2 weeks, the prodromal phase of measles begins with fever, rhinorrhea, cough, and conjunctivitis. Koplik's spots, which are small, irregular red spots with a bluish-white speck in the center, appear on the buccal mucosa in 50% to 90% of cases shortly before rash onset. An erythematous maculopapular rash begins on the face 3 to 4 days after prodromal symptoms and usually spreads to the trunk and extremities. The symptoms gradually resolve, with the rash lasting for approximately 6 days, fading in the same order as it appeared.

Although recovery is rapid and complete in most cases, complications can arise as a result of continued and progressive virus replication, bacterial or viral superinfections, or abnormal host immune response.[204,206,207] The most common complications are secondary bacterial pneumonia and otitis media.[206] Other complications include febrile convulsions, encephalitis, liver function abnormalities, chronic diarrhea, and sinusitis. Several pulmonary and central nervous system (CNS) syndromes that are often fatal have been described. Death occurs in about 1 of every 1000 measles cases; however, the risk of death and other complications is substantially increased in infants, adults, malnourished and immunocompromised individuals, persons with underlying illnesses, and nonimmunized populations in underdeveloped countries.[208–212]

The first step in measles infection is attachment of the virus to CD46 cell surface receptors on the respiratory epithelium.[213] Adhesion and fusion of the virus to the respiratory epithelium is mediated by both the H and F viral glycoproteins.[214] This stage is followed by local replication in respiratory mucosa and draining lymph nodes. A primary viremia follows, with dissemination
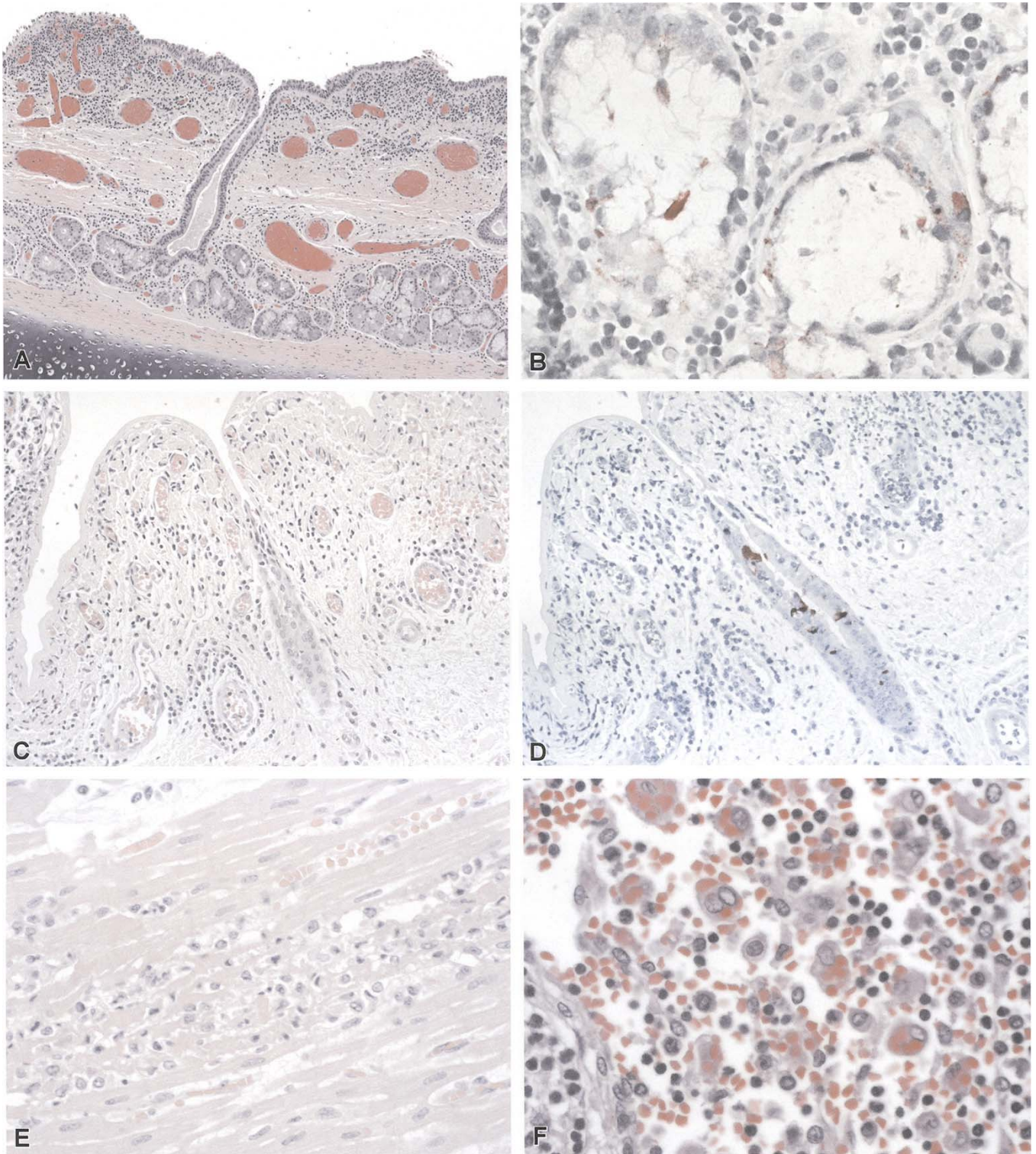
FIGURE 11.9. Influenza B. **A**. A cross-section of trachea from a child with fatal influenza B showing extensive vascular congestion and predominantly mononuclear inflammatory cell infiltrates in the lamina propria and submucosa. **B**. Immunohistochemical staining of influenza B antigens in epithelium of the tubuloalveolar submucosal glands. **C**. A bronchus of a child with fatal influenza B showing extensive denudation of the respiratory epithelium. **D**. Immunohistochemical staining of influenza B antigens in epithelial cells lining a bronchial gland duct (same field as in **C**). **E**. Focus of myocyte necrosis accompanied by a mixed inflammatory cell infiltrate in the heart of a patient with fatal influenza B. **F**. Extensive hemophagocytosis in a parabronchial lymph node in a patient with fatal influenza B. **A,C,E,F,** H&E; **B,D,** immunoalkaline phosphatase staining, naphthol fast-red, and hematoxylin counterstain.
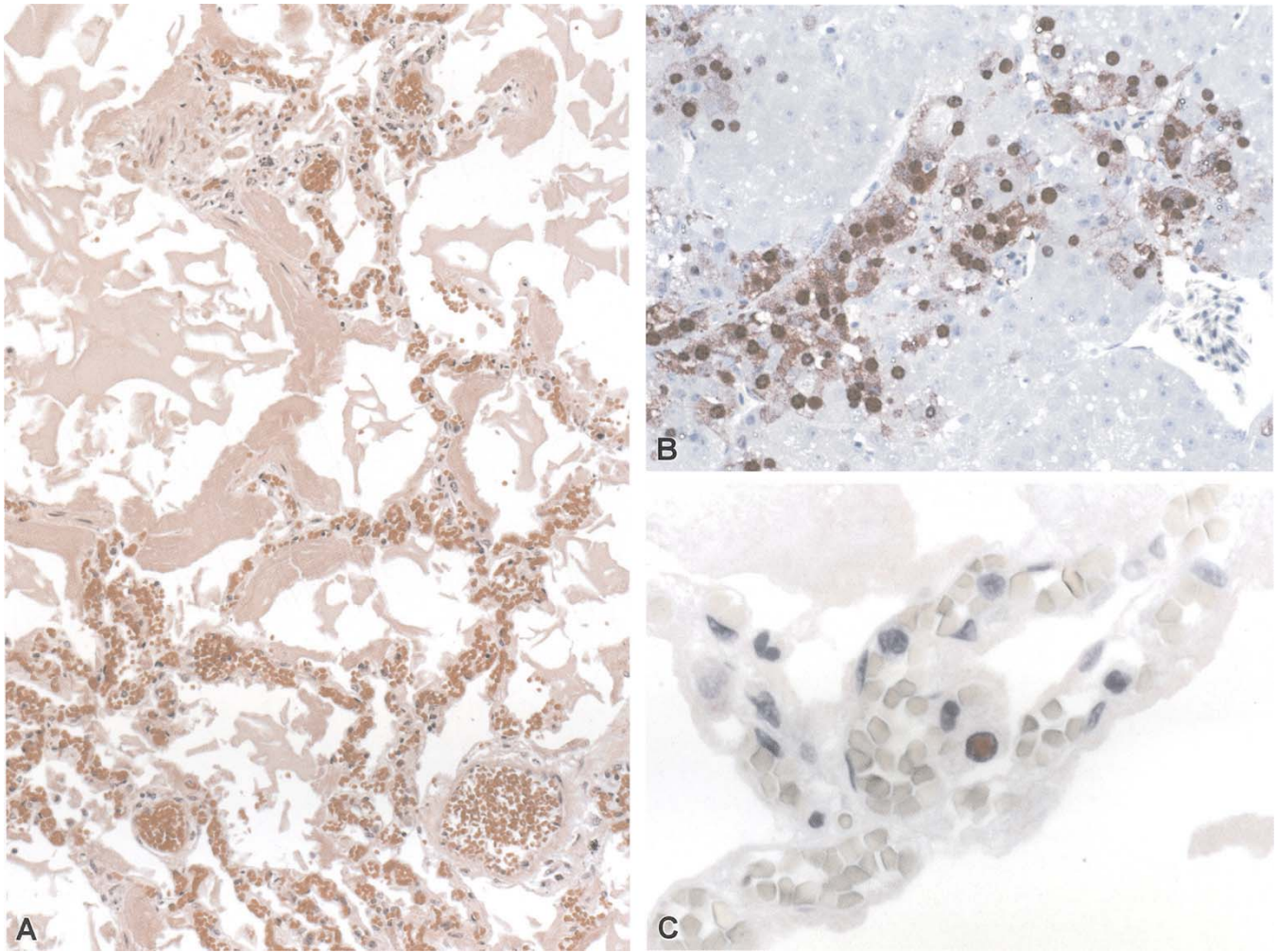
FIGURE 11.10. Avian influenza. **A.** Diffuse alveolar damage in a patient with fatal avian influenza (H5N1) showing extensive hyaline membrane formation and congestion. **B.** Influenza virus A (H5N1) antigens in the nuclei of avian hepatocytes. Influenza A is a zoonosis and aquatic birds represent the largest natural reservoir of the virus. **C.** Influenza virus A (H5N1) antigens in the nucleus of a pneumocyte in the lung of a patient with fatal avian influenza. Unlike other influenza viruses that cause disease in humans, H5N1 preferentially infects alveolar epithelial cells, and causes relatively minimal pathology in the upper airway. **A,** H&E; **B,C,** immunoalkaline phosphatase stain, naphthol fast-red, and hematoxylin counterstain.

throughout the reticuloendothelial system. Replication of virus at the secondarily infected sites leads to lymphoid hyperplasia and the formation of characteristic reticulo-endothelial multinucleated giant cells. A stage of secondary viremia soon develops, with widespread dissemination of virus to different tissues by infected lymphocytes and monocytes. At this point in the infection, prodromal symptoms, such as cough, coryza, and conjunctivitis, become more severe, and virus replication in the respiratory tract predisposes the infected individual to complications such as pneumonia and otitis media.

Two types of multinucleated giant cells have been described in patient tissues during measles infection.[215,216] The reticuloendothelial giant cell (Warthin-Finkeldey) appears first during the incubation period and is seen in different lymphoid tissues throughout the body. The second type is the epithelial giant cell, which has been observed in the epithelium of essentially every major organ.

The onset of the rash temporally coincides with the appearance of detectable serum antibody to measles virus. Interestingly, virus replication and giant cell formation cease with the appearance of rash. T-cell immunity is essential in the process of viral clearance from lymphoid tissue and respiratory tract. While children with congenital agammaglobulinemia respond normally to measles virus infection, patients with cell-mediated immunodeficiency develop severe disease that presents as giant-cell pneumonia or encephalopathy in the absence of an exanthem.[208–211,217,218]

Immunity to the F surface glycoprotein is necessary to prevent the spread of measles infection.[219] An atypical measles syndrome characterized by pulmonary consolidation with pleural effusions and hilar adenopathy has been reported in children exposed to wild-type measles virus who had previously received the killed-measles virus vaccine.[220-222] Recipients of the formalin-inactivated vaccine have a good antibody response to the H protein, but antibodies to the functional region of the F protein and to the nucleoprotein are weak or absent. It has been suggested that the lack of a functional F antibody response may play a role in virus spread.[223]

The pathologic features of measles have been well described and several references containing detailed morphologic descriptions are recommended.[131,209,224-230] The typical morbilliform skin lesions, Koplik's spots, and measles lymphadenitis are seldom seen by the surgical pathologist since the clinical diagnosis is usually apparent. Histopathologic changes in the skin include mild congestion, edema, and a predominantly mononuclear infiltrate surrounding small vessels of the dermis, as well as other nonspecific features. Occasional diagnostic multinucleated epithelial giant cells with eosinophilic cytoplasmic and nuclear inclusions are observed.[228,231] Pathognomonic reticuloendothelial multinucleated giant cells can be observed in appendix specimens from patients mistakenly operated on for acute appendicitis before the emergence of diagnostic Koplik's spots and rash. These cells, which have been reported in various lymphoreticular tissues throughout the body, are typically large and contain from a few to occasionally up to 100 nuclei. These cells do not usually contain viral inclusions. The lymphoid tissues are typically hyperplastic, and the architecture is partially or totally obliterated by diffuse proliferation of immunoblasts.[232-234]

A focal or generalized interstitial pneumonitis, similar to that seen in many other viral infections, is seen in the lungs of measles patients. Histopathologic features seen include various degrees of peribronchial and interstitial mononuclear cell infiltrates, squamous metaplasia of bronchial endothelium, proliferation of type II pneumocyte alveolar lining cells, and intraalveolar edema with or without mononuclear cell exudates and hyaline membranes. Secondary changes, such as bacterial or viral superinfection, or organizational changes may alter the original pathology.

The hallmark of the disease is the formation of multinucleated epithelial giant cells (Fig. 11.11A,B). These cells, which are often numerous, are formed by fusion of bronchiolar or alveolar lining epithelial cells (Fig. 11.11A). In contrast to the reticuloendothelial giant cells, these cells generally contain characteristic nuclear and cytoplasmic inclusions. The intranuclear inclusions are homogeneous, eosinophilic, and surrounded by a slight indistinct halo (Fig. 11.11C,D). The cytoplasmic inclusions are deeply eosinophilic, vary in size, and some form large masses with a "melted tallow" appearance (Fig. 11.11D). These giant cells may undergo degenerative changes with progressive loss of cytoplasm, increasing basophilia, and shrinkage of nuclei. The presence of measles virus in these giant cells may be demonstrated by immunofluorescent,[235,236] IHC,[210,237] and ISH techniques (Fig. 11.11E,F). These giant cells can also be seen in extrapulmonary tissues (Fig. 11.11G,H).

The diagnosis of typical cases of measles can usually be made on the basis of clinical signs and symptoms. Other causes of a similar rash, but without other features of measles, include rubella, dengue virus, enteroviruses, and drug reactions, especially to ampicillin. The typical case of measles giant-cell pneumonia generally presents little diagnostic difficulty for the surgical pathologist. The presence of giant cells with both intranuclear and intracytoplasmic inclusions in a setting of interstitial pneumonitis is highly specific for measles infection. However, multinucleated giant cells are not seen in all cases of measles pneumonia and their absence should not exclude the

FIGURE 11.11. Measles pneumonia. **A.** Several multinucleated giant cells are seen in the epithelial cells lining a bronchiole. **B.** Low-power photomicrograph showing interstitial pneumonitis, hemorrhage, and abundant giant cells. **C.** Multinucleated giant cells generally line the alveoli, although some are found lying free within alveolar spaces. **D.** High-power of multinucleated giant cells showing numerous eosinophilic cytoplasmic inclusions. The inclusions may be large in size and have a characteristic "melted tallow" appearance. Intranuclear inclusions are also seen, but are ill-defined, eosinophilic, and lack clear circumscription. **E.** Viral antigens in cytoplasm of giant cells as seen by immunohistochemical staining. The multinucleated giant cells can be seen to originate by fusion of infected alveolar lining epithelial cells. **F.** Numerous measles giant cells are seen in lung by colorimetric in-situ hybridization using digoxigenin-labeled probes. **G.** Multinucleated epithelial and endothelial giant cells in adrenal gland of a fatal case of measles. **H.** Measles antigens in the same giant cells as seen in **G. I,J.** Measles antigen in nuclei of a giant cell (**I**) detected by immunohistochemistry. Intranuclear measles virus nucleocapsids as seen by electron microscopy (EM) (**J**) in the neuron of a patient who died from subacute sclerosing panencephalitis (SSPE) associated with measles. (**J:** courtesy of Sylvia Whitfield, Centers for Disease Control and Prevention, Atlanta, GA.) **A–D,G,** H&E; **E,H,I,** immunoalkaline phosphatase staining, naphthol fast-red, and hematoxylin counterstain; **F,** digoxigenin-labeled probes followed by immunoalkaline phosphatase staining, naphthol fast-red, and hematoxylin counterstain; **J,** uranyl acetate and lead citrate stain.
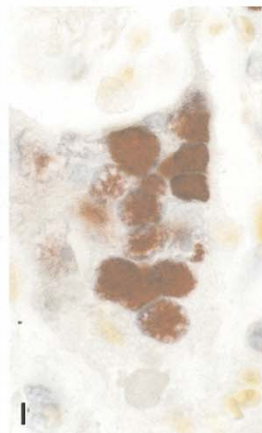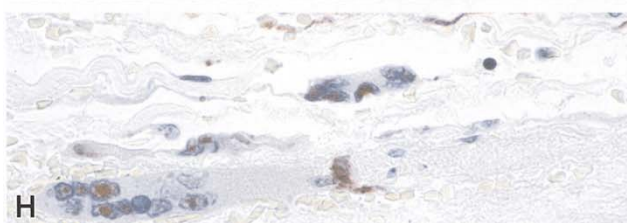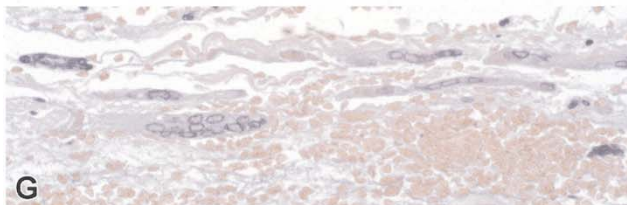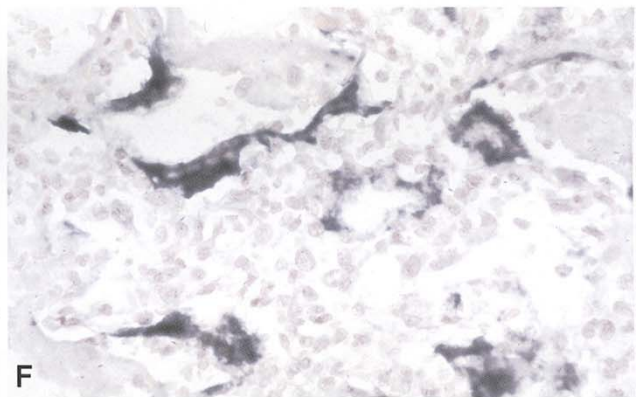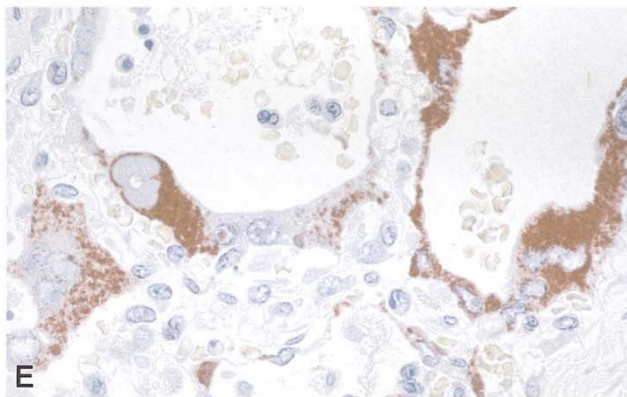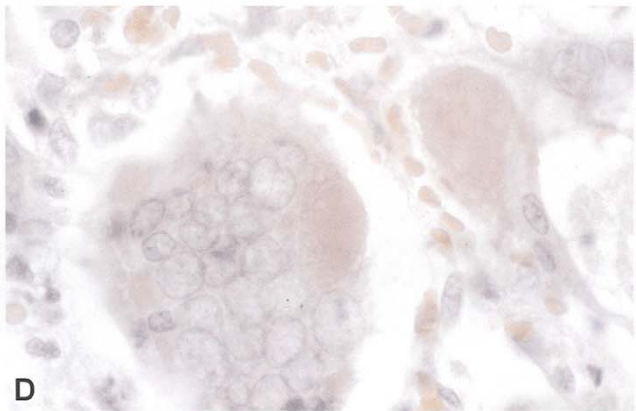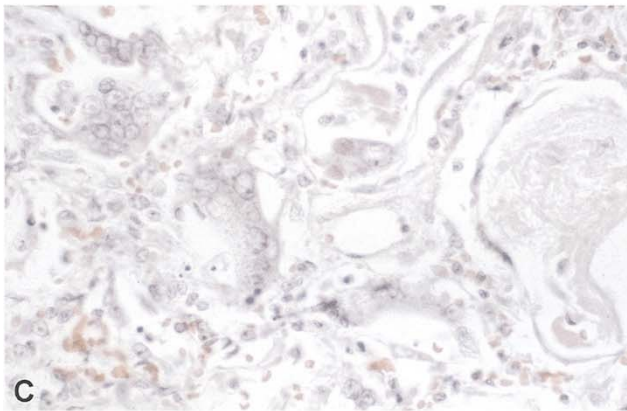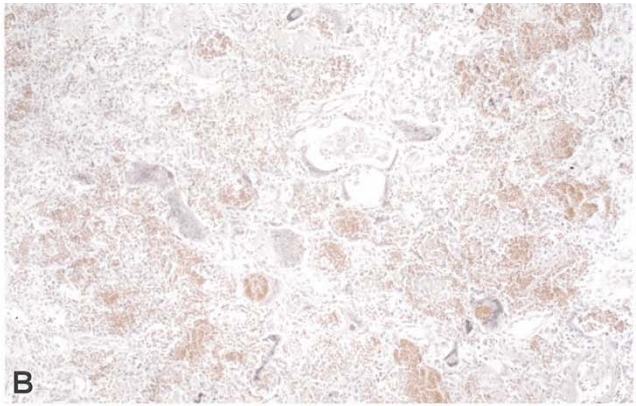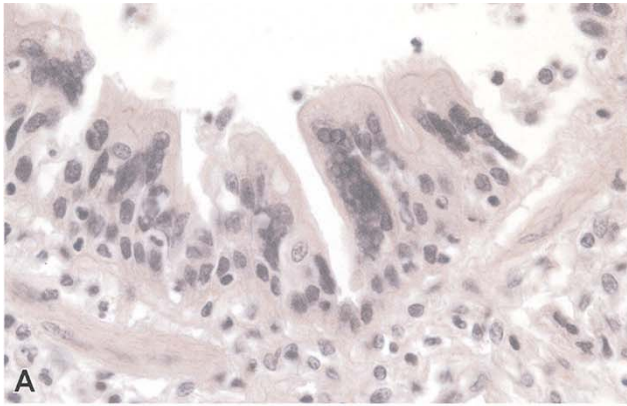
diagnosis. Other viral and rickettsial agents may also cause a similar interstitial pneumonitis, but without the typical giant cells, and should be differentiated.[229] As previously noted, the histopathologic features in measles pneumonia can be somewhat variable,[226] and secondary bacterial and viral infections may modify the histology, further complicating the pathologic diagnosis (Fig. 11.5D).[207,229] Other viral pathogens, such as respiratory syncytial virus,[238] parainfluenza,[239,240] VZV,[241] and a recently discovered Hendra virus,[242] as well as granulomatous diseases of the lung, may give rise to pneumonia with giant cells and should also be considered in the differential diagnosis. However, these clinical entities can be distinguished by history, histopathologic features, and laboratory tests. Immunohistochemistry[237,243,244] or ISH[243,244] tests demonstrate viral antigens or nucleic acids in the majority of cases.

Laboratory confirmation is useful to avoid possible confusion with other rash-causing illnesses. Diagnostic laboratory procedures consist of direct detection of either the virus or the viral antigens, usually by indirect immunofluorescence or by serologic methods using hemagglutination inhibition, neutralization, or enzyme immunoassay. Specimens for serologic testing consist of acute- and convalescent-phase serum pairs. Antibody appears within 1 to 2 days after onset of rash, and titers peak approximately 2 weeks later. Alternatively, the presence of specific immunoglobulin M (IgM) antibody can be used to diagnose recent infection.[245]

Human Parainfluenza Viruses

Human parainfluenza viruses (HPIVs) are second only to RSV as a cause of lower respiratory tract disease in young children. Human parainfluenza viruses are negative-sense, nonsegmented, single-stranded, enveloped RNA viruses that possess fusion and hemagglutinin-neuraminidase glycoprotein "spikes" on their surface (Fig. 11.1G). The four serotypes of HPIV belong in the family Paramyxoviridae, subfamily Paramyxovirinae, and genera *Respirovirus* (HPIV-1 and -3) and *Rubulavirus* (HPIV-2 and -4). The virions are variable in shape and size, ranging from 150 to 300nm.[246]

Human parainfluenza viruses are spread from respiratory secretions through close contact with infected persons or contact with contaminated surfaces or objects. Infection can occur when infectious material contacts mucous membranes of the eyes, mouth, or nose, and possibly through the inhalation of droplets generated by a sneeze or cough. Human parainfluenza viruses are unstable in the environment (surviving a few hours on environmental surfaces), and are readily inactivated with soap and water. They are ubiquitous, and infect most

people during childhood. The highest rates of serious HPIV illnesses occur among young children. Serologic surveys have shown that 90% to 100% of children aged 5 years and older have antibodies to HPIV-3, and about 75% have antibodies to HPIV-1 and -2. The different HPIV serotypes differ in their seasonality, with HPIV-1 causing biennial outbreaks of croup in the fall and HPIV-2 causing annual or biennial fall outbreaks. Human parainfluenza virus-3 peak activity occurs during the spring and early summer months each year, but the virus can be isolated throughout the year.

Similar to RSV, HPIVs can cause repeated infections throughout life, usually manifested by an upper respiratory tract illness (e.g., cold and sore throat). Human parainfluenza viruses can also cause serious lower respiratory tract disease with repeat infection (e.g., pneumonia, bronchitis, and bronchiolitis), especially among the elderly, and among patients with compromised immune systems. Each of the four HPIVs has different clinical and epidemiologic features. The most distinctive clinical feature of HPIV-1 and HPIV-2 is croup (i.e., laryngotracheobronchitis); HPIV-1 is the leading cause of croup in children, whereas HPIV-2 is less frequently detected. Both HPIV-1 and -2 can cause other upper and lower respiratory tract illnesses. Human parainfluenza virus-3 is more often associated with bronchiolitis and pneumonia. Human parainfluenza virus-4 is infrequently detected, possibly because it is less likely to cause severe disease. The incubation period for HPIVs is generally from 1 to 7 days.[247]

Most HPIV infections cause a mild, self-limited illness; however, HPIV-3 infections are an important cause of bronchiolitis, croup, and pneumonia that may be life-threatening in infants and newborns.[247] Human parainfluenza virus infections are also increasingly being recognized as an important cause of severe morbidity and mortality in immunocompromised patients.[20,248-252] The mortality of bone marrow transplant patients with HPIV-3 infection has been reported to be as high as 60%.[249,253,254] In patients with severe HPIV infection, multinucleated giant cells derived from the respiratory epithelium may be seen in association with an interstitial pneumonitis and organizing changes (Fig. 11.12A,B).[239,240,249,255-260] These giant cells, which may contain intracytoplasmic eosinophilic inclusions (Fig. 11.12C), have also been reported in extrapulmonary tissues such as kidney, bladder, and pancreas.[258] Other viral causes of giant cell pneumonia, including measles, RSV, VZV, and HSV, should be considered in the histopathologic differential and laboratory testing, including IHC, can be useful in making this differentiation possible (Fig. 11.12D). Diagnosis of infection with HPIVs can also be made by virus isolation, direct detection of viral antigens by enzyme-linked immunoassay (EIA) or immunofluorescent assay (IFA) in clinical specimens, detection of viral RNA by RT-PCR, demonstration of a rise in
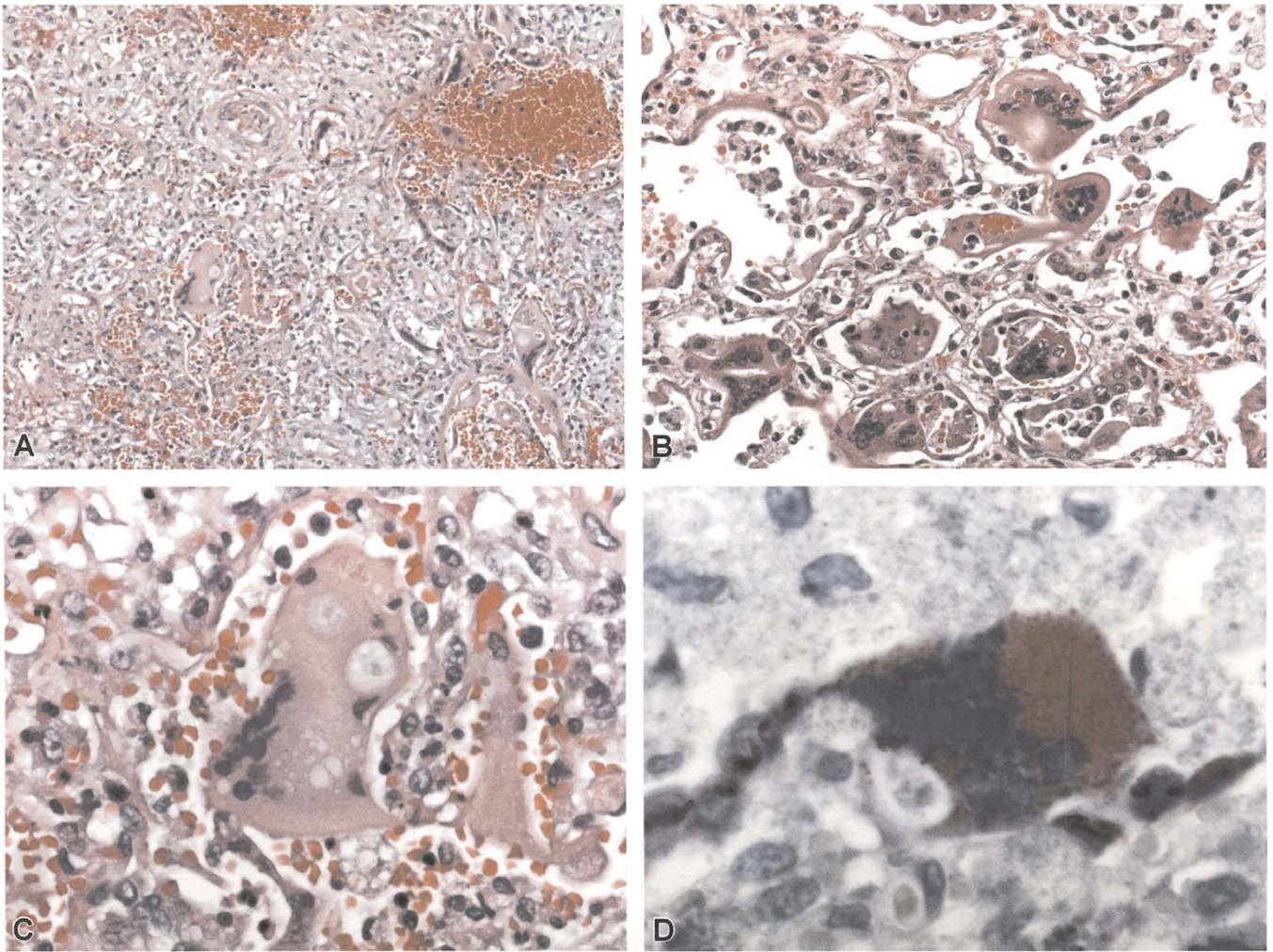
FIGURE 11.12. Parainfluenza pneumonia. **A.** Giant cell pneumonia associated with parainfluenza virus infection with numerous giant cells, interstitial pneumonitis and hemorrhage. **B.** Multinucleated giant cells that originate from respiratory alveolar epithelium. **C.** High-power magnification of a giant cell containing poorly defined cytoplasmic eosinophilic inclusions. **D.** Parainfluenza virus antigens in giant cells localized by using immunohistochemistry. **A–C,** H&E; **D,** immunoalkaline phosphatase staining, naphthol fast-red, and hematoxylin counterstain.

specific serum antibodies, or a combination of these approaches.[261–264]

Respiratory Syncytial Virus

Respiratory syncytial virus (RSV) is the most common cause of bronchiolitis and pneumonia among infants and children under 1 year of age. The causative agent is a negative-sense, nonsegmented, single-stranded, enveloped RNA virus. The virion is variable in shape and size and ranges from 120 to 300 nm. Respiratory syncytial virus is a member of the family Paramyxoviridae, subfamily Pneumovirinae, in the genus *Pneumovirus*, and can be

further distinguished genetically and antigenically into two subgroups, A and B. The subgroup A strains are usually associated with more severe infections. Two surface glycoproteins, G and F, are present in the envelope and mediate attachment and fusion with respiratory epithelium. The F protein also mediates coalescence of neighboring cells to form the characteristic multinucleated syncytial giant cells for which the virus name is derived.[265]

Respiratory syncytial virus is spread from respiratory secretions through close contact with infected persons or contact with contaminated surfaces or objects.[266] Infection can occur when infectious material contacts mucous membranes of the eyes, mouth, or nose, and possibly
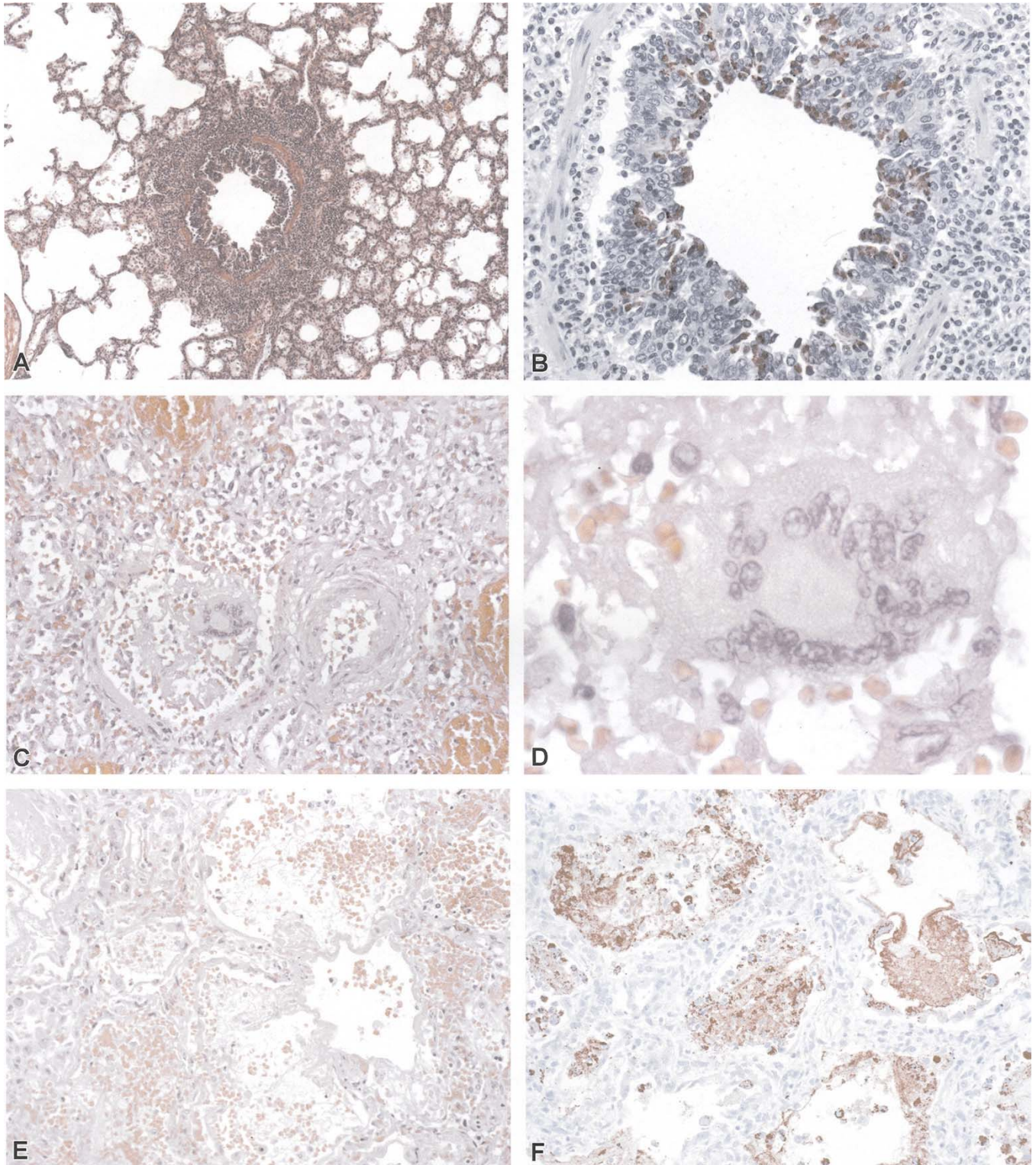
FIGURE 11.13. Respiratory syncytial virus (RSV) pneumonia. **A.** Bronchiolar and peribronchiolar inflammation in RSV infection. **B.** Respiratory syncytial virus antigens are seen in lining epithelial cells of same bronchiole by using immunohistochemistry. **C.** Diffuse interstitial pneumonitis, hemorrhage, and giant cells in RSV pneumonia. **D.** Higher-power magnification showing giant cells lacking unequivocal viral inclusions. **E.** Diffuse alveolar damage with septal thickening and hyaline membranes in RSV pneumonia. No giant cells can be seen. **F.** Respiratory syncytial virus antigens associated with hyaline membranes and necrotic debris within alveolar spaces. **A,C–E,** H&E; **B,F,** immunoalkaline phosphatase staining, naphthol fast-red, and hematoxylin counterstain.

through the inhalation of droplets generated by a sneeze or cough. Respiratory syncytial virus is unstable in the environment, surviving a few hours on environmental surfaces, and is readily inactivated with soap and water. In temperate climates, RSV infections usually occur during annual community outbreaks, often lasting several months, during the late fall, winter, or early spring months. The timing and severity of outbreaks in a community vary from year to year. Respiratory syncytial virus spreads efficiently among children during the annual outbreaks, and most children will have serologic evidence of RSV infection by 2 years of age.

Illness begins most frequently with fever, runny nose, cough, and sometimes wheezing. During their first RSV infection, between 25% and 40% of infants and young children have signs or symptoms of bronchiolitis or pneumonia, and 0.5% to 2% require hospitalization. Most children recover from illness in 8 to 15 days. The majority of children hospitalized for RSV infection are under 6 months of age. Respiratory syncytial virus also causes repeated infections throughout life, usually associated with moderate-to-severe cold-like symptoms; however, severe lower respiratory tract disease may occur at any age, especially among the elderly or among those with compromised cardiac, pulmonary, or immune systems.

The major histopathologic changes described in fatal RSV infections include necrotizing bronchiolitis and interstitial pneumonia (Fig. 11.13A,B).[238,267-272] Bronchial lumina and airways are usually filled with necrotic debris and inflammatory cells. These findings may be accompanied by various degrees of diffuse alveolar damage (Fig. 11.13E), organizational changes, and secondary bacterial superinfection. Giant cell pneumonia is a feature seen in some cases (Fig. 11.13C,D). The multinucleated giant cells contain irregular, intracytoplasmic, eosinophilic inclusions surrounded by a clear halo. These inclusions are extremely difficult to identify with any degree of certainty and are only seen in about half the cases (Fig. 11.13D). Other viral causes of giant cell pneumonia should be considered in the histopathologic differential and laboratory testing, including IHC,[271,273,274] can be useful in making this differentiation possible (Fig. 11.13B,F). Diagnosis of RSV infection can also be made by virus isolation, direct detection of viral antigens in clinical specimens by EIA or IFA, detection of viral RNA by RT-PCR, demonstration of a rise in RSV-specific serum antibodies, or a combination of these approaches (see also Fig. 7.43, Chapter 7).[264,275-281]

Human Metapneumovirus

In 2001, van den Hoogen et al.[282] described the identification of this new viral agent from clinical specimens obtained from patients with respiratory illness, which they designated human metapneumovirus (HMPV). It is a negative-sense, nonsegmented, single-stranded, enveloped RNA virus. The virion is variable in shape and size, ranging from 150 to 300 nm (Fig. 11.1F). It has been categorized in the family Paramyxoviridae, subfamily Pneumovirinae, genus *Metapneumovirus*, based on genomic sequence and gene constellation. Human metapneumovirus can be further distinguished genetically and antigenically into two subgroups, A and B.

Similar to RSV, HMPV infection is ubiquitous and occurs during infancy and early childhood, with annual epidemic peaks occurring in the winter and spring months in temperate regions. Seroprevalence studies reveal that 25% of all children aged 6 to 12 months have antibodies to HMPV; by age 5 years, 100% of patients have evidence of past infection.

Like RSV, HMPV has been associated with a wide spectrum of respiratory illnesses.[283-287] The patient may be asymptomatic, or symptoms may range from mild upper respiratory tract illness to severe bronchiolitis and pneumonia. Although RSV, HPIV-1, and HPIV-3 have been definitively linked to cases of lower respiratory tract disease in infants and young children, the relative contribution of HMPV remains undetermined. Like RSV and the HPIVs, studies suggest that HMPV may also contribute to respiratory disease in elderly adults and the immunocompromised.[288-290]

Histopathologic descriptions of features of HMPV infections are few and have not been well described.[291-294] This is partly related to interpreting the clinical significance of virus detection in context with the ubiquitous nature of the virus. Virus detection in such cases is usually made by culture isolation of the virus from upper airways or by PCR assays performed on nasopharyngeal aspirates or BAL washings. In nonhuman primates viral antigens are observed in ciliated epithelial cells, type 1 pneumocytes, and alveolar macrophages. This distribution is associated with mild, multifocal, erosive, and inflammatory changes in airways, and an increased number of foamy macrophages in alveoli.[291] The BAL specimens collected from patients within a few days of a positive HMPV assay show degenerative changes and cytoplasmic inclusions within epithelial cells, multinucleated giant cells, and histiocytes. The intracytoplasmic inclusions are ill-defined, eosinophilic structures that measure 3 to 4 μm.[293] Lung biopsy or autopsy tissue obtained and examined later in the disease show chronic airway inflammation, intraalveolar foamy and hemosiderin-laden macrophages, and acute and organizing lung injury including areas of diffuse alveolar damage with hyaline membrane formation and foci of a bronchiolitis obliterans/organizing pneumonia like reaction (Fig. 11.14). In such cases, typical multinucleated giant cells or viral inclusion cannot be identified.[292-294] In-situ hybridization studies on limited number of human cases
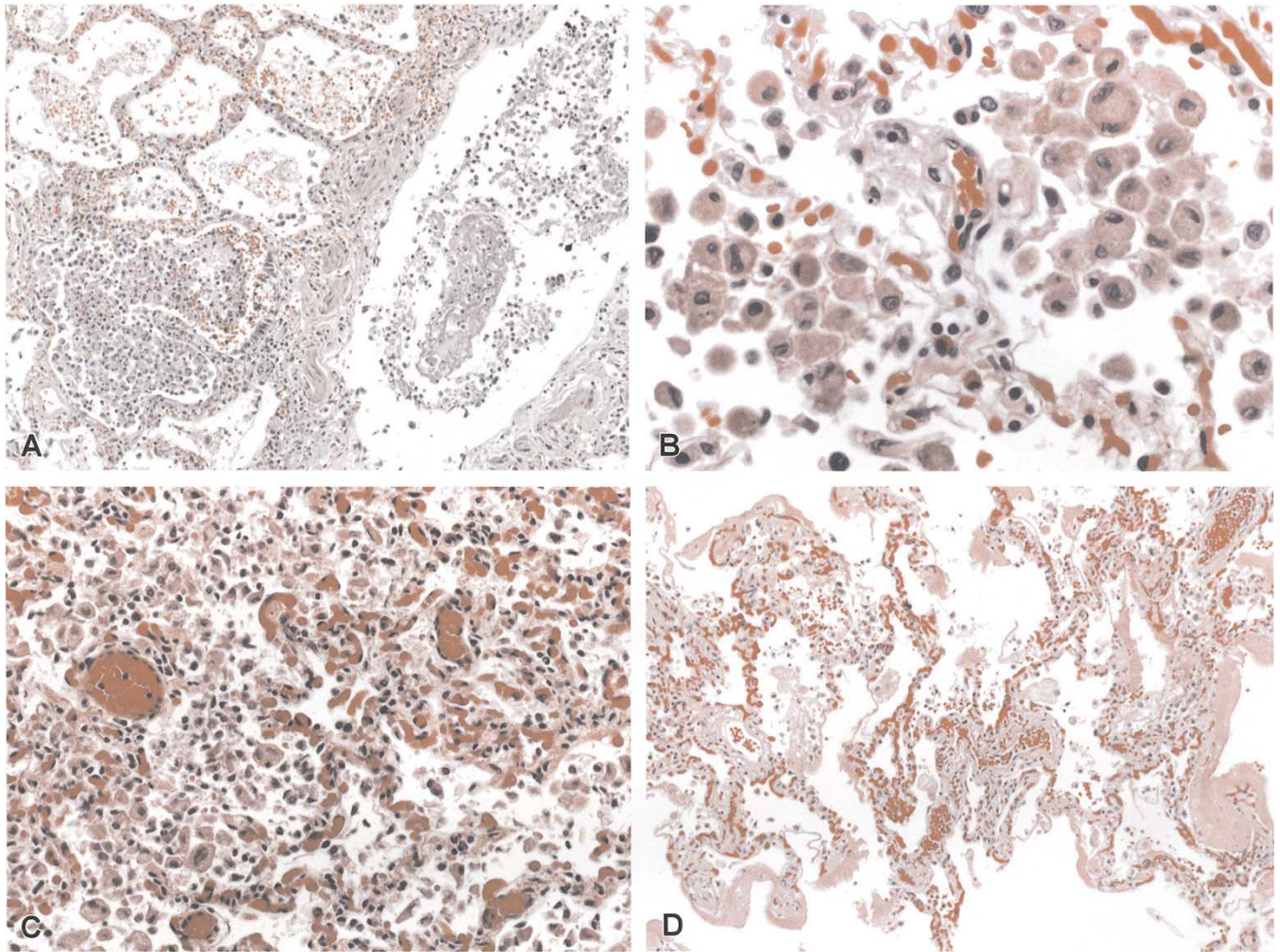
FIGURE 11.14. Human metapneumovirus (HMPV) infection. **A.** Marked bronchiolitis, intraalveolar macrophages, and hemorrhages are seen in this photomicrograph. The bronchiolar epithelium is focally ulcerated. **B.** Higher-power magnification showing accumulation of intraalveolar foamy macrophages. (Patient bronchoalveolar lavage [BAL] specimen positive for HMPV by polymerase chain reaction [PCR].) **C.** Pulmonary congestion, edema, and mild interstitial lymphocytic inflammation. (Patient lung positive for HMPV by PCR.) **D.** Organizing pneumonia with marked interstitial thickening and hyaline membrane formation. HMPV was detected. (Patient BAL positive for HMPV by PCR.) **A–D,** H&E.

suggest infection of alveolar and bronchial epithelial cells.[292]

Human metapneumovirus is difficult to identify with commonly used viral diagnostic procedures. The virus replicates slowly in primary and tertiary monkey kidney cell lines, and the cytopathic effect can be difficult to discern. Commercial monoclonal antibody reagents to HMPV are not widely available. Most HMPV studies have been conducted using RT-PCR assays or by demonstration of a rise in HMPV-specific serum antibodies.

Henipah Viruses

Two novel paramyxoviruses, Hendra and Nipah, have been recently identified in Australia and Malaysia; these viruses have been associated with acute febrile encephalitis and respiratory tract disease. Both infections are zoonotic. Hendra was first identified in 1994 when patients who came in close contact with sick horses developed an influenza-like illness. Two patients died with pneumonitis and multiorgan failure.[30] The closely related Nipah virus was identified during an outbreak in

Malaysia and Singapore during 1998–1999 that included more than 250 patients. Patients presented with a severe acute encephalitic syndrome, but some also had significant pulmonary manifestations.[22,295-302] Most of the patients had a history of contact with pigs, most of them being pig farmers. In Bangladesh in 2001 and 2003, outbreaks of Nipah encephalitis occurred.[303,304] Similar to the Malaysian outbreak, the most prominent symptoms were fever, headache, vomiting, and an altered level of consciousness. Respiratory illness was much more common in the Bangladesh cases, however, with 64% having cough and dyspnea. The reason for increased involvement of the respiratory tract in this outbreak is not known. Epidemiologic and laboratory investigations identified fruit bats of the *Pteropus* genus as asymptomatic carriers of Hendra and Nipah viruses and possible animal reservoirs.[305-309]

Hendra and Nipah viruses belong to the recently designated genus *Henipavirus* within the family Paramyxoviridae, subfamily Paramyxovirinae. Both viruses are nonsegmented, negative-stranded RNA viruses composed of helical nucleocapsids enclosed within an envelope to form roughly spherical, pleomorphic virus particles.[310-312] The structure of their genome is consistent with the other members of the subfamily.[30]

Histopathologic findings in fatal cases of Hendra and Nipah infections are similar with varying degrees of CNS and respiratory tract involvement.[104,313-315] Findings include a systemic vasculitis with extensive thrombosis, endothelial cell damage, necrosis, and syncytial giant cell formation in affected vessels (Fig. 11.15A,B,F). Plaques with various degrees of necrosis, in association with inclusion-bearing neurons, can be found in both the gray and white matter of the CNS (Fig. 11.15E). Multinucleated giant cells with intranuclear inclusions can occasionally be seen in lung, spleen, lymph nodes, and kidneys (Fig. 11.15C,G). In the lung, vasculitis and fibrinoid necrosis can be seen in majority of cases. Fibrinoid necrosis often involves several adjacent alveoli and is frequently associated with small vessel vasculitis. The multinucleated giant cells with intranuclear inclusions are usually noted in alveolar spaces adjacent to necrotic areas. Histopathologic changes of bronchiolar epithelium are uncommon; rarely, the large bronchi may show transmural inflammation and ulceration. Widespread presence of Nipah virus antigens can be seen by IHC in endothelial and smooth muscle cells of blood vessels as well as in various parenchymal cells (Fig. 11.15D).

The diagnosis of Nipah virus infection, suspected by patient history and clinical manifestations, can be supported by characteristic histopathologic findings. From a diagnostic standpoint, perhaps the most unique histopathologic finding is the presence of syncytial and parenchymal multinucleated endothelial cells. This feature occurs in only approximately one fourth of the cases and cannot be used as a sensitive criterion for the diagnosis of Henipah virus infections; furthermore, these cells can also be seen in measles virus, RSV, HPIV, herpesviruses, and other infections. Unequivocal diagnosis can be made only by laboratory tests such as IHC, cell culture isolation, PCR, or serology.[104,304,316-319]

Other Unusual Infections

Parvoviruses

The parvoviruses are small (18 to 26 nm) naked viruses that possess a single-stranded DNA genome and require actively dividing cells to complete the viral replication cycle. In 2005, Allander et al.[320] identified a new parvovirus (genus *Bocavirus*) associated with lower respiratory tract infections in children; however, there is no information as yet that describes specific pulmonary pathology attributed to this newly identified agent. On the other hand, human parvovirus B19, a member of the genus *Erythrovirus*, has long been known to cause human disease and has been well studied.[321,322]

The most commonly recognized manifestation of B19 infections is erythema infectiosum, and approximately one third of the cases of maternal parvovirus infections result in intrauterine parvovirus B19 infections. This places the fetus at increased risk for severe anemia, hydrops, and death. Hydrops fetalis is the most commonly recognized complication of intrauterine parvovirus infection, accounting for 4% to 18% of all cases of nonimmune hydrops. Cases tend to be clustered during community outbreaks of erythema infectiosum.[321,322] Pathophysiologic effects and histopathologic findings are a result of the tropism of B19 parvovirus for erythroid precursor cells. Villi from placentas from patients with B19–associated nonimmune hydrops are edematous, and fetal capillaries show numerous nucleated erythroid precursors, some containing parvovirus inclusions. The infected cells with eosinophilic "ground glass" intranuclear inclusions and ring-like margination of nuclear chromatin are easily recognized and in the context of a hydropic fetus are pathognomonic of B19 infection. The liver is the major site of blood production in the fetus and the principal organ affected by intrauterine B19 infection. Inclusion-bearing nucleated erythrocytes can also be frequently identified in the lung, making histopathologic examination of this organ a worthwhile endeavor for confirming the diagnosis of intrauterine B19 infection (Fig. 11.16A,B).[323-332] However, these cells may be infrequent and irregularly distributed, requiring examination of multiple sections and use of IHC to confirm the diagnosis (Fig. 11.16C,D).
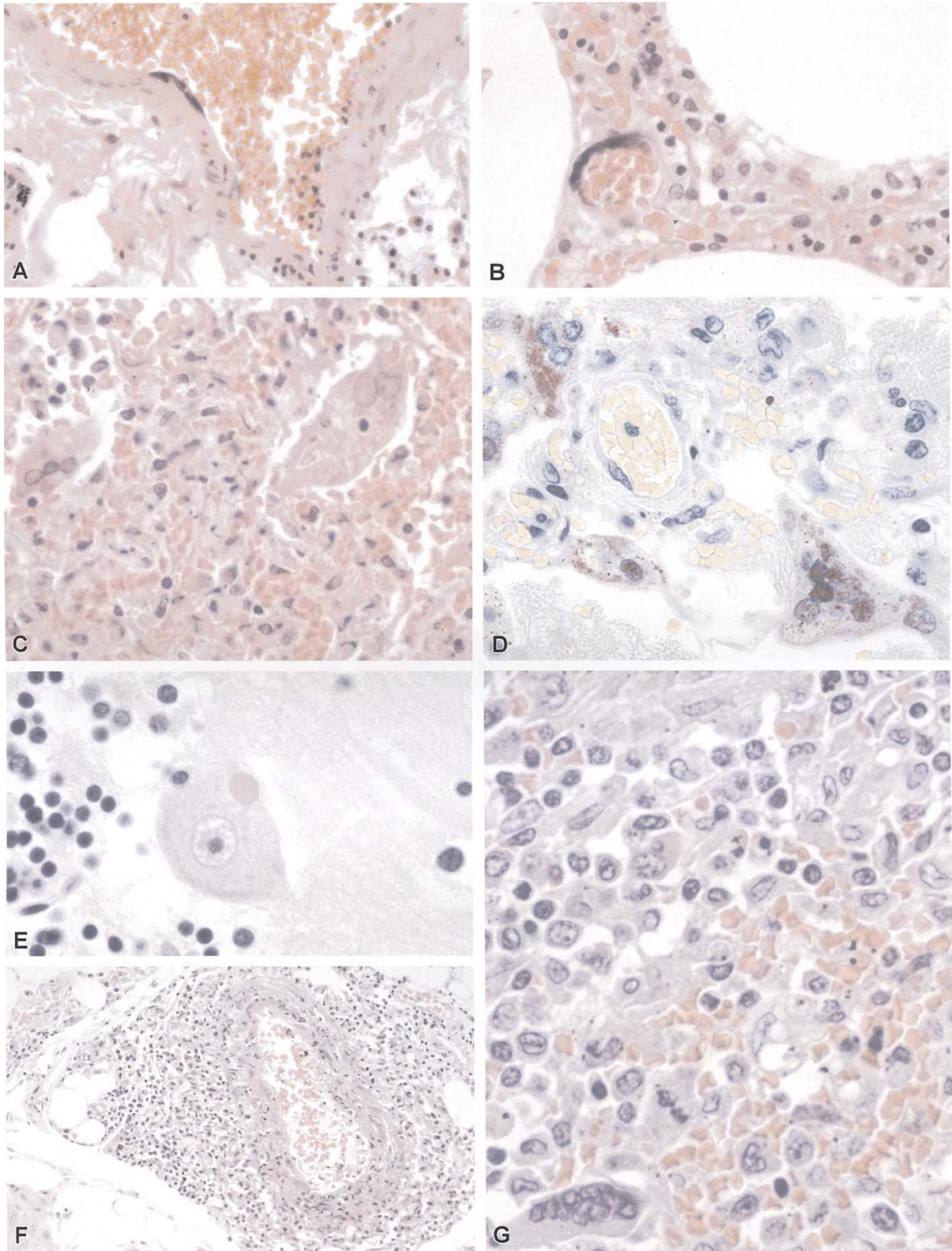
FIGURE 11.15. Nipah virus. **A.** Pulmonary vessels showing a mild vasculitis and a multinucleated endothelial syncytium. **B.** Interstitial pneumonitis. Note small vessel lined by a multinucleated endothelial cell. **C.** Interstitial pneumonitis with intraalveolar multinucleated giant cells with nuclear inclusions. **D.** Viral nature of giant cells, in **C,** as evidenced by immunostaining for Nipah viral antigens. **E.** Typical eosinophilic viral inclusion ("Negri-like") in cytoplasm of a neuron. **F.** Moderate vasculitis in soft tissue in a fatal case of Nipah virus infection. **G.** Multinucleated giant cell as seen in the splenic parenchyma. Besides in lung, giant cells can also be seen in other organs such as kidney and lymphoid organs. **A–C,E–G,** H&E; **D,** immunoalkaline phosphatase staining, naphthol fast-red, and hematoxylin counterstain.
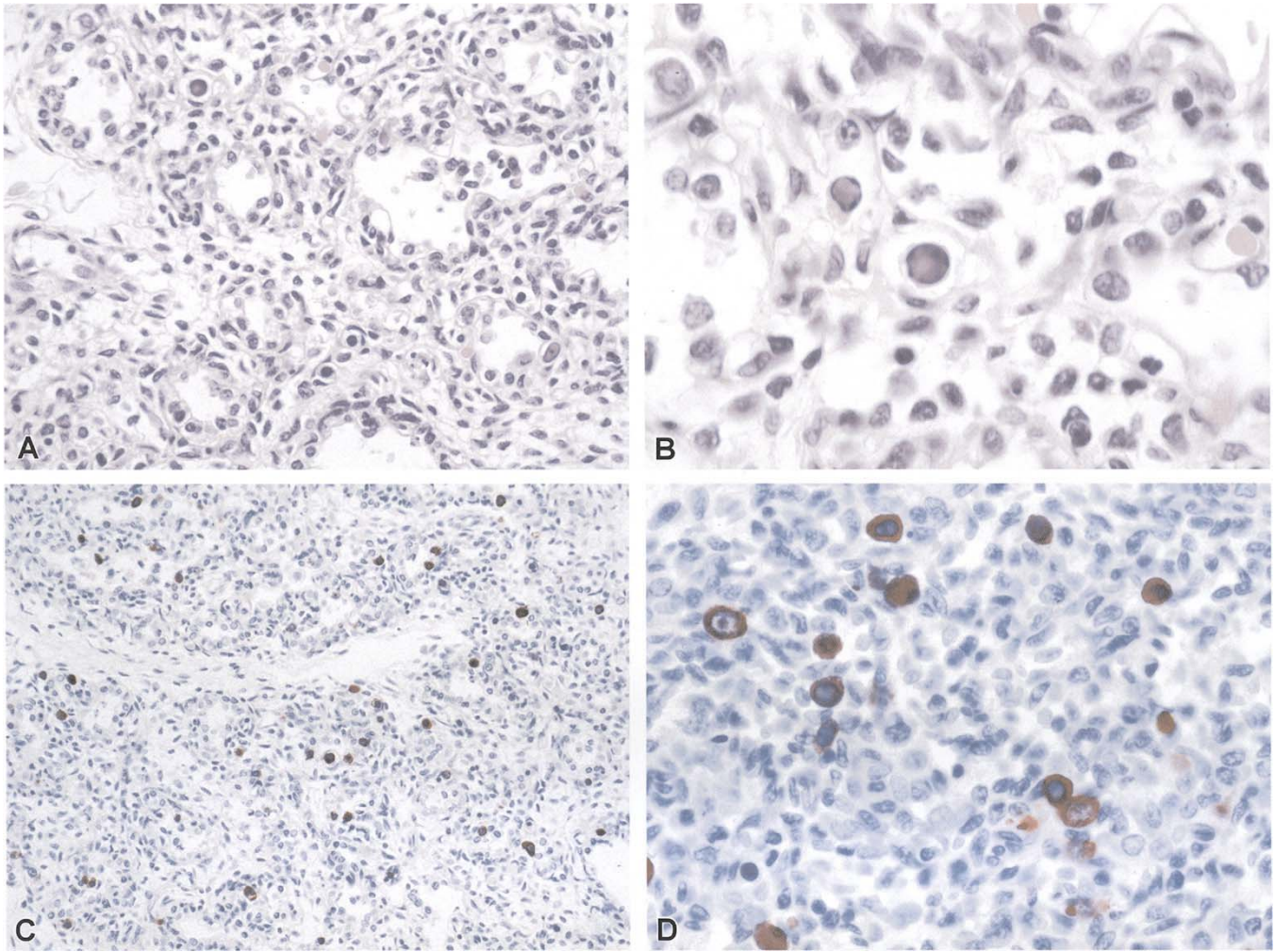
FIGURE 11.16. Parvovirus. **A,B.** Histopathologic findings in the lung of a B19–infected hydropic fetus. Several erythroid precursors contain ground-glass inclusions typical of parvovirus B19. Inclusions-bearing erythrocytes can frequently be identified in the vascular spaces. **C,D.** Localization of parvovirus antigens in the nucleus and cytoplasm of erythroid precursors by using immunohistochemistry (IHC) (same patient as in **A,B**). **A,B,** H&E; **C,D,** immunoalkaline phosphatase staining, naphthol fast-red, and hematoxylin counterstain.

Hemorrhagic Fever Viruses

The combination of fever and hemorrhage can be caused by different viruses, rickettsiae, bacteria, protozoa, and fungi. However, the term *viral hemorrhagic fever* (VHF) is usually reserved for systemic infections characterized by fever and hemorrhage caused by a special group of viruses transmitted to humans by arthropods and rodents. The VHFs are febrile illnesses characterized by abnormal vascular regulation and vascular damage and are caused by small, lipid-enveloped RNA viruses. This syndrome can be caused by viruses belonging to four different families that differ in their genomic structure, replication strategy, and morphologic features (Table 11.4). Arenaviruses, bunyaviruses, and filoviruses are negative-stranded,

whereas flaviviruses are positive-stranded RNA viruses. Hemorrhagic fever viruses are distributed worldwide, and the diseases they cause are traditionally named according to the location where they were first described. The oldest and best known is yellow fever virus; others include Lassa fever, lymphocytic choriomeningitis, Ebola, and Dengue viruses.

Viral hemorrhagic fevers share many common pathologic features, although the overall changes vary among the different diseases. The similar pathologic and immunopathologic findings in cases of VHF suggest that microvascular involvement and instability is an important common pathogenic pathway leading to shock and bleeding in many instances. Infection of the mononuclear phagocytic system and endothelium are thought to play

TABLE 11.4. Viruses associated with the hemorrhagic fever syndromes

| Arenaviridae	Bunyaviridae	Flaviviridae	Filoviridae
Junin	*Nairovirus*	Mosquito-borne	Marburg
Machupo	Crimean-Congo	Yellow fever	Ebola (four subtypes)
Guanarito	Hemorrhagic fever	Dengue viruses 1–4	
Sabia	*Phlebovirus*	Tick-borne	
Lassa	Rift Valley fever	Kyasanur Forest disease	
	Hantavirus	Omsk hemorrhagic fever	
	Hantaan, Puumala,		
	Seoul, Sin		
	Nombre, Black Creek		
	Canal, Bayou, others		
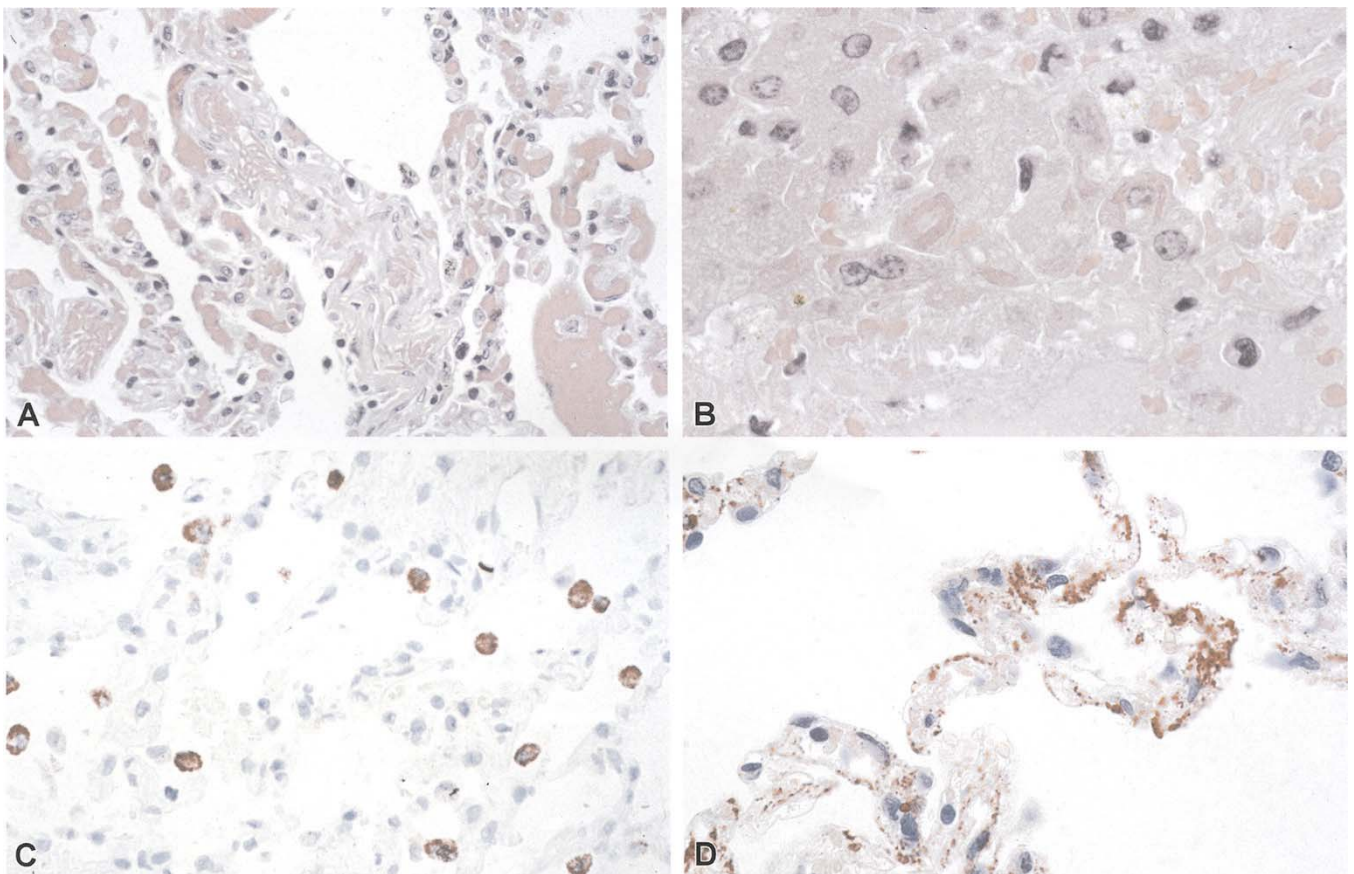
FIGURE 11.17. Ebola virus hemorrhagic fever. **A.** Pulmonary congestion and lack of inflammation. **B.** Numerous filamentous Ebola virus inclusions are seen within hepatocytes in association with hepatocellular necrosis. **C.** Ebola virus–infected intraalveolar macrophages as seen by colorimetric in-situ hybridization using digoxigenin-labeled probes. **D.** Viral anti-gens are seen in endothelial cells and other interstitial cells in this lung section. **A,B,** H&E; **C,** digoxigenin-labeled probes followed by immunoalkaline phosphatase staining, naphthol fast-red, and hematoxylin counterstain; **D,** immunoalkaline phosphatase staining, naphthol fast-red, and hematoxylin counterstain.
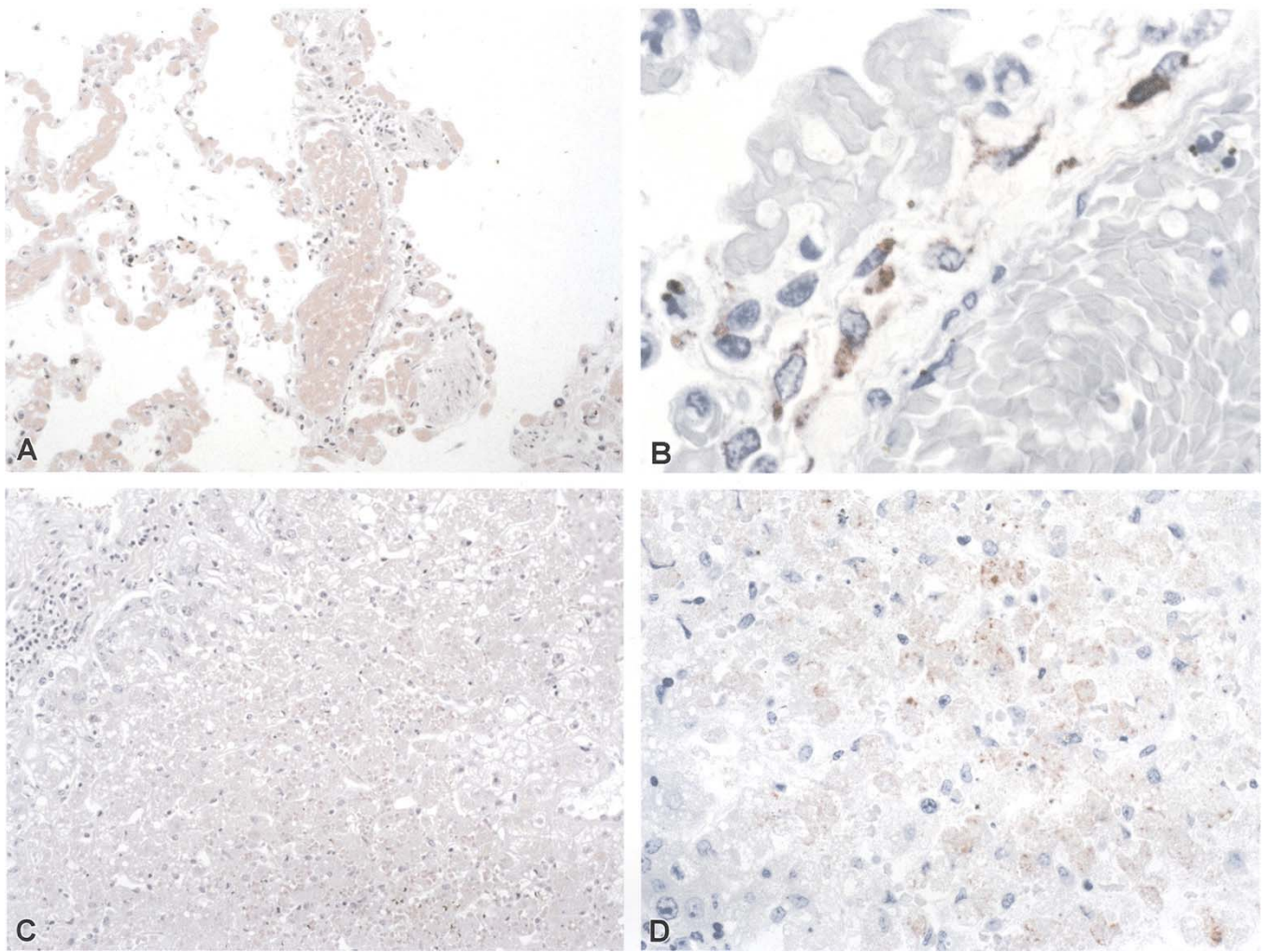
FIGURE 11.18. Yellow fever. **A.** Pulmonary congestion in fatal case of yellow fever associated with vaccination. **B.** Yellow fever antigens in the pulmonary interstitium of the same patient. In natural infections, yellow fever antigens are usually not seen outside the liver in fatal cases. In contrast, in vaccine-associated cases viral antigens can be found in a variety of extrapulmonary organs, including heart, lung, and spleen. **C.** Extensive midzonal hepatic necrosis characteristic of yellow fever. **D.** Abundant antigens of yellow fever virus are observed in midzonal area of hepatic lobule in this immunohistochemical preparation. **A,C,** H&E; **B,D,** immunoalkaline phosphatase staining, naphthol fast-red, and hematoxylin counterstain.

a critical role in the pathogenesis of VHFs through the secretion of physiologically active substances, including cytokines and other inflammatory mediators (Figs. 11.17C,D, 11.18B, 11.19B,C, and 11.20B). At autopsy common findings include widespread petechial hemorrhages and ecchymoses involving skin, mucous membranes, and internal organs. However, in many HF patients manifestations of bleeding may be minimal or absent. Effusions, occasionally hemorrhagic, are also frequently seen. Widespread, focal, and sometimes massive necrosis can be commonly observed in all organ systems and is often ischemic in nature. Necrosis is usually most prominent in the liver and lymphoid tissues. The most consistent microscopic feature is found in the liver and consists of multifocal hepatocellular necrosis with cytoplasmic eosinophilia, Councilman bodies, nuclear pyknosis, and cytolysis (Figs. 11.17B and 11.18C). Inflammatory cell infiltrates and necrotic areas are usually mild and, when present, consist of neutrophils and mononuclear cells. Commonly observed histopathologic changes in the lung include various degrees of hemorrhage, intraalveolar edema, interstitial pneumonitis, and diffuse alveolar damage (Figs. 11.17A, 11.18A, 11.19A, 11.20A, and 11.21A). Several references containing detailed pathologic descriptions in human cases are recommended.[29,72,333–350]
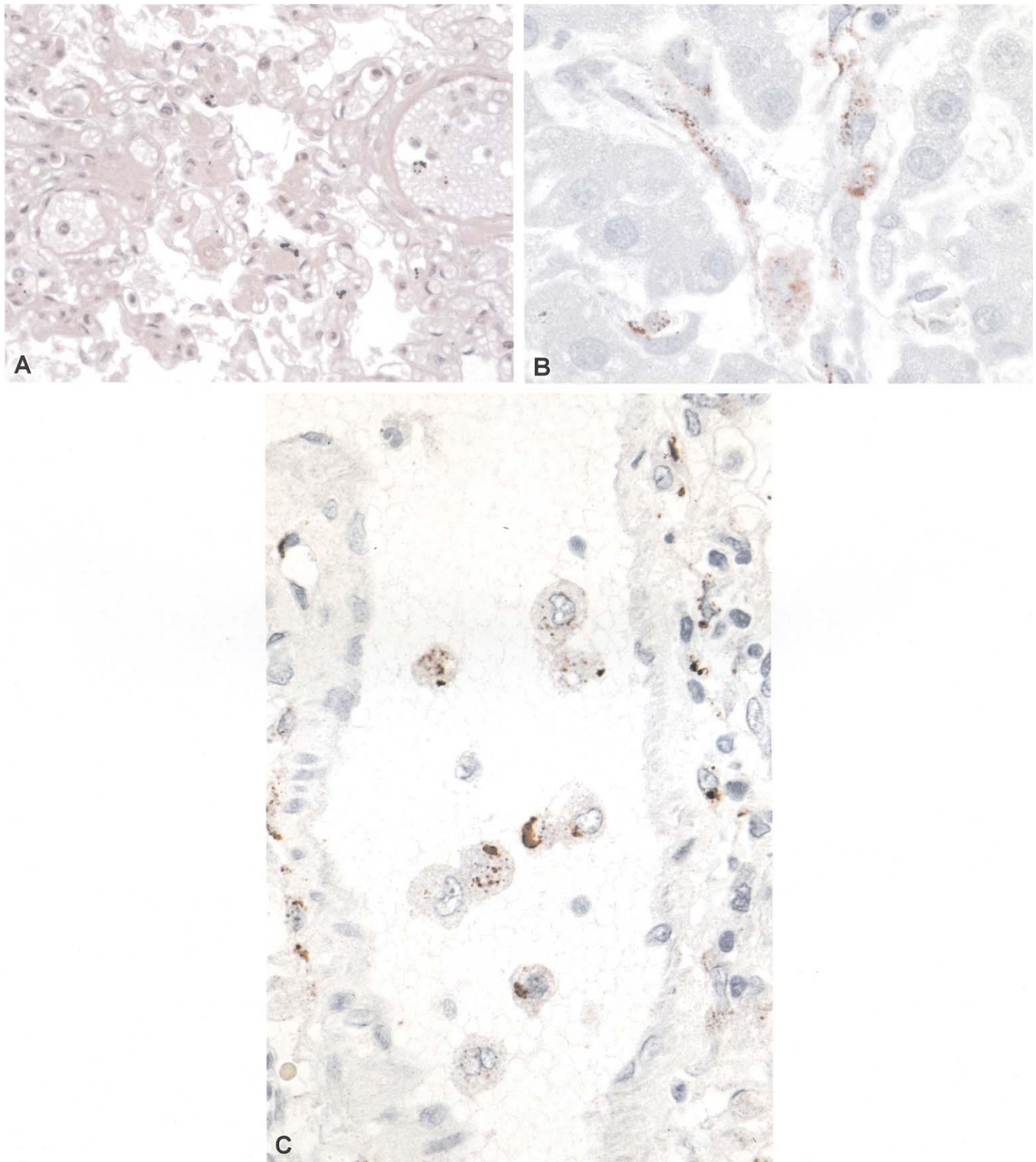
FIGURE 11.19. Dengue hemorrhagic fever. **A.** Lung showing severe congestion and a mild mononuclear interstitial pneumonitis. **B.** Immunostaining of liver showing viral antigens predominantly within sinusoidal Kupffer cells. Note absence of staining of hepatocytes. **C.** Numerous viral antigen-containing circulating mononuclear cells in a pulmonary vessel. **A,** H&E; **B,C,** immunoalkaline phosphatase staining, naphthol fast-red, and hematoxylin counterstain.
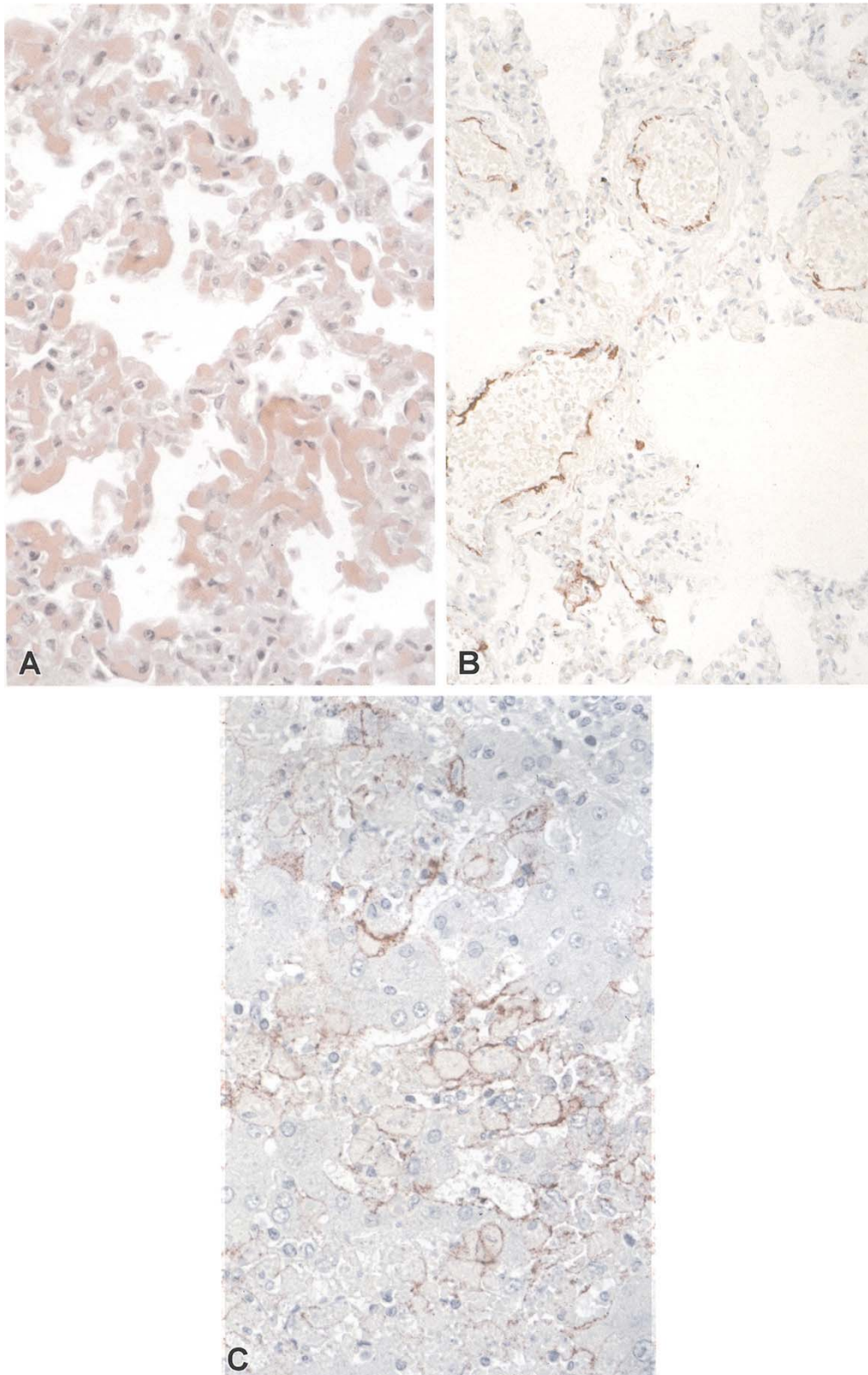
FIGURE 11.20. Lassa fever. **A.** Pulmonary congestion and an absence of significant inflammatory response. **B.** Lassa viral antigens are seen in endothelial cells lining medium-sized vessels in this section of the lung. **C.** Using immunohistochemistry, abundant Lassa virus antigens are seen within cytoplasm of hepatocytes and sinusoidal lining cells in association with areas of hepatocellular necrosis. **A,** H&E; **B,C,** immunoalkaline phosphatase staining, naphthol fast-red, and hematoxylin counterstain.
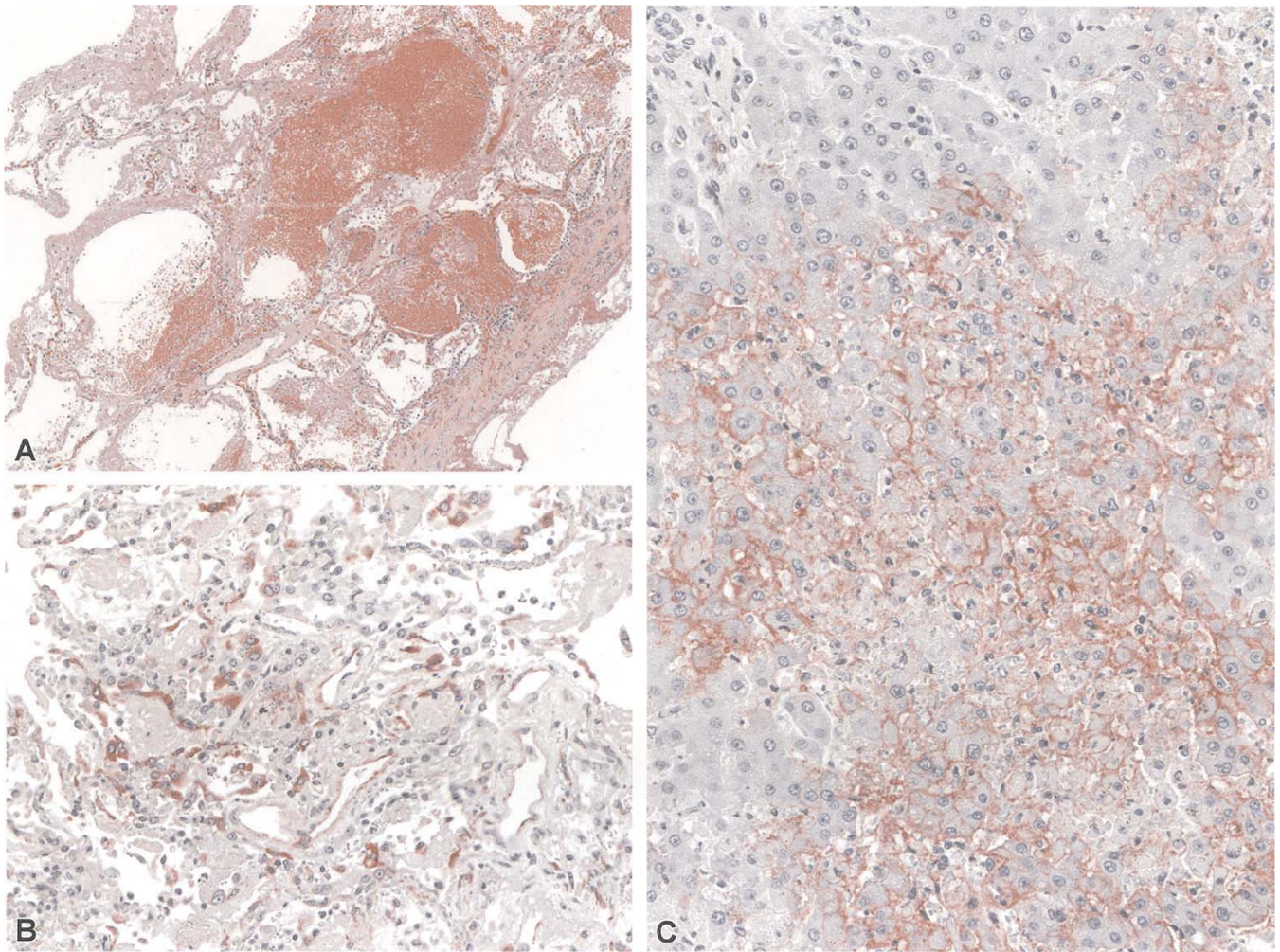
Figure 11.21. Lymphocytic choriomeningitis virus (LCMV). **A.** Pulmonary hemorrhage and diffuse alveolar damage in a patient who was infected through organ transplantation. Note that, unlike this case, which occurred due to immunosuppression, LCMV infections are rarely fatal and usually resolve with no specific treatment. **B.** Abundant LCMV antigens in areas of lung showing diffuse alveolar damage. **C.** Using immunohistochemistry, abundant LCMV antigens are seen within cytoplasm of hepatocytes and sinusoidal lining cells in association with areas of hepatocellular necrosis. **A,** H&E; **B,C,** immunoalkaline phosphatase staining, naphthol fast-red, and hematoxylin counterstain.

The diagnosis of VHF should be suspected in patients with appropriate clinical manifestations returning from an endemic area, particularly if there is travel to rural areas during seasonal or epidemic disease activity. The diagnosis suspected by history and clinical manifestations can also be supported histopathologically, and the overall pattern of histopathologic lesions may suggest a specific diagnosis. However, because of similar pathologic features seen in VHF and a variety of other viral, rickettsial, and bacterial infections, unequivocal diagnosis can only be made by laboratory tests such as cell culture isolation, serology, PCR, and IHC (Figs. 11.18D, 11.20C, and 11.21B,C).

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