CHAPTER 9

Viral Infections

WASHINGTON C. WINN, JR. and DAVID H. WALKER

Viral infections are among the most common afflictions of man. It has been estimated that children experience two to seven respiratory infections each year; adults are afflicted with one to three such episodes.[1]

Many viral infections, such as chickenpox and measles, are contracted through the respiratory tract but usually manifest themselves in other organ systems. Despite the frequency with which viruses infect respiratory epithelium, very few viral pneumonias come to the attention of the anatomic pathologist. The reasons for this paradox are multiple. Most viral respiratory infections, excluding epidemic influenza, produce acute morbidity but little mortality in healthy individuals. The specific etiologic agent may not be known, but the likely identity of the culprit as viral is often suspected from clinical or epidemiologic data. Invasive procedures to procure tissues are unlikely under these conditions, except in those few cases that have unusual or severe clinical presentations.

With rare exceptions the specific identification of a viral infection must come from the clinical virology laboratory. The cytopathic effect of cytomegalovirus in tissue is pathognomonic, and the cytopathic effect of certain other viruses, such as adenovirus and herpes simplex virus, strongly suggests the specific diagnosis. However, definitive identification must come from virologic and/or immunologic investigations. What, then, is the role of the anatomic pathologist? First and most importantly, he should be at the center of the diagnostic action once a specimen of tissue has been obtained. If the clinician has suspected a viral etiology, the pathologist must ensure that specimens are preserved for culture and immunologic studies. If the correct diagnosis has not been suspected and if fresh tissue in sufficient volume has been submitted to the laboratory, the pathologist has a chance to salvage an etiologically specific diagnosis before the tissue is irreversibly immersed in fixative. If, as happens all too often, tissue has been immersed in chemical fixatives, the anatomic pathologist may be able to provide a specific diagnosis by molecular or immunologic means. It should be emphasized, however, that the optimal approach to diagnosis of viral infections includes attempts to recover the infectious agent in culture.

General Features

Viruses Causing Lower Respiratory Tract Infection

The number of viral immunotypes that produce upper respiratory tract infection is large, primarily because more than 100 types of rhinovirus can produce the common cold. A relatively restricted number of viruses account for almost all infections below the level of the larynx. With few exceptions, serious viral infections of the trachea, bronchi, and lung are diseases of infants, children, and immunocompromised adults. To a considerable extent, discrete clinical syndromes in defined age groups can be associated with specific viruses. These syndromes and associated viruses are detailed below and summarized in Table 9–1.

Clinical Syndromes

In neonates and infants less than 2 years old, respiratory syncytial virus (RSV) is the most important cause of viral bronchiolitis and pneumonia[1] (see Chapter 4). Parainfluenzavirus 3 is second only to RSV as a cause of bronchiolitis in this age group. Although much less

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Table 9–1. Viral causes of Respiratory Disease Syndromes[a]

Syndrome	*Common agents*	*Less common agents*
Coryza; "cold"	Rhinovirus Coronavirus Adenovirus Parainfluenza 3	Influenza A or B Parainfluenza 1 or 2 RSV Enterovirus
Pharyngitis	Adenovirus EBV Enterovirus Herpes simplex	Influenza A or B RSV Parainfluenza 1 or 2 Rhinovirus Coronavirus
Croup	Parainfluenza 1–3	Influenza A RSV
Bronchiolitis	RSV Parainfluenza 3	Adenovirus Parainfluenza 1 or 2 Influenza A or B Rhinovirus
Pneumonia	RSV Parainfluenza 3 Adenovirus Influenza A or B CMV (compromised host)	Parainfluenza 1 or 2 Rhinovirus EBV

[a] Adapted from McIntosh.[28]

common, adenoviruses may also produce severe bronchiolitis and fatal pneumonia in very young children.[2,3] The bronchiolitis may be obstructive and apnea may occur. Viruses have been established as etiologic agents in some cases of sudden infant death syndrome,[4] particularly those associated with a lymphocytic bronchiolar infiltrate.

Parainfluenza viruses 1 and 2 are important causes of croup (infectious laryngotracheobronchitis) and pneumonia in older children (aged 2–6 years). These infections occur primarily in epidemics, and most children have been infected by the time they are 5 years old.

Immunity to these pediatric respiratory viruses is incomplete. Infections recur throughout life, usually as mild upper respiratory disease. It is now recognized, however, that severe lower respiratory tract disease is more common in adults than was previously appreciated.[5,6]

A common manifestation of severe respiratory infection in children and adults is the influenza syndrome—the abrupt onset of fever, headache, malaise, severe myalgias, and prostration in addition to cough, nasal congestion, and sore throat. The etiology of approximately half of these infections is unknown. Influenzaviruses A and B are responsible for one-third of cases; the remainder of the influenza syndromes are produced by a variety of agents, including coronaviruses, which usually cause upper respiratory tract disease.[1] Adenoviruses are particularly likely to infect military recruits.

Infection of the lower respiratory tract may occur as a complication of disseminated infection by viruses that usually cause disease in other organs. The most common examples of this phenomenon are the pneumonias that occur in patients with the cutaneous viral infections, measles and varicella.

As many as 10% of influenza virus infections are associated with preexistent lower respiratory tract disease.[7] Other respiratory viruses, such as adenovirus, may also infrequently produce pneumonia in normal adults.[8,9] On very rare occasions, upper respiratory pathogens, such as rhinoviruses, may cause infection of the airspaces. The respiratory viruses function as opportunistic pathogens when they produce serious, life-threatening disease in adults. Even influenzavirus, a potent pathogen in its own right, produces pneumonia more frequently in patients with chronic cardiopulmonary disease, in elderly persons, and in women who are in the late stages of pregnancy.[1,7,10]

The predisposing factors can take a variety of forms. The patients at highest risk are those whose cellular immune system has been compromised, usually by immunosuppressive disease or chemotherapy.[11] Not unexpectedly, the most common viral infections in these patients are those produced by endogenous viruses that are periodically reactivated, especially cytomegalovirus (CMV) and herpes simplex virus.

Local host defense mechanisms are also important, as demonstrated by the predisposition to severe influenza infection in patients with chronic cardiac and pulmonary disease.[10] Herpes simplex infections often occur when the integrity of the respiratory tract has been breached by an endotracheal tube or a tracheostomy.[12] Viral pneumonia itself, usually from measles, may even serve as the predisposing factor for other viral infections.[13]

Radiologic and Pathologic Features of Viral Pneumonia

There are several patterns of inflammatory reaction in viral infections of the lower respiratory tract; they are not mutually exclusive.

The first of these is necrotizing bronchitis or bronchiolitis, which is produced most commonly by the influenza viruses, RSV, adenovirus, and herpes simplex virus. The inflammatory reaction to each of these agents consists primarily of mononuclear inflammatory cells, but neutrophilic leukocytes (PMNs) also participate in the inflammatory response.

On occasion, the response to a pure viral infection includes many PMNs, simulating the response to a bac-

terial infection.[14] The inflammatory exudate in some viral pneumonias undergoes extensive karyorhexis and karyolysis, producing fragmented cells and nuclear dust that make determination of the cell types difficult. A similar phenomenon may be observed in some bacterial pneumonias, such as those produced by *Legionella* spp.[15]

A second common histologic pattern is interstitial pneumonitis with diffuse alveolar damage. Interstitial edema and lymphocytic infiltration are mixed to varying degrees with destruction and regeneration of the alveolar lining cells, intraalveolar edema and hemorrhage, and hyaline membranes. This pattern of reaction can be seen with virtually any viral infection of the lung. Unless diagnostic inclusions are present, the differential diagnosis is extensive and includes noninfectious damage to the alveolar membrane.[16]

Finally, there are focal inflammatory lesions, often centered around bronchioles or small blood vessels. These lesions may be necrotizing with an acute or chronic inflammatory response or they may resemble ischemic necrosis, perhaps because of virally induced damage to vascular endothelium. Varicella-zoster and herpes simplex viruses are the preeminent causes of this pattern, probably following hematogenous dissemination of the virus. A micronodular pattern has also been described for pulmonary infections by cytomegalovirus in immunosuppressed patients.[17]

The macroscopic and radiographic appearance of the lung is as varied as the microscopic pathology.[18] Patchy or diffuse infiltrates may be present. The chest radiograph is classically described as suggesting interstitial disease, but a pattern that indicates alveolar filling is often present.

The lungs are usually heavy and airless when visualized at surgery or at autopsy. Interstitial thickening may be evident. Hemorrhage is frequently noted. Nodular or micronodular lesions have been described pathologically. On occasion, infiltrates are segmental or even lobar, resembling bacterial pneumonia.[7,10,18]

Methods for Specific Diagnosis of Viral Infections

Infections may be diagnosed by isolation of the agent in culture, by demonstration of antigens or nucleic acid in clinical specimens, or by documenting a serological response to the virus (Table 9–2). The interested reader should consult recent textbooks of diagnostic microbiology for complete details.[19–22]

Viral Culture

Viral diagnostic facilities, which were once the province of the research laboratory, are increasingly available in clinical laboratories of academic medical centers and even in community hospitals.[23] A specimen should be submitted for culture as soon as possible, because a decrease in the number of infectious particles can be expected after storage. The rapidity with which viruses deteriorate varies, being greatest with cytomegalovirus and respiratory syncytial virus (RSV). Although some centers prefer to inoculate cell cultures at the bedside when RSV is considered,[24] acceptable results have been achieved even with this labile virus when delays in processing did not exceed 6 hr and when specimens were refrigerated during transportation.[25,26] It is quite feasible, therefore, to use a regional reference laboratory for viral cultures if facilities for culture of viruses are not available locally.[27]

In general, maintenance of the specimen at 4°C is preferable to freezing, which results in the loss of many

Table 9–2. Diagnostic modalities for Viral Respiratory Disease

Virus	*Culture*	*Antigen*	*Molecular*	*Serology*	*Comments*
Influenza	++	+	−	+	Culture primary; antigen detection not widely practiced
Adenovirus	++	+	+	+	Culture primary
Herpes simplex	++	+	+	−	Serology not useful
Measles	+	−	−	++	Culture difficult; diagnosis clinical
RSV	++	++	−	+	Antigen detection thoroughly evaluated
Parainfluenza	++	+	−	+	Serology hard to interpret
Cytomegalovirus	++	+	+	−	Molecular probes promising; serology hard to interpret

infective particles. Once again, cytomegalovirus and RSV are particularly vulnerable to freezing and thawing. If a long delay between collection of the specimen and processing is inevitable, the specimen should be frozen quickly and maintained at −70°C. The definition of "long" is a matter of some dispute. McIntosh[28] has suggested, most reasonably, that specimens should be refrigerated unless more than 4 days will elapse before they are cultured. The worst temperature for storage of most viruses is −20°C; use of a frost-free freezer, which produces repetitive cycles of freezing and thawing, is tantamount to murder.

The major advantage of virologic diagnosis is that the procedure is relatively "broad minded," if a variety of cell cultures are infected. That is, most of the likely etiologic agents will be recovered and the prejudices of the clinician or pathologist are unlikely to misdirect the diagnostic efforts. The major disadvantage of virologic diagnosis is the length of time required for the recovery of some important pathogens, particularly cytomegalovirus and respiratory syncytial virus. Innovative approaches to viral diagnosis are likely to minimize this difficulty. For example, centrifugation of the inoculum onto a monolayer of cells and subsequent detection of viral growth by immunofluorescence have resulted in recovery of most isolates of several herpes viruses, including cytomegalovirus, within 48 hr.[29,30]

Morphologic Diagnosis

Demonstration of viral inclusions in clinical material is the time-honored method for diagnosing viral infections rapidly (Table 9–3). All viruses produce biochemical alterations in infected cells and morphologic abnormalities can be detected in many instances. In a few instances, the cytologic abnormalities can be visualized with the light microscope as viral inclusions. In general, the DNA-containing viruses are assembled in the nucleus and produce intranuclear inclusions, whereas the RNA-containing viruses are assembled in the cytoplasm and produce cytoplasmic inclusions.

A presumptive etiologic diagnosis can be made from the typical morphology of the inclusions. Part of the characteristic light microscopic appearance of the inclusions derives from artifacts of fixation. Strano[31] recommends fixation of material in Zenker's acetic acid or Bouin's fixative and also in formalin, if viral infection is suspected. The extent to which these fixatives are superior to formalin has not been documented, however. It is fortunate that formalin appears to be an adequate fixative, because the nature of the process is often not suspected before the tissue is fixed. Special stains for the demonstration of inclusions have been developed, but they offer no advantage over a well-done hematoxylin and eosin stain.[31]

Intranuclear inclusions must be differentiated from large nucleoli and from invaginations of cytoplasm; the differential diagnosis of cytoplasmic inclusions includes large phagolysosomes or other focal cellular abnormalities and phagocytized matter, such as red blood cells. Among the viruses that produce lower respiratory infections, herpes simplex, varicella-zoster, and adenovirus produce intranuclear inclusions; respiratory syncytial virus and parainfluenza viruses may cause cyto-

Table 9–3. Viral inclusions

Virus	*Intranuclear*	*Cytoplasmic*	*Comments*
Influenza	−	−	Occasional reports of small cytoplasmic inclusions in exfoliated cells
Parainfluenza	−	+	Inclusions not common
Respiratory syncytial	−	+	Eosinophilic inclusions common in cell culture, less frequent in tissue
Measles	+	+	Nuclear inclusions resemble herpes; cytoplasmic inclusions may be very large
Herpes simplex, varicella-zoster	+	−	Eosinophilic; may have halo; may be multinucleate
Adenovirus	+	−	Early inclusions resemble herpes; late inclusions "smudge" cells
Cytomegalovirus	+	+	Early nuclear inclusions resemble herpes; late inclusions large and basophilic; cytoplasmic inclusions may be present

plasmic inclusions; both nuclear and cytoplasmic inclusions may be seen in cells infected by measles and cytomegalovirus. The inclusions may be detected in tissue sections or, in some instances, in cytologic preparations.[32,33] They will be discussed under the individual infections.

Less specific cytologic alterations may also be detected in expectorated sputum or lavaged bronchial contents. Fragmented cells with enlarged, hyperchromatic nuclei and prominent nucleoli represent nonspecific responses to epithelial damage.[33] Virally induced damage to ciliated respiratory epithelial cells has been well documented.[34] Ciliocytopthoria, originally described by Papanicolaou, is a pattern of cellular degeneration in which free ciliated tufts without nuclei, nuclear degeneration, and acidophilic cytoplasmic inclusions may be found.[35] The phenomenon has been associated with viral respiratory tract infection, but may also be observed in other pulmonary diseases.[35]

The electron microscope has also been used for diagnostic purposes, although less frequently for diseases of the respiratory tract than for those of other organs such as the gastrointestinal tract. Viral particles may be demonstrated directly in clinical material or after amplification in cell culture by negative-staining electron microscopy.[36] Virions may also be demonstrated in thin sections.[37] The diagnosis may be definitive, as in the case of adenovirus. In other instances the morphology of the agents, such as those of the herpesvirus group, is identical, and electron microscopy may be of less differential value than light microscopy.

The advantages of morphologic diagnosis are that the detection of viruses is not limited by narrow specificity of reagents and that cellular or tissue damage can be documented at the time the virus is identified. However, it is often very difficult to associate viruses that produce latent infections, particularly cytomegalovirus, with clinical disease. Demonstration of cytopathology, and especially histopathology, may be a much better indicator that the agent is clinically important than the recovery of the virus in cell culture or the demonstration of viral nucleic acid in cells that appear normal structurally.

The disadvantages of morphologic diagnosis are the limited number of viruses that produce specific morphologic changes in infected cells and even in those that do, the limited number of inclusions compared to the large number of infected cells. For example, the sensitivity of detection of cytomegalic inclusions in lungs from which CMV is isolated may be as low as 38%.[38] Electron microscopy is infrequently used as a routine diagnostic tool because of the restricted availability of the equipment and the expense of examining ultrathin sections. Sampling errors are magnified when tissue is examined at the ultrastructural level. Fortunately, infectious agents are sturdy. Although the tissue may not be well preserved, the viruses are usually well preserved even after formalin fixation and paraffin embedding. Pinkerton and Carroll[39] have demonstrated that portions of a paraffin block may be successfully re-embedded for electron microscopy after inclusions are identified with the light microscope.

Immunologic and Molecular Diagnosis

Immunologic and molecular diagnostic techniques can be applied to antigens and nucleic acids in solution or in tissue sections (in situ). Immunofluorescence and immunoenzymatic techniques have been used in the diagnosis of viral infections for several decades, but have received a boost in recent years from a supply of reagents that has steadily improved in quality. Monoclonal antibodies are now available for most of the viruses that infect the respiratory tract. In some instances the monoclonal antibodies represent a major improvement over their polyclonal forebears.[40] It is essential that the efficacy of the monoclonal reagent be demonstrated in clinical studies, because the restricted antigenic site to which the monoclonal antibodies react may not be uniformly present on infecting strains.[41]

An immunoassay may be performed on imprint smears from tissue, on centrifuged fluid, or on tissue sections. If the specimen contains large amounts of mucus, which produces nonspecific fluorescence, washing of the exudate has been recommended to facilitate evaluation of the fluorescent smear,[42] but this may not be necessary to detect most infections.[43] In recent years, experience has been most extensive with RSV infections; the availability of ribavirin as effective antiviral chemotherapy has greatly stimulated the interest in rapid diagnostic tests.[44,45] If effective therapy becomes available for other agents, the impetus for expedited diagnosis will be increased. Although herpes simplex infections have been treated successfully with acyclovir in some instances, there are as yet no reports of therapy of herpes simplex virus infections of the lower respiratory tract.[46] The efficacy of therapy for cytomegalovirus infections has not yet been unequivocally demonstrated.

In recent years nucleic acid hybridization has been increasingly used for diagnosis of viral infections in the research laboratory.[47] Probes have been developed for a variety of viruses, including cytomegalovirus, herpes simplex, and adenovirus.[48–50] To date, most investigators have concentrated on the detection of nucleic acid in body fluids after fixation to a solid phase such as nitrocellulose.[51] Sandwich assays, in which the specimen remains in solution, are useful for testing complex biological specimens but may have reduced

sensitivity.[52] Jansen and colleagues[53] approached the problem innovatively, combining molecular and immunologic methods; by absorbing the clinical specimen to a monoclonal antibody-coated surface before hybridization they eliminated much of the nonspecific activity in highly contaminated specimens such as stool.

In situ hybridization, using tissue sections as the substrate, has been applied less frequently to the diagnosis of respiratory infections. The feasibility of the technique was demonstrated by Myerson and co-workers,[54] who detected cytomegalovirus DNA in 13 of 14 lung biopsies from which the virus was cultured; hybridization was not demonstrated in sections that contained varicella-zoster virus, herpes simplex virus, or adenovirus. One drawback of the first-generation hybridization procedures was the need to label the probe with a radiotracer, usually ^{32}P. Replacement of the radiolabel with an enzymatic colorimetric assay has been accomplished and will make the procedure more generally applicable if adequate sensitivity can be obtained. Unger and colleagues[55] have described a simplified colorimetric in situ hybridization test for CMV, which can be performed on formalin-fixed, paraffin-embedded sections; an avidin-alkaline phosphatase label can be visualized within 8 hr of cutting the histologic section.

If tissue has been refrigerated or snap-frozen, there is greater flexibility in the selection of immunologic and molecular studies. In many instances enzyme immunoassays and in situ hybridization can be performed on fixed, embedded tissue. Immunofluorescence for some viral antigens can be accomplished only after the tissue section has been treated with a proteolytic enzyme such as trypsin.[56,57] Enzymatic digestion of tissue sections has been demonstrated to facilitate in situ hybridization also.[55]

These immunologic and molecular techniques facilitate the provision of more rapid etiologic diagnoses. In most instances they require such intensive or prolonged effort that it is difficult to offer them as "stat" procedures unless a therapeutic decision for a critically ill patient hangs in the balance. Even if not performed on an emergency basis, however, these tests offer greatly expedited diagnostic capability for infectious agents that are difficult to isolate, such as varicella-zoster virus, or grow slowly in isolation systems, such as respiratory syncytial virus and cytomegalovirus. There is little doubt that the nucleic acid probes will be useful diagnostically, just as immunodiagnostic procedures have been in selected situations. Adequate sensitivity and specificity must be combined with a procedure that can be performed on a routine basis before they will receive wide acceptance. The selection of the optimal nucleic acid sequence is essential for both adequate sensitivity and specificity. Although monoclonal antibodies are immunologically specific and nucleic acid probes are genetically specific, freedom from false-positive results cannot be assumed when complex clinical specimens are tested.

Approach to the Specimen in the Pathology Laboratory

If the clinician suspects an infectious etiology, many of the diagnostic procedures will have been initiated in parallel with submission of the tissue to the pathology laboratory. If an infectious process has not been suspected clinically, unfixed tissue is not likely to be reserved unless macroscopic lesions suggest an inflammatory process or a frozen section suggests an infectious cause. Increasingly, small biopsies, bronchial brushings, or bronchial washings are submitted to the laboratory, and often there is not enough residual material to refrigerate an aliquot. It is good practice, however, to preserve tissue for special studies routinely when the patient has been subjected to the risk and trauma of a lung biopsy. Tissue fixed for electron microscopy, frozen for immunofluorescence, or refrigerated for culture is probably not needed often, but is invaluable on those few occasions.

Individual Viral Pathogens

Influenza Virus

Influenza has been and continues to be one of the great scourges of man. Influenza viruses and RSV alone produce epidemic disease annually. Irregularly, but with all-too-great frequency, widespread epidemics of influenza occur, occasionally producing a pandemic that involves virtually the whole world. Epidemics attributed to the influenza viruses have occurred throughout recorded history. In the past century, major epidemics occurred in 1890, 1900, 1918, 1957, and 1968. The great pandemic of influenza in 1918–1919 is estimated to have killed 20–40 million people and accounted for 80% of the deaths in the United States Army during World War I.

The influenza viruses are classified as orthomyxoviruses.[58] They contain RNA, measure 80–120 nm, have a pleomorphic shape with long filamentous forms, and acquire a lipid envelope after maturing ("budding") through the plasma membrane of an infected cell. Three antigenic types are defined by the ribonucleoprotein core of the virus. Type A is the primary pathogen of this group and is responsible for pandemic disease. Influenzavirus B is also an important pathogen and produces epidemic disease. Influenzavirus C is associated serologically with a human infection that appears to be mild and usually goes unrecognized.

Influenzaviruses A and B are additionally classified by the antigenic composition of virally specified surface projections.[59,60] Both viruses contain hemagglutinin and neuraminidase, which are important for attachment of the virus to cells and subsequent penetration of the cells. The hemagglutinin, in particular, is useful for detection of the virus in cell cultures and as a stimulus for a serologic response in infected patients. Minor changes in antigenic composition of the influenza viruses happen regularly (antigenic drift), and major changes (antigenic shift) occur periodically. Strains are described as follows: antigenic group/geographic source/host of origin if other than man/isolate number/year, followed by the antigenic designation of the hemagglutinin and neuraminidase [e.g., Influenza-virus A/Singapore/1/57 (H2N2) or A/swine/Iowa/31 (Hsw1N1)].

Influenzavirus was first isolated from swine in 1920 and from man in 1933. Analysis of subsequent isolates and retrospective evaluation of sera from earlier epidemics has suggested that major shifts in antigenic structure are associated with pandemic disease and that strains may recirculate. Until the 1970s, a single strain or a closely related group of strains circulated at any one time. Since 1977, however, strains with antigenic composition H3N2 and H1N1 have produced human disease concurrently.

Occasional cases of influenza infection may occur at any time of the year, but most are seen in the winter and spring months. In the northern hemisphere, most epidemics occur from January through April; from a global perspective, influenza occurs during every month of the year. There is no evidence for persistent or latent infection by influenza viruses. Although transmission of a virus from an animal to man may occur, it is presumed that the primary means for maintenance of the infection is by person-to-person transmission. As with other respiratory agents, transmission is facilitated by the crowding that occurs during the winter months. Young children are frequently responsible for dissemination of the infection to their families. It is probable that coughing and sneezing are the means of transmission of these viruses, but direct spread by contact cannot be excluded.

Experimental infection may be produced after inoculation of a variety of species, most frequently ferrets or mice. Ferrets, the species in which the initial human isolate was made, are the most susceptible host; in that species infection is usually limited to the upper respiratory tract, but the pathology of viral pneumonia appears similar to human disease.[61] Viral isolates must often be adapted to mice before damage to the tracheobronchial tree and lung results. Raut and colleagues[62] suggested that the ability of isolates to multiply in alveolar cells may confer virulence. They analyzed sections of lung that had been reacted with antiinfluenza antiserum in an indirect immunofluorescence test and suggested that alveolar macrophages were the site of viral replication. In contrast, Rodgers and Mims[63] were unable to detect any differences in the replication of virulent and avirulent isolates of influenzavirus A in macrophages. They examined macrophages that had been washed from the lungs of normal mice by bronchoalveolar lavage and infected in vitro as well as lavaged macrophages from the lungs of mice that had been previously infected in vivo. Influenzavirus A produced a cytolytic infection in alveolar macrophages, but the infection was not productive of infectious viral particles.

Influenza varies from asymptomatic infection to fatal pneumonia. The classic influenza syndrome includes the abrupt onset of headache, chills, and dry cough, followed by high fever, myalgias, malaise, and anorexia. Physical findings are usually minimal, but the patient appears toxic.[59] Fry[7] describes the syndrome as follows:

> The onset was dramatically sudden, the patients often being able to recall the time almost to the minute. The initial symptoms were shivering, a sensation of being chilled, headache, backache, pains in the arms and legs. Later, in a few hours, there developed symptoms referable to irritation of the respiratory tract, with nasal obstruction, rawness of the throat and chest and an irritating cough predominating. The patients felt extremely ill, with marked depression and feelings of impending doom.

The symptomatology of influenzavirus B infection is indistinguishable from that of influenzavirus A.

The symptoms in children are similar, but fever tends to be higher, febrile convulsions may occur, and gastrointestinal symptoms are more common.

Primary influenza pneumonia was first recognized during the pandemic of "Asian" influenza in 1957–1958.[10,64,65] A spectrum of pathologic lesions was recognized in that epidemic. Hers and colleagues[65] in the Netherlands described the bacteriology and pathology of 148 virologically confirmed cases of fatal Asian influenza. Pure viral lesions were present in approximately 25% of patients. Tracheobronchitis was present in all cases and was the only lesion in 4% of individuals; viral pneumonia without bacterial superinfection was demonstrated in 20%.

Primary viral pneumonia occurs predominantly in elderly individuals, in those with chronic cardiopulmonary disease, and in infants, but as many as 25% of individuals may have no recognized underlying disease.[10,66] In the typical case there is a rapid progression from the sudden onset of the influenza syndrome to a severe pneumonia with tachypnea and hypotension. Infiltrates are often found in multiple lobes by chest radiograph. Cyanosis and frothy pulmonary edema are poor prognostic signs.

Viral infection of the respiratory epithelium impairs the function of the mucociliary escalator. Impaired clearance of radiolabeled particles could be demonstrated in patients for as long as 1 month after an acute influenzavirus A infection.[67] The mechanism of the impaired clearance was presumed to be destruction of ciliated epithelial cells. Indeed, investigators have found ultrastructural evidence of ciliary damage during viral upper respiratory infection, even in epithelial cells that were not destroyed.[34]

In addition, functional impairment of phagocytes has been demonstrated in man and in experimental animals. Abramson and associates[68] found decreased middle ear pressure, decreased chemotaxis of neutrophils, and decreased chemiluminescence of stimulated leukocytes in chinchillas that had been infected with influenzavirus A. Although the phagocytic capability of alveolar macrophages is not altered after infection with influenzavirus A in vitro, an immunologically mediated defect in phagocytosis has been observed in vivo.[69] Larson and Blades[70] demonstrated that both chemotaxis and the ability to phagocytize staphylococci were impaired in infected human neutrophils.

Oropharyngeal bacteria are the main particulates of concern in a patient with influenza infection. Bacterial infection, most commonly by *Haemophilus influenzae,* was well recognized during the 1919 pandemic, although the underlying viral agent had not been documented at the time. During the 1957 pandemic *Staphylococcus aureus* was the most frequently isolated secondary pathogen in most locations, followed by *Streptococcus pneumoniae,* and, much less frequently, *Haemophilus influenzae* or *Streptococcus pyogenes.*[10,65]

Bacterial superinfection has also been demonstrated in animal models of influenzavirus infection. An increased frequency of pneumococcal otitis media occurred in chinchillas that had been experimentally infected with influenzavirus A if tympanostomy tubes were not placed in the ear.[68]

Bacterial superinfection was observed in the remaining three-quarters of the Dutch cases, bacterial tracheobronchitis in addition to a viral lesion in 5%, bacterial tracheobronchitis and pneumonia in the presence of viral tracheobronchitis in 40%, and mixed bacterial and viral pneumonia in 31%. Hers et al.[65] noted that the mixed pneumonia category might have been underrepresented, because viral lesions could have been obscured by necrotizing bacterial superinfection. Once the pathologic lesions associated with pure viral pneumonia and mixed infection were defined by bacterial and viral cultures, the presence of pathologically pure viral pneumonia during the 1918 pandemic could be recognized.

It may be very difficult to differentiate clinically the pure viral pneumonia from a mixed bacterial and viral infection unless cavitary lesions are demonstrated on chest radiographs or Gram stains of expectorated sputum suggest bacterial infection. When a bacterial superinfection complicates a resolving viral infection, there is a period of clinical improvement followed by the abrupt reappearance of fever, chills, pleuritic chest pain, and a cough productive of bloody or purulent sputum.[64,66] The mortality appears to be less than when the bacterial and viral infections are concurrent. It may be difficult to isolate the influenzavirus in this situation.

Pathologic Anatomy

In many descriptions of influenza pneumonia it is difficult to separate the viral component of the pathology from damage inflicted by secondary bacterial invaders.[71–73] Most of our knowledge about the pathology of virologically documented influenza pneumonia comes from the Asian and Hong Kong influenzavirus A epidemics of the 1950s and 1960s. There is very little information about the pathology of influenzavirus B disease, which is associated with morbidity but only rarely with mortality. Hers and Mulder[74] described a case of fatal influenzavirus B infection in which pneumonia was absent. There was a widespread degenerative change in the tracheal and bronchial epithelium without an inflammatory response, but mitotic activity was observed. These findings are compatible with more extenisve data from influenzavirus A infections, but difficult to interpret in autopsy material.

Several authors have commented on the similarity of histopathology among cases from epidemics as far back as the great 1918 pandemic.[65,73] Feldman and colleagues[73] were able to compare directly the microscopic abnormalities that they observed in 1968 with those that Winternitz et al.[73a] had observed at the same hospital in 1918. It appears therefore that changes in antigenic structure of the virus have not affected the interactions of virion and man in a major way.

Influenzavirus tracheobronchitis was studied by Walsh[74a] and colleagues, who performed tracheal and bronchial biopsies on six young adults who had acute uncomplicated influenzavirus A disease without secondary bacterial infection. The damage to the mucosal surface was more prominent in the bronchus than in the trachea. Histological changes ranged from cytologic abnormalities to extensive necrosis and ulceration of the epithelium. Columnar cells were vacuolated, nuclei were hyperchromatic, and cilia were absent. Desquamation of the epithelium extended to a basal cuboidal layer of cells or even to the basement membrane. Most

specimens that were taken more than 24 hr after onset of symptoms also demonstrated some degree of metaplasia.

The histopathology of bronchiolitis and alveolitis produced by influenzavirus A has been documented by open lung biopsy[75–77] and at autopsy.[10,71–73,78] If the cases in which bacterial superinfection occurred are eliminated, a consistent but diverse pattern of damage is evident.

The morphologic damage to the pulmonary parenchyma may be minimal even when the virus has been isolated from the lung. Hers and colleagues mentioned 13 cases between 1941 and 1953 in which the patient died in the acute stage of the infection and influenzavirus type A was isolated from the lung, but specific histologic changes were not present. They hypothesized that most of the virus had been aspirated into the distal airspaces ante mortem. Engblom and colleagues,[79] however, described a patient with an influenza syndrome followed by influenzavirus A myocarditis (proved by isolation of the virus from the myocardium) who had clear lung fields by chest radiograph and congestion of the organs at autopsy. We have observed a similar case in which the lungs showed only congestion despite isolation of influenzavirus A in pure culture from the lung. Extensive myocarditis was unsuspected clinically. It appears, therefore, that replication of the virus and serious systemic disease can occur in the absence of extensive pulmonary histopathology.

Hers and colleagues[65] described seven features of pure influenzavirus A pneumonia: (1) cytopathology of the respiratory epithelium down to the alveolar ducts; (2) cytologic damage to the alveolar lining cells; (3) capillary thrombosis and focal necrosis with leukocytic exudate; (4) capillary aneurysms and hemorrhage; (5) after 3–4 days, the appearance of a plasmatic exudate that lines the alveolar ducts and includes hyaline membranes; (6) regeneration of the epithelium of the respiratory bronchioles and alveolar ducts into a pseudostratified metaplastic epithelium after 5–7 days; and (7) regeneration of the alveolar epithelium into a continuous monolayer covering the alveolar wall. The lesions were always focal and sometimes clearly lobular in distribution.

Most recent descriptions of the pathology of influenza pneumonia have concentrated on the bronchiolitis, the interstitial pneumonia, and the reparative process. There are no viral inclusions to help distinguish this viral disease from others. In patients who died early in the course of the disease, necrosis of the ciliated and goblet cells of the epithelium was prominent.[73] Damaged epithelial cells were enmeshed in a luminal fibrin mass.[78] Even 2 weeks after onset of symptoms, patchy membranous bronchitis and bronchiolitis could be seen. The bronchiolitis is often described as necrotizing and may be observed as early as the second day of illness.[73] An acute inflammatory response in the bronchial and bronchiolar epithelium may be seen in the absence of bacterial infection and has been documented in a lung biopsy from which influenzavirus A was recovered in pure culture.[75]

The interstitium of affected areas is congested and edematous and contains a mixed cellular infiltrate. Edema fluid, fibrin, blood, and inflammatory cells fill the airspaces to a varying degree (Figs. 9–1 and 9–2). The contribution of neutrophils to the cellular response varies from scanty to extensive.[73,75,78,80] A dense, purulent inflammatory response is characteristic of bacterial superinfection. There may be areas in a pure influenza pneumonia, however, in which neutrophils are prominent. There may also be extensive fragmentation of cells, leaving a dusting of nuclear debris and making determination of the cell types more difficult (Fig. 9–1).

Hyaline membranes, which stain with periodic acid–Schiff reagent, are a prominent part of the alveolar reaction to influenzavirus A. They are apposed directly to the damaged alveolar walls or to the epithelium of the alveolar ducts and terminal bronchioles (Fig. 9–2). The hyaline membranes have been seen as early as the second day and as late as the third week of illness.[73] As a nonspecific response to alveolar damage, they have been noted to be more prominent when other potentially damaging influences, such as oxygen therapy, have been present.

In patients who died or were biopsied after the first week of illness, reparative changes have been prominent. Metaplastic regeneration of the epithelium has been frequent and sometimes coexistent with residual acute damage. On occasion the proliferation has produced masses of epithelium that are sometimes described as "tumorlets."[78] Interstitial fibrosis has been observed in conjunction with residual inflammation,[76] and may become prominent as the lesion heals. Restrictive pulmonary function defects have been demonstrated in uncomplicated influenza[81] and resolution of the abnormalities may take many weeks.[82] Similarly, interstitial fibrosis and inflammation, obliterative bronchiolitis, and metaplasia may persist for weeks or months after an acute influenzavirus A infection.[83] Influenzavirus A has been isolated from the lung of one child 8 weeks after the acute infection, at a time when interstitial inflammation and mild fibrosis were present.[83] The extent to which influenza infection contributes to focal or diffuse pulmonary fibrosis is unknown.

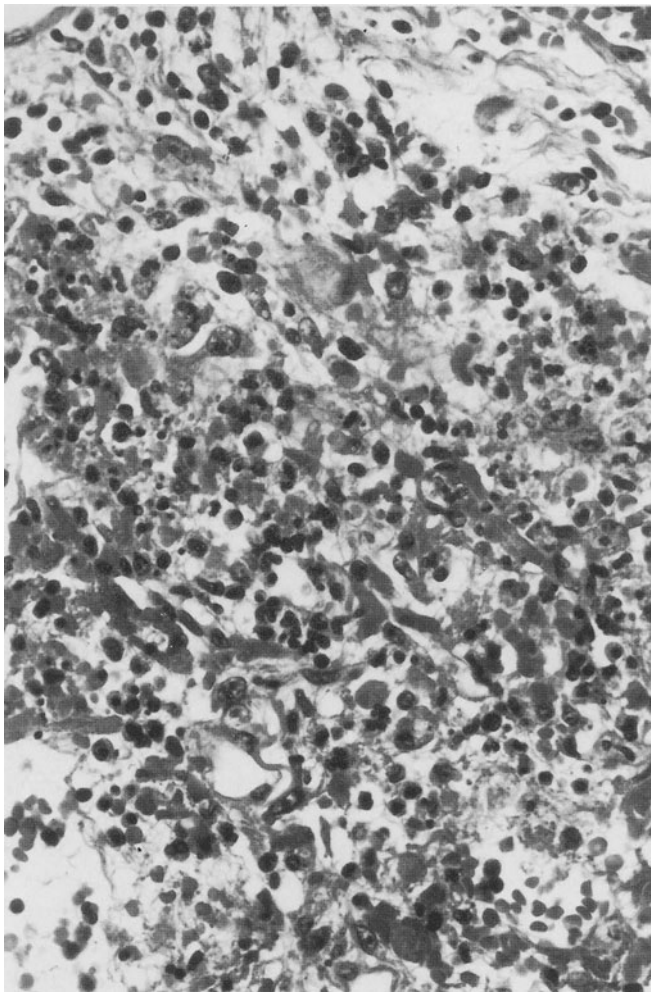


Fig. 9–1. Influenzavirus A pneumonia. Boundaries between interstitium and airspace are obscured by intense inflammatory infiltrate of mononuclear cells and polymorphonuclear neutrophils. Karyolysis and karyorrhexis produce cellular debris and nuclear fragments. Influenzavirus A isolated from lung in pure culture. H and E, ×300.

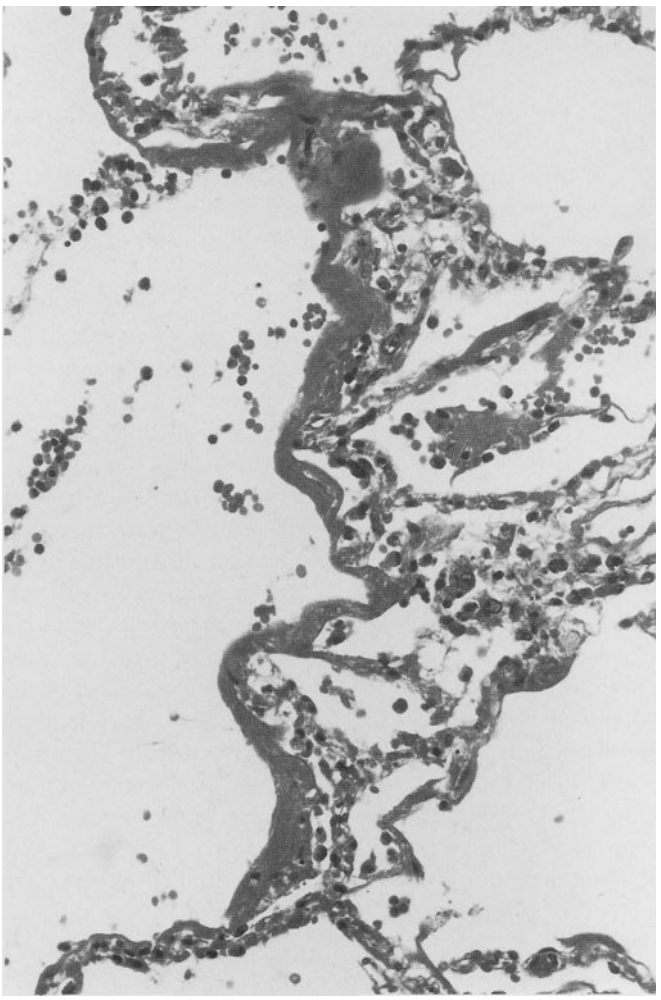


Fig. 9–2. Influenzavirus A pneumonia. Dense laminated hyaline membrane lines respiratory membrane of distal airspace. Interstitium is edematous and contains scant mononuclear cell infiltrate. Red blood cells, fibrin, and few mononuclear leukocytes present in airspace. Influenzavirus A isolated from lung in pure culture. H and E, ×150.

The diagnosis of influenza infection can be made by isolation of the virus, by demonstration of antigen in respiratory specimens, or by documenting a seroconversion. Isolation of influenzavirus A is made with equal facility in monkey kidney cell cultures and in embryonated eggs; influenzavirus B is more efficiently recovered in cell culture. Traditionally, washings or swabs from the nose or throat have been the specimens of choice. With improvements in virologic techniques, sputum may also provide an acceptable specimen. Antigen has been demonstrated in nasal specimens and sputum by immunofluorescence, and monoclonal antibodies have been developed, but the sensitivity of antigen detection is less than that of culture.[84,85] A common type-specific antibody to nucleoprotein antigen may be detected by complement fixation, providing a useful serologic screening tool. Strain-specific antibodies must be detected by hemagglutination inhibition.

Extrapulmonary symptoms are prominent during influenza, but inflammatory lesions outside of the respiratory tract are uncommon and identification of virus in extrapulmonary sites is even more rare. The best documented extrapulmonary inflammatory lesion is acute myocarditis, from which influenzavirus A has been isolated.[79]

Adenovirus

Adenoviruses were first recognized when spontaneous degeneration occurred in cell cultures that had been

derived from tonsils and adenoidal tissue.[86] These viruses produce a variety of diseases in man and may persist for many months in a normal human host after an acute infection. Forty-one types of adenovirus have been identified. Ocular infections (including epidemic keratoconjunctivitis), acute hemorrhagic cystitis, and acute gastrointestinal disease in young children have been associated with adenoviruses. Rarely, meningoencephalitis and intussusception have been ascribed to these agents.

The most common adenovirus respiratory infections, comprising some 5% of acute respiratory disease in young children, are an exudative pharyngitis and a flu-like syndrome;[87] these diseases may be accompanied by ocular disease (pharyngoconjunctival fever). Epidemic respiratory disease has been concentrated in populations of military recruits during the winter months. Person-to-person spread by the respiratory route is presumed. Epidemics of adenovirus infection in military camps have been distinguished from influenza epidemics by limitation of disease to new recruits, other personnel presumably having become immune.[86] Similar epidemics have not occurred in civilian populations, such as college students. Nosocomial transmission of adenovirus infection has been demonstrated, however.[9]

Severe, even fatal pneumonia has occurred in recruits,[88–90] in children,[2,3,39,91,92] in immunosuppressed civilians,[93] and rarely in apparently normal adults.[8,9,94] A variety of adenovirus types has been associated with pneumonia, including types 1,2,3,4,5,6,7,7-A, 11,21, 31,35, and strains that were intermediate between two serotypes in antigenic composition. There do not appear to be any histopathologic changes that are type specific. Infection in immunosuppressed patients is similar to that in normal hosts, but is more severe and more frequently disseminated.[86,95]

Two patterns of damage have been described consistently in adenoviral infections of the lower respiratory tract: destructive infection of the bronchi or bronchioles and interstitial pneumonitis.

A necrotizing inflammation affects the bronchial and bronchiolar mucosa, which is partially or completely sloughed into an obstructive intraluminal coagulum of fibrin, necrotic tissue, and inflammatory cells. The necrosis may extend completely through the epithelium, but the muscularis is usually preserved (Fig. 9–3). Specific mention is made in multiple reports of necrotizing inflammation involving the submucosal glands of the bronchi. With time, reparative proliferative changes become evident in the surviving mucosa.

An alveolitis, which may appear necrotizing, often accompanies the bronchiolitis. Edema and cellular infiltration of the interstitium are accompanied by an alveolar exudate of fibrin, edema fluid, and cells (Fig. 9–3). Hyaline membranes are prominent in some cases. Focal necrosis of the alveolar walls has been described.

Pyknotic nuclear debris provides a necrotizing appearance to the airspaces, even in the absence of abundant inflammatory cells. Neutrophils may be virtually absent from the lesion (Fig. 9–4)[9,91] or may be a prominent part of the inflammatory response (Fig. 9–3).[3,96]

These histologic changes are not specific for adenovirus infection. A presumptive etiologic diagnosis is provided by the distinctive intranuclear inclusions that are produced by this virus (Fig. 9–5; Color Plate 9–I, front). Two types of inclusions have been described.[31,97] The first, which is eosinophilic and surrounded by a clear halo, resembles the inclusions of the herpes viruses. In its earliest form, there are multiple, small eosinophilic masses intermixed with basophilic chromatin and a prominent nucleolus. The intermixture of chromatin, eosinophilic inclusion, and clear halo may give the nucleus a honeycomb appearance. These structures are Feulgen negative.

The second type of inclusion is often more numerous in tissue sections. This inclusion is basophilic or amphophilic and Feulgen positive. It may be surrounded by a small halo, but often fills the entire nucleus and obscures the nuclear envelope. Cells with such inclusions are often referred to as "smudge" cells. They are quite distinctive and characteristic of adenovirus infection, but must be differentiated from enlarged, hyperchromatic nuclei in regenerating epithelium. It has been suggested from study of experimentally infected human tracheal epithelium[97] and human tissue[31] that the smudge cells are the mature form of inclusion, whereas the eosinophilic inclusion represents an earlier form. Other investigators have noted that the two types of inclusions may be physically separate in tissue sections and have suggested that they are developmentally disparate.[3]

Ultrastructurally, adenovirus inclusions consist of masses of virions packed in a paracrystalline array within the nucleus (Fig. 9–6). Adenovirus proteins are manufactured in the cytoplasm, where antigen may be demonstrated by immunofluorescence, but inclusions are not seen there. Fibrillar nuclear inclusions have been seen in both adenovirus and influenza[80] infections in vivo, but do not have their counterpart in infected tissue culture cells.

Although adenovirus accounts for only 5% of bronchiolitis in the United States,[98] the acute inflammatory damage to the airspaces is impressive. Becroft[99] has described an equally impressive incidence of chronic complications after an epidemic of type 21 adenovirus infection. A distinctive feature of the epidemic was the protracted course of the infection, which waxed and waned over a period of several weeks; in some

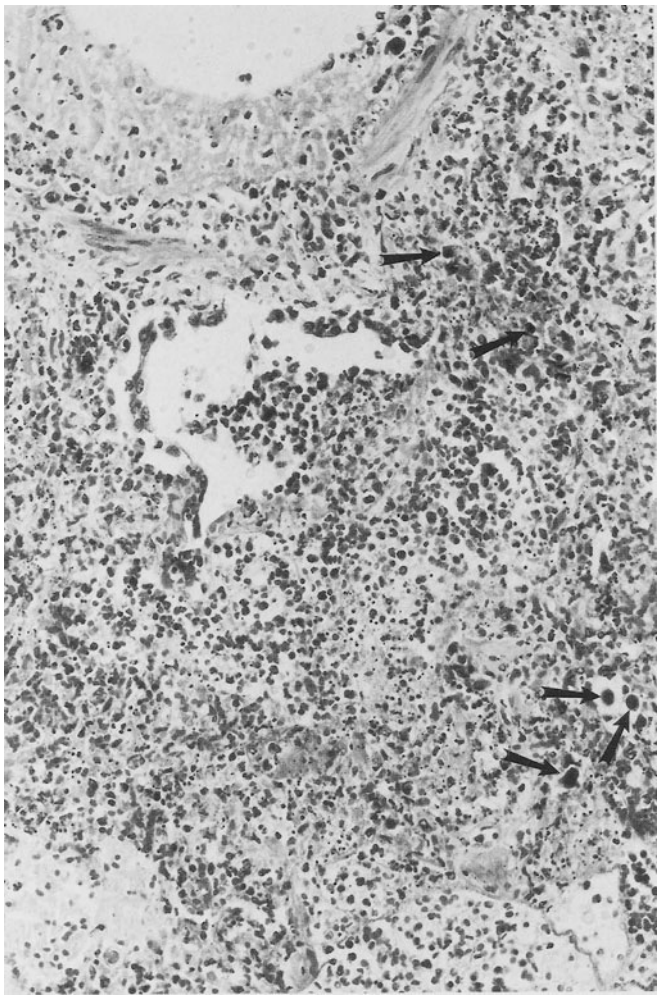


Fig. 9–3. Adenovirus pneumonia. Intensely inflammatory alveolitis in which mononuclear cells, polymorphonuclear neutrophils, and red blood cells are enmeshed in coagulum of fibrin. Extensive fragmented nuclear debris; dense adenovirus inclusions (smudge cells) can be noted even at this magnification (*arrows* indicate some obvious examples). Terminal bronchiole in left center is inflamed. At top, wall of larger, distal bronchiole is necrotic but muscularis remains intact. H and E, ×150.

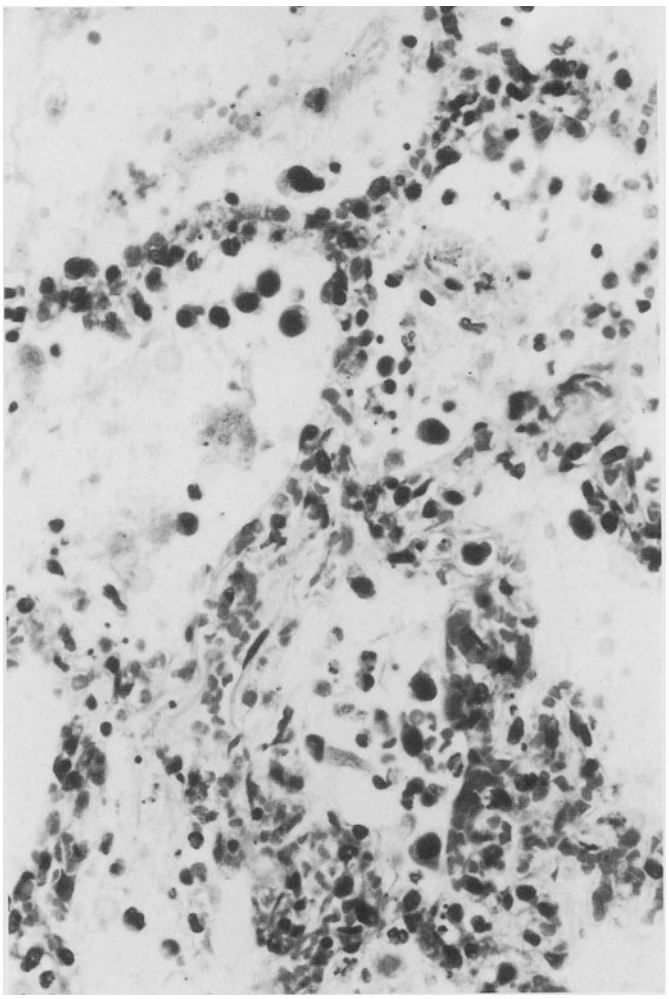


Fig. 9–4. Adenovirus, type 7 pneumonia with alveolar damage. Interstitium has increased numbers of cells; alveoli contain red blood cells, proteinaceous material, and scattered cells. Many epithelial cells appear dense because they contain mature adenovirus inclusions in nuclei. Although histopathologic damage is present, this lesion is relatively noninflammatory in comparison to that pictured in Fig. 9–3. H and E, ×300.

instances complete recovery never resulted. Similarly, Wohl and Chernick[98] have noted the protracted course of adenovirus infections and development of chronic pulmonary disease in as many as 60% of infants.

Bronchiectasis, bronchiolitis obliterans, and the unilateral hyperlucent lung syndrome have been reported as serious chronic sequelae to well-documented cases of adenoviral bronchiolitis or pneumonia. The rarity of cartilage in the walls of obliterated or stenotic bronchioles suggested to Becroft[99] that the primary lesion was in the terminal bronchioles rather than the small bronchi. The mucosa and submucosa were replaced by vascular connective tissue, the nature of the structures being clarified by the residual muscularis. Distal to the obstructive lesions, the alveolar ducts were dilated.

It has been suggested that certain populations, such as those studied by Becroft,[99] are highly susceptible to severe adenovirus infection.[100] Bronchiolitis obliterans has been documented after measles, whooping cough, influenza in infants, and ingestion of foreign bodies or toxic chemicals[98] (see Chapter 5).

The diagnosis of adenovirus infections can be made by isolation of the virus from respiratory specimens,

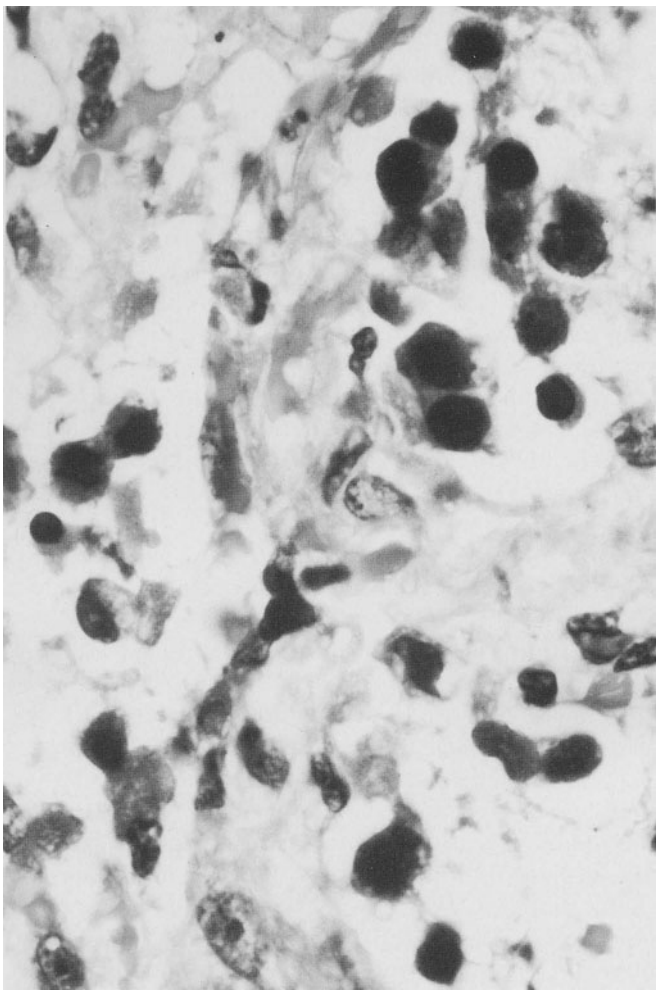


Fig. 9–5. Adenovirus pneumonia. Virtually all cells contain mature adenovirus inclusions. Nuclei are densely basophilic, making it difficult to "see into" them. In some cases boundary between nucleus and cytoplasm is obscured. These "smudge" cells must be distinguished from nonspecifically damaged or reactive epithelial cells. (See Color Plate 9–I, front.) H and E, ×750.

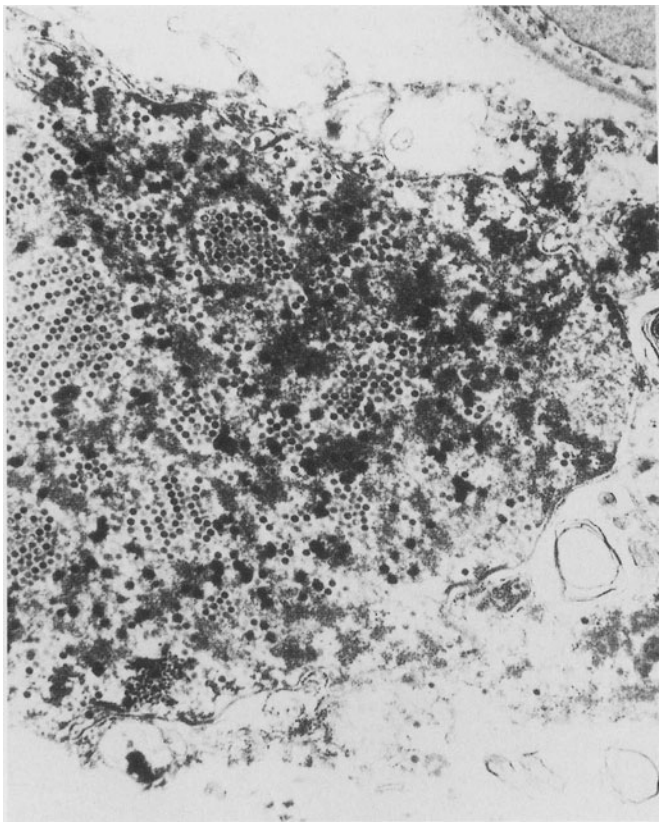


Fig. 9–6. Adenovirus pneumonia. Nucleus has been largely replaced by adenovirus virions packed in paracrystalline array. Portions of residual nuclear chromatin are pushed against nuclear membrane. Formalin-fixed lung tissue. ×12,500.

by demonstration of antigen in respiratory secretions, or by demonstration of a serological response to a common viral antigen. Inclusions have been demonstrated only infrequently in exfoliated respiratory cells.[32] Ciliocytophthoria has been described in exfoliated respiratory cells from patients with adenovirus infections.[35]

Herpes Simplex

Herpes simplex virus is the virus most commonly isolated in most hospital laboratories.[101] Two serological types have been defined, and either type of virus can produce any clinical syndrome. Gingivostomatitis, pharyngitis, esophagitis, encephalitis, and respiratory infections in adults are caused primarily by type 1 virus. Type 2 virus is the primary cause of genital infections, and may produce meningitis and disseminated neonatal infection, including pulmonary lesions. Antibody to type 1 develops during the first decade of life, whereas antibody to type 2 herpes simplex begins to appear in the teenage years.

Herpes simplex viruses occur only in man, and both types are spread by contaminated secretions. Once they have produced a primary infection, they may become latent in ganglia, from which site reactivation can occur repetitively. Asymptomatic shedding of virus is well documented in both the oral cavity[102] and in the genital tract, so that an active lesion is not required for transmission of an infection.

Herout and colleagues[103] first suggested that herpetic tracheobronchitis and pneumonia might be more common than suspected. It is unclear whether the respiratory disease is of recent occurrence or whether the diagnosis was missed previously. Nash[14] found that 9

of 10 cases of respiratory infection by herpes simplex had been missed clinically and pathologically.

Most herpetic infections of the lower respiratory tract occur in individuals whose defense mechanisms are compromised in some manner. The defects may be systemic or local. Newborn infants,[104] patients with burns,[105,106] and those with immunosuppressive diseases or treatments[107] are at increased risk of developing herpetic respiratory infection. Patients with tracheostomies or endotracheal tubes also have a greater risk of infection, although intubation is not a prerequisite for the infections.[12] The source of virus is usually the maternal vagina in neonates and the oropharynx in older children and adults. Mucocutaneous herpesvirus infection preceded respiratory disease in 17 of 20 individuals from whom herpes simplex virus was isolated at autopsy.[107]

The distribution of the pathologic lesions, prominence of tracheobronchial pathology, and association of infection with conditions that favor contamination of the lower tract all suggest that aspiration of oral secretions is the most common pathogenic mechanism.[12,107] Ramsey and colleagues[107] noted that diffuse intersitial pneumonia was frequently associated with disseminated infection, whereas focal pneumonia was more commonly associated with lesions in the trachea and bronchi. Focal lesions, often of a miliary nature, may also occur after disseminated infection and viremia, such as occurs in disseminated herpes simplex infections. The miliary lesions cannot be differentiated from disseminated varicella-zoster infection on morphologic grounds alone.

Several patterns of pulmonary damage occur. Ulcerative tracheobronchitis, which may also be accompanied by necrotizing pneumonia, is the most common manifestation of infection.[107] The surface of the ulcerated area is covered with a fibrinopurulent exudate containing necrotic cells, nuclear debris, fibrin, and inflammatory cells (Fig. 9–7). The infection may extend into the submucosal mucous glands. Polymorphonuclear neutrophils are a prominent part of the inflammatory response.[14] Necrosis of large portions of the epithelium may lead to sloughing of the mucosa and formation of a thick pseudomembrane, which may obstruct the airway.[12]

The accompanying pneumonitis is usually patchy, reflecting the focal endobronchial source of the infection. A necrotizing pneumonia results in a microscopic appearance that resembles the tracheal ulcers. The airspaces are filled with a coagulum of fibrin, necrotic cells, prominent fragmented nuclear debris, and inflammatory cells. The necrotizing appearance, prominence of neutrophils in the exudate, and bronchial distribution of the lesions may lead the unsuspecting pathologist to classify the lesion as bacterial in origin (Fig. 9–8). Nash[14] found 10 cases of herpetic lower respiratory infection in 1,000 consecutive autopsies at the Massachusetts General Hospital. Only 1 of the 10 cases had been diagnosed originally as a viral infection, presumably because the pathologist did not scrutinize the tissue for the characteristic inclusions.

Diffuse interstitial pneumonitis, which may also be necrotizing and hemorrhagic,[108] and miliary lesions without obvious relationship to the bronchial tree appear to be less common manifestations of herpetic lower respiratory infection (Fig. 9–9). Tuxen and colleagues,[109] however, demonstrated morphologic and virologic evidence of herpes simplex infection in the lungs of 14 patients (30%) with adult respiratory distress syndrome (ARDS).[109] The herpetic infection was associated with an increased need for prolonged respiratory support and an increased frequency of late mortality. The nature of the relationship between the herpes infection and the ARDS was not clear.

A presumptive etiologic diagnosis of herpes simplex infection may be rendered if the characteristic intranuclear inclusions are demonstrated[31] (Fig. 9–10; Color Plate 9–II, front). The inclusions of both types of herpes simplex virus and varicella-zoster virus, also a member of the herpesviridae, are identical. Initially, the nucleus enlarges and rarefies, the nucleolus is dispersed, and multiple masses of light-staining, amphophilic material are outlined by strands of basophilic chromatin. The classical Cowdry type A inclusion forms by coalescence and condensation of the smaller masses. The type A inclusion is a central, eosinophilic nuclear mass, which is surrounded by a halo and peripherally marginated, beaded chromatin. As the inclusion becomes senescent, the central mass decreases in size, the halo widens, and the beaded character of the peripheral chromatin is lost. Ultrastructurally, the inclusion consists of viral deoxyribonucleoprotein and nucleocapsids. The naked nucleocapsids acquire a glycolipid envelope from the host cell as they pass from the nucleus to the cytoplasm (Fig. 9–11).

Multinucleation is a characteristic cellular reaction to both herpes simplex and varicella-zoster infection. Multinucleated giant cells with characteristic intranuclear inclusions are virtually diagnostic of infection by one of these viruses. It should be remembered that parainfluenza, respiratory syncytial, and measles viruses can also produce multinucleated giant cells. Although multinucleated cells are not characteristic of cytomegalovirus (CMV) infection, their presence has been reported.[32] It has been suggested that the classical giant cells are less frequent in herpetic respiratory infection than in genital infection,[110] but they are, nevertheless, commonly present both in expectorated sputum

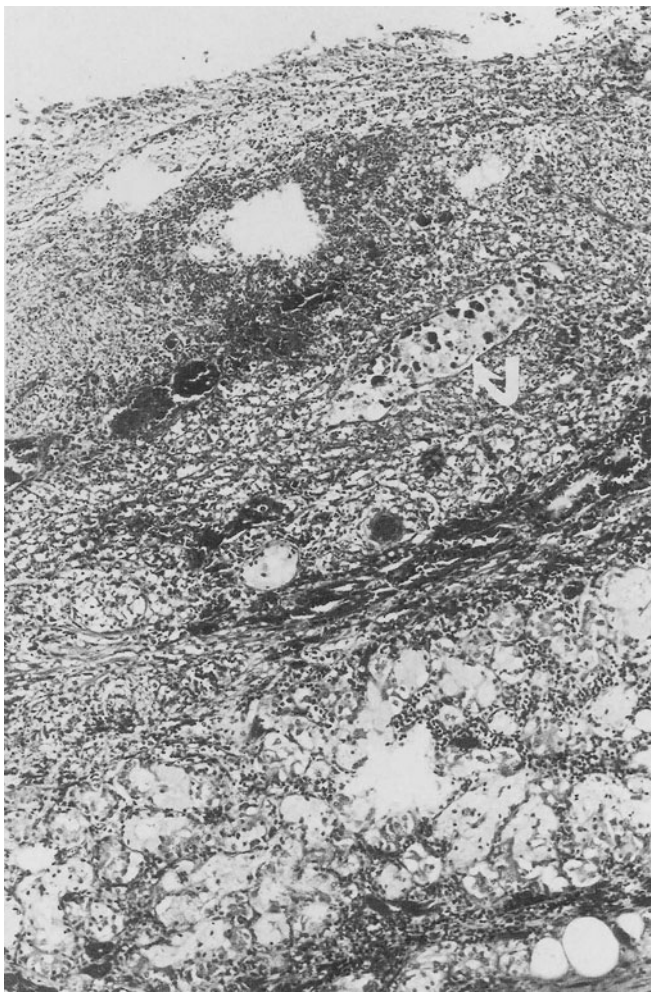


Fig. 9–7. Herpes simplex bronchitis. Mucosa and submucosa of this major bronchus are necrotic. Epithelium is replaced with coagulum of necrotic tissue, fibrin, red blood cells, mononuclear cells, and polymorphonuclear neutrophils (top). Dense, inclusion-bearing cells are visible even at this magnification (*white arrows*). Inflammation has extended from lumen through submucosal mucous glands to muscularis. H and E, ×150.

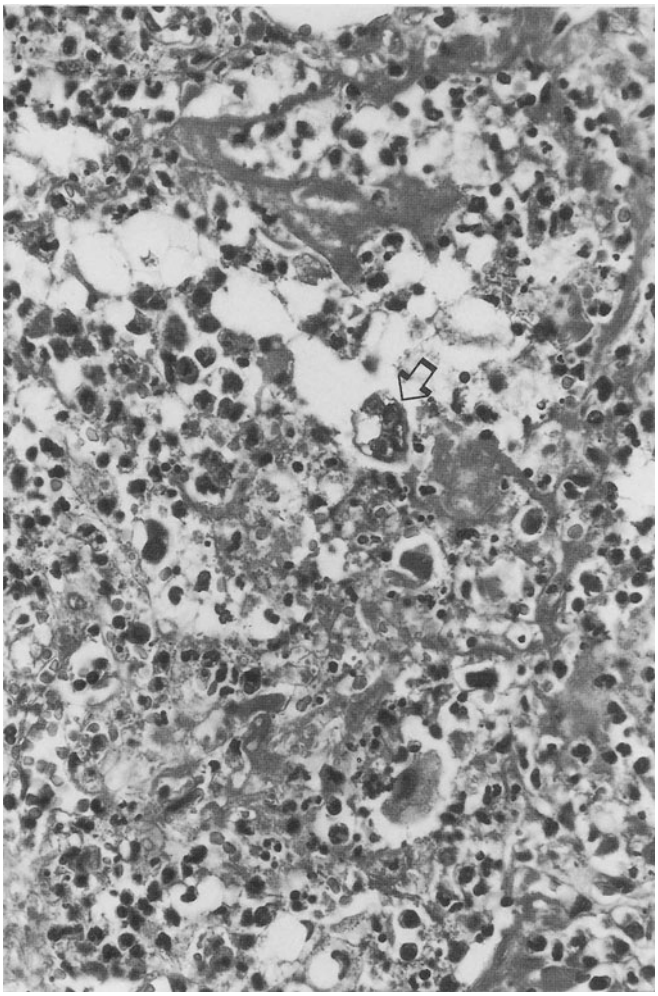


Fig. 9–8. Herpes simplex pneumonia. Alveolar structure is obliterated by necrotizing inflammation, which includes many polymorphonuclear neutrophils, fragmented nuclear debris, and fibrin. Such viral inflammatory lesions have been erroneously attributed to bacterial pathogens because of necrotizing character of inflammation. Multinucleated giant cell contains intranuclear inclusions that are surrounded by clear halo (*arrow*). Herpes simplex virus isolated from lung in pure culture. H and E, ×300.

and in histological sections.[12,32,111] The inclusions may be difficult to locate, particularly when there is a large amount of necrotic material present; they are best sought in intact cells at the periphery of ulcers or necrotic foci.

The diagnosis of herpes simplex infections is best made by a combination of viral culture or antigen detection and morphologic methods. Recovery of the virus from respiratory secretions is not of itself sufficient for a diagnosis of herpetic disease, because 1–5% of the population excretes virus in the oropharynx.[12,102] On the other hand, demonstration of characteristic infected cells provides only presumptive identification of the etiologic agent. Serology is of little use in the diagnosis of herpes infections.

Measles Virus

Measles is a highly contagious disease that can spread only from acutely infected individuals to susceptible contacts, probably by means of respiratory secretions. The frequency of measles has decreased dramatically since the introduction of vaccines in the 1960s. Outbreaks continue to occur in populations that are inadequately immunized. The diagnosis is usually so obvious

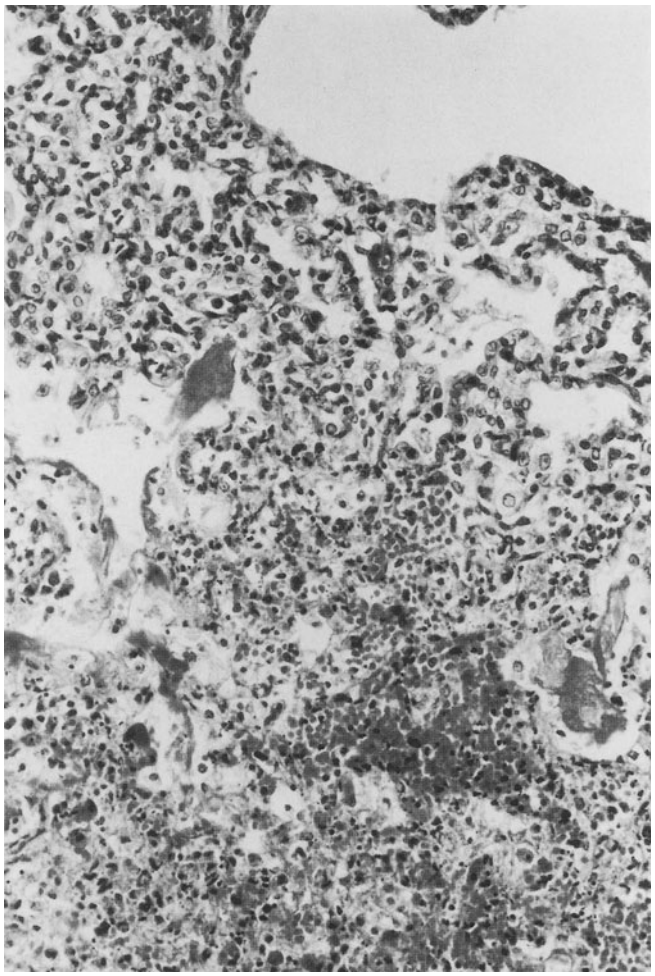


Fig. 9–9. Neonatal herpes simplex pneumonia. Edge of miliary lesion. Lung tissue is necrotic (below) with exudate of fragmented cells, hemorrhage, and proteinaceous material. Note dilated distal airway, possibly alveolar duct (top right). Interstitium is cellular but tissue is viable. Herpes simplex isolated in pure culture. H and E, ×150.

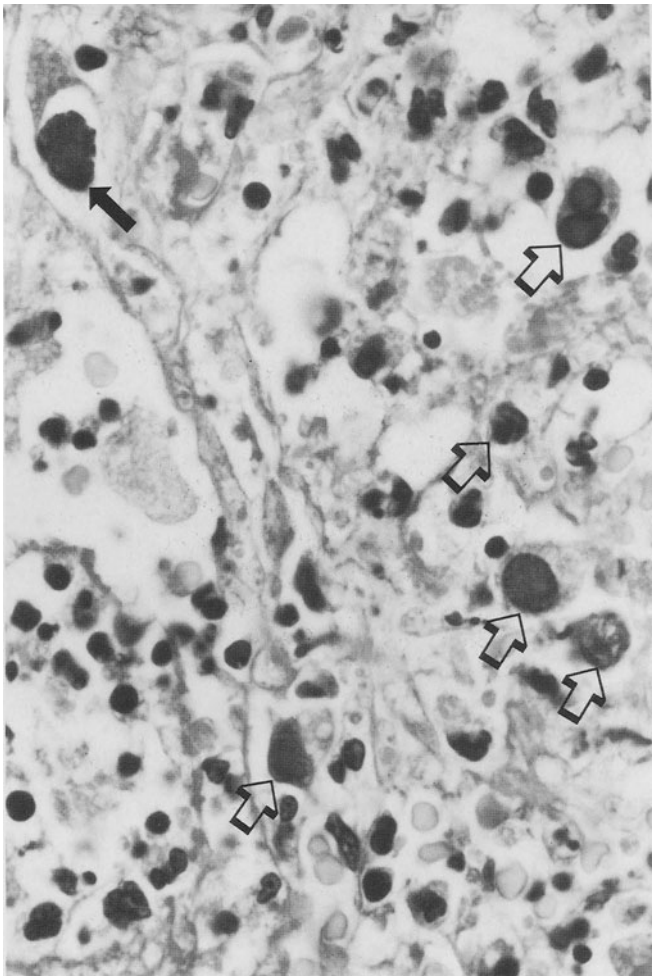


Fig. 9–10. Herpes simplex pneumonia. Multiple cells in inflammatory exudate contain intranuclear inclusions. Inclusions are eosinophilic, homogeneous, and fill virtually entire nucleus. *Open arrows* indicate several more easily identified inclusions in several phases of formation; one shows up better in color (*solid arrow*) (see Color Plate 9–II, front). Nuclear chromatin has been pushed to edge of nuclear membrane and is beaded in some cells. Herpes simplex virus isolated from lung in pure culture. H and E, ×750.

clinically that specimens are not submitted to the laboratory for confirmation, but atypical cases occur, particularly in patients who received the initial killed vaccine.[112]

The most serious complications of acute measles infection are progressive infection, including pneumonia, and central nervous system disease, including postinfectious encephalitis and subacute sclerosing panencephalitis.[113] The progressive measles syndrome, in which pulmonary infection figures prominently, occurs in individuals who are immunologically compromised by underlying disease or therapy.[114] The development of the characteristic skin rash in measles coincides with the appearance of the earliest immune response; in these immunodeficient patients the rash is absent or atypical.[115] Isolation of the virus from normal individuals is virtually impossible within 48 hr after development of the rash,[114] whereas the virus persists in those with pneumonia. Measles pneumonia has been described, however, in apparently normal individuals who developed a rash.[116]

Measles may also be complicated by bacterial pneumonia[13] or by other viral infections.[116] *Haemophilus influenzae* was considered the cause of viral influenza in 1918 because of its frequent occurrence as a secondary invader. Similarly, a small gram-negative bacillus, possibly also Haemophilus, was suggested as a

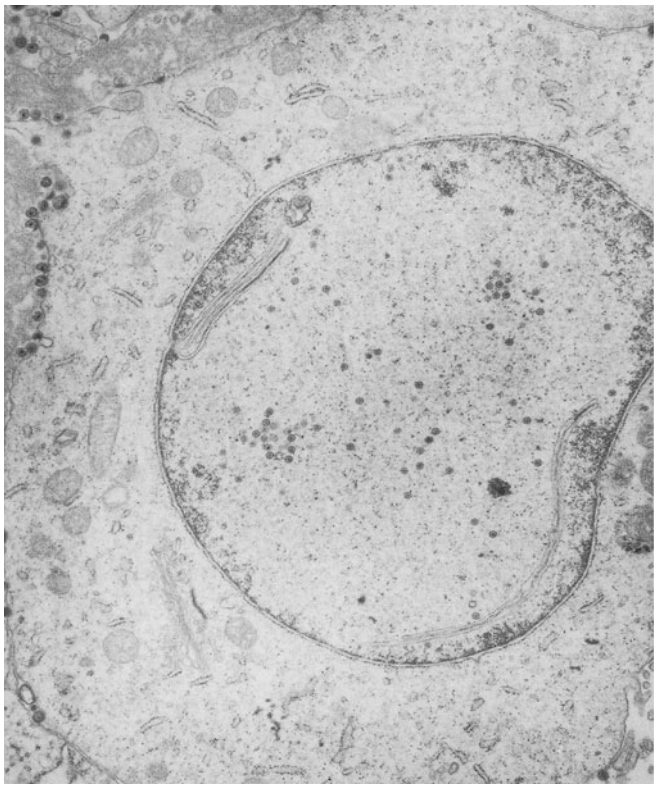


Fig. 9–11. Herpes simplex virus-infected MRC-5 fibroblast. Nucleus of tissue culture cell has been replaced by viral nucleoprotein. Scattered naked virions are evident in nucleus and nuclear chromatin has been pushed to nuclear membrane. Fully enveloped virions that have passed through nuclear membrane are evident in extracellular space at upper left of photomicrograph. ×12,000.

possible etiologic agent for measles because it was found in the lungs of patients who died shortly after the onset of the rash and had giant cell pneumonia.[117] In South Africa adenovirus and herpes simplex virus were common secondary pathogens in fatal cases of measles.[13,116] Goodpasture and associates[118] described a diverse group of viral pneumonias in infants. It is probable that the lesions observed in the cases of measles were caused by herpes simplex virus or by adenovirus. Cytoplasmic inclusions and giant cells were not described by these accomplished investigators; the illustrations depict structures that are compatible with or strongly suggest viral superinfection.

Macroscopically, the infiltrates of measles pneumonia are diffuse, patchy, or even nodular.[115] The nodular lesions have a distribution that is primarily peribronchial and peribronchiolar.[117] A hemorrhagic component may be present.[119] Microscopically, the small airways and alveoli are primarily affected. There is an interstitial pneumonia in which mononuclear cell infiltration predominates (Fig. 9–12). The airspaces contain fibrin and inflammatory cells; hyaline membranes may be present. The bronchioles show epithelial hyperplasia and extensive squamous metaplasia occurs, even with the formation of nodular epithelial masses. Macroscopic and microscopic pulmonary thromboemboli have been described.[120]

The most distinctive abnormality in measles pneumonia is the multinucleated giant cell, which contains eosinophilic intranuclear and intracytoplasmic inclusions (Fig. 9–13). Ultrastructurally, the inclusions consist of fibrillar viral ribonucleoprotein.[120] The giant cells are often numerous. They may be flattened against the respiratory membrane or heaped up in masses. The intranuclear inclusions are eosinophilic and resemble those of herpes viruses.[31] The cytoplasmic inclusions are brightly eosinophilic and may form very large masses[121] (Fig. 9–13; Color Plate 9–III, front).

In 1910 Hecht described giant cell pneumonia in children. The similarities between Hecht's pneumonia and the lesion seen in both measles and distemper infections was recognized in 1945,[122] but the virologic association of giant cell pneumonia and measles virus was first made by Enders and colleagues.[123]

There are many other causes of giant cells in pulmonary lesions—infectious and noninfectious, viral and nonviral. The virologic differential diagnosis of interstitial pneumonia with giant cells includes respiratory syncytial virus and parainfluenza virus. A diagnosis of measles pneumonia can be made confidently if the characteristic intranuclear and intracytoplasmic inclusions are present, if there is a clinical history of measles, if the virus is isolated or identified immunologically, if a serological response to the virus is documented, or if there are systemic abnormalities, such as lymphoid hyperplasia with Warthin-Finkeldey giant cells.

In the early 1960s a formaldehyde-inactivated measles vaccine was introduced. Subsequently, it was recognized that children who received the vaccine developed an atypical measles syndrome in which the rash was accentuated peripherally and segmental pneumonia was prominent. This vaccine failed to induce immunity to a viral surface glycoprotein (F protein) that is essential to prevent spread of the infection.[113] Very little is known about the pathology of the atypical reaction. Annunziato and colleagues[124] described 17 patients, of whom 15 had dense lobar or segmental pulmonary consolidation. Hilar or mediastinal lymphadenopathy and pleural effusions were seen in a minority of cases. Some infiltrates persisted and resolved into large nodular masses.[125] The differential diagnosis of nodular pulmonary infiltrates is extensive, but the lesions are sufficiently distinctive that the diagnosis of atypical measles syndrome can be suggested.[126]

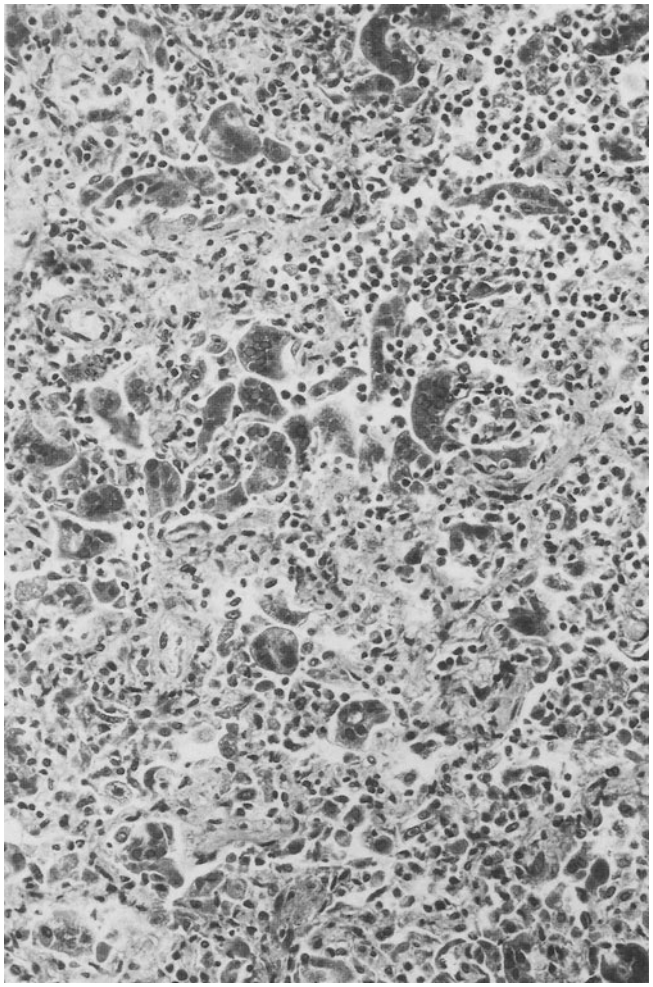


Fig. 9–12. Measles pneumonia. Architecture of airspaces has been obliterated by mixed inflammatory infiltrate, proliferation of alveolar epithelial cells, and formation of multinucleated epithelial cells. Intranuclear inclusions are easily seen in syncytial giant cells, even at low magnification; cytoplasmic inclusions also evident. H and E, ×150.

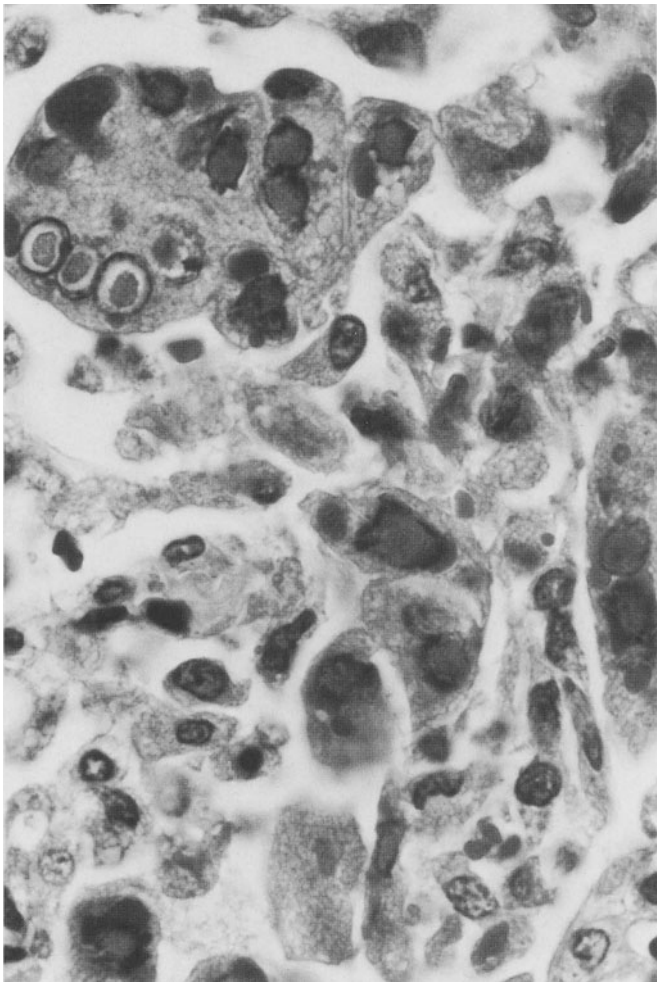


Fig. 9–13. Measles pneumonia. Many cells in airspaces, both uninucleate and multinucleate, contain inclusions. Intranuclear inclusions resemble those of herpes simplex virus; they are homogeneous and fill nucleus, pushing chromatin to periphery of nuclear membrane. Artefactual halo separates several intranuclear inclusions in multinucleated giant cells from residual chromatin. Cytoplasmic inclusions are brightly eosinophilic and vary in size. (See Color Plate 9-III, front). H and E, ×750.

Respiratory Syncytial Virus

Infection with respiratory syncytial virus occurs repeatedly throughout life including infancy to old age. Epidemics, which occur regularly in most communities, are separated by alternating long (13–16 months) and short (7–12 months) intervals.[98] Virtually all seronegative infants exposed to their first epidemic become infected. In a study of intrafamily transmission of viral infection, the secondary attack rate for RSV infection within families was 27% for all children and 45% for infants.[127] This virus is an important cause of nosocomial infection on pediatric hospital wards.[128]

Immunity to RSV is short lived and incomplete, although recurrent infections tend to be milder. The clinical syndrome varies from upper respiratory infection to croup, bronchitis, bronchiolitis, and interstitial pneumonia. The initial infection usually occurs in infants between 6 weeks and 6 months of age, 25–40% of whom suffer lower respiratory tract infection (See also Chapter 4). Infection in infancy is associated with greater severity of illness (Color Plate 9–IV, front), shows the severe involvement of bronchioles that accounts for "croupy cough"). Among children from whom RSV was isolated, 38% had bronchiolitis and 25%, pneumonia. Although RSV is the cause of 75% of cases of bronchiolitis, adenoviruses, rhinoviruses, parainfluenza virus (particularly type 3), mumps virus,

influenza viruses, and *Mycoplasma pneumoniae* may also cause this syndrome.

RSV is usually transmitted to the upper respiratory mucosa by the fingers. After an incubation period of 4–5 days, patients develop rhinorrhea and anorexia. Viral replication occurs in the nasopharynx, and during the next 1–3 days virus spreads along the airways to the lower respiratory tract. Then cough, tachypnea, low-grade fever, rales, and rhonchi occur. Signs of bronchiolar obstruction and air trapping, including expiratory wheezes, intercostal and substernal retractions, atelectasis, and hyperresonance may also develop.[129–131] Severe illness is further characterized by dyspnea and cyanosis. The pathophysiology of bronchiolitis includes reduced pulmonary compliance, increased pulmonary resistance and end expiratory volume, and hypoxemia that is caused by decreased ventilation in the presence of normal perfusion.

Newborn infants and adults infected with RSV usually have upper respiratory infections; elderly patients often have prolonged bronchitis with tachypnea, wheezing, cough, and fever.[5,132] Hospitalization with RSV infection is most likely to occur during the first year of life. The mortality rate is 1% in normal children and up to 37% in those with underlying diseases, particularly congenital heart disease and bronchopulmonary dysplasia. RSV was the most common virus isolated in a series of sudden infant deaths.[4]

RSV infection is diagnosed best by viral culture or by demonstration of RSV antigen in respiratory secretions. Among infants and young children with lower respiratory tract infections, RSV is the most frequent viral isolate. Viral culture and immunologic identification of RSV antigens in washings from the nasopharynx are similar in sensitivity and specificity. Several immunologic approaches have been used, including immunofluorescence (Color Plate 9–V, front), and immunoenzyme tests of aspirated epithelial cells and enzyme or radioimmunoassays on the nasopharyngeal washings.[41,133–135] Recently circulating RSV antigen was also demonstrated in mononuclear cells in the peripheral circulation.[136] Serologic diagnosis may be achieved by demonstration of a fourfold or greater rise in titer of antibodies by complement fixation, neutralization, and enzyme immunoassay.

The cytoplasm of respiratory epithelial cells is the site of RSV replication. Final assembly of viral matrix proteins, nucleocapsids, and surface glycoproteins occurs at the cell membrane, which is converted into the viral envelope by the process of budding. Immunofluorescence with monoclonal antibodies to nucleocapsid proteins demonstrates cytoplasmic antigen that varies from small particles to larger inclusions.[41] These structures correspond to the cytoplasmic inclusions that are seen when infected cells are stained with H and E or by the Giemsa method (Fig. 9–14; Color Plate 9–IV, front). The eosinophilic inclusions in epithelial cells often have a clear halo and sometimes have vacuoles (Fig. 9–15).[31,129,131]

RSV-infected cells may have a single nucleus, but are commonly multinucleated. The virus has a fusion protein that mediates entry of virus into the host cell and also causes coalescence of adjacent cells to form multinucleate syncytial giant cells (Fig. 9–14; Color Plate 9–IV, front). This phenomenon, which is remarkable in cell cultures infected with RSV, suggested the name of the virus. Both multinucleate syncytial and uninuclear cells show effects of cell injury, including necrosis. The syncytial giant cells are observed in vivo, and RSV infection must be included in the differential diagnosis of giant cell pneumonia. Bronchiolitis may be produced by RSV without formation of giant cells, in which case immunologic or virologic studies must be performed to elucidate the etiology of the infection.[137]

Lungs from patients with RSV bronchiolitis are overexpanded and do not collapse when the thorax is opened.[138] Chest radiographs may show a depressed diaphragm, patchy areas of atelectasis, and air trapping, which is manifested by hyperlucency.[129] Microscopic examination reveals nipple-like projections of epithelial cells into the lumens of the airways, necrosis of the ciliated epithelium of bronchioles and bronchi, and inflammatory infiltration of the epithelium and submucosa (Fig. 9–16). The inflammation consists predominantly of lymphocytes and macrophages, which extend into adjacent alveolar septa. Necrotic cellular debris, fibrin, and mucus accumulate in the bronchiolar lumina. In the airspaces themselves there is hyperplasia of the alveolar lining cells, alveolar edema, and on occasion hyaline membranes.[129,131,137–140] Other bronchial and bronchiolar changes include a cuboidal-to-flat epithelium that represents early regeneration and a multilayered epithelium that is characteristic of regenerative hyperplasia.

Interstitial pneumonia is characterized by an infiltrate of mononuclear cells in alveoli and alveolar septa, alveolar edema, and hyaline membranes. Bronchiolar injury may also be present. RSV antigen and infectious virus are more abundant in the lungs of patients with interstitial pneumonia than with bronchiolitis.[140,141]

The pathogenesis of the bronchiolar lesions and of cellular injury by RSV is unclear. RSV does not shut off host cell protein synthesis as do most cytopathic viruses. Yet, RSV-infected cells undergo fusion and suffer direct cytopathic injury in vitro and in vivo (Fig. 9–14). Bronchiolitis results in part from the proclivity of the virus to infect small, easily obstructed bronchioles

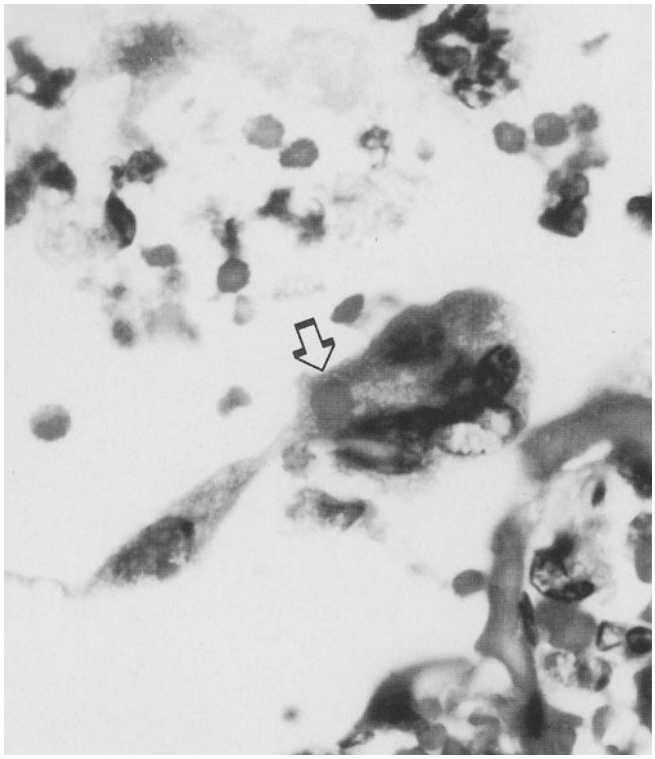


Fig. 9–14. Respiratory syncytial virus pneumonia. Airspace contains proteinaceous material, red blood cells, and scant inflammatory cells. Multinucleated (syncytial) epithelial giant cell contains single, discrete cytoplasmic inclusion (*arrow*). (See Color Plate 9–IV, front). H and E, ×750.

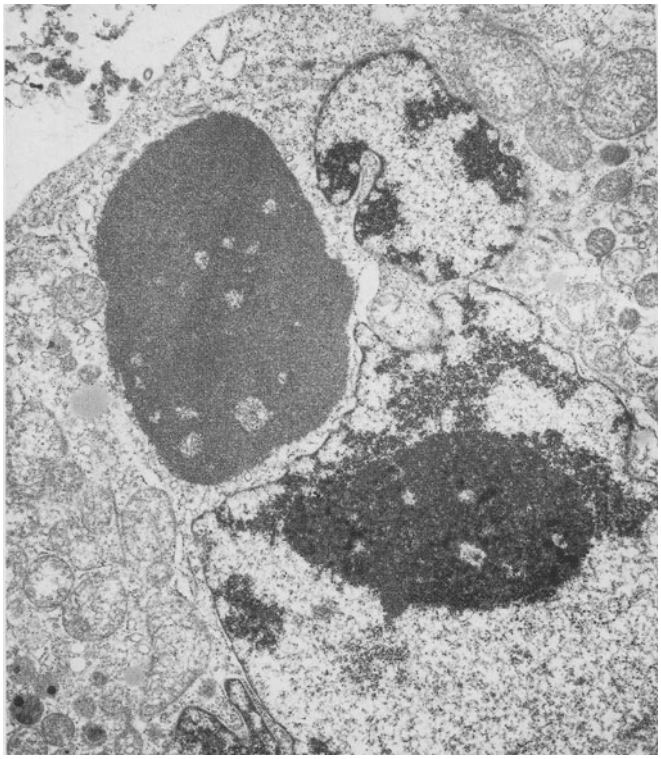


Fig. 9–15. Respiratory syncytial virus-infected HEp-2 cell. This human cell line was derived from laryngeal carcinoma and is preferred means for cultivating RSV. Large cytoplasmic inclusion is composed of fibrillar ribonucleoprotein, but difference in density between viral RNA and nucleolar RNA is evident. Remnants of cytoplasm within inclusion correspond to vacuoles in light microscopic sections. ×9,500.

during the first year of life, in part from direct viral injury to respiratory epithelium, and partially because mucus accumulates in the absence of ciliary action.[142]

RSV bronchiolitis is associated with RSV-specific IgE that is bound to nasal epithelial cells, histamine, and fewer suppressor T lymphocytes.[143–145] These findings suggest that a component of the bronchiolar obstruction may be immunopathologic; IgE and histamine-mediated bronchospasm could result from a failure of the suppressor subset of T lymphocytes to limit IgE production. An additional, age-dependent pathophysiologic factor is the presence of poor collateral ventilation in infant lungs, which favors atelectasis.

Acute complications of RSV infection include otitis media and bacterial superinfection, particularly by *Haemophilus influenzae*. Long-term sequelae in survivors apparently occur frequently as chronic disease of the airways. (See Bronchiectasis, Chapter 5.)

Parainfluenza Virus

Human parainfluenza viruses comprise the antigenically distinct types, 1, 2, 3, 4A, and 4B.[146] All types cause infections of the respiratory system; the attack rates and the frequency with which the lower respiratory tract is affected vary among the five serotypes. Parainfluenza viruses cause 8% of acute respiratory disease in hospitalized children.[147]

The clinical manifestations include upper respiratory infection, laryngotracheobronchitis (croup), bronchitis, bronchiolitis, and interstitial pneumonia. Parainfluenzavirus 3 accounts for the greatest portion of pneumonia, bronchiolitis, and bronchitis. Parainfluenzaviruses 1 and 2 are the major etiologic agents of croup. Signs and symptoms correlate with the anatomic location of the viral infection; they may include low-grade fever, rhinorrhea, hoarseness, cough, pharyngitis, stridor, rhonchi, wheezing, rales, and retractions.[148,149]

Transmission by person-to-person contact or large droplets requires only a small inoculum of this virus, which survives very poorly on environmental surfaces. After an incubation period of 2–6 days, infection of nasopharyngeal epithelium may be followed by spread

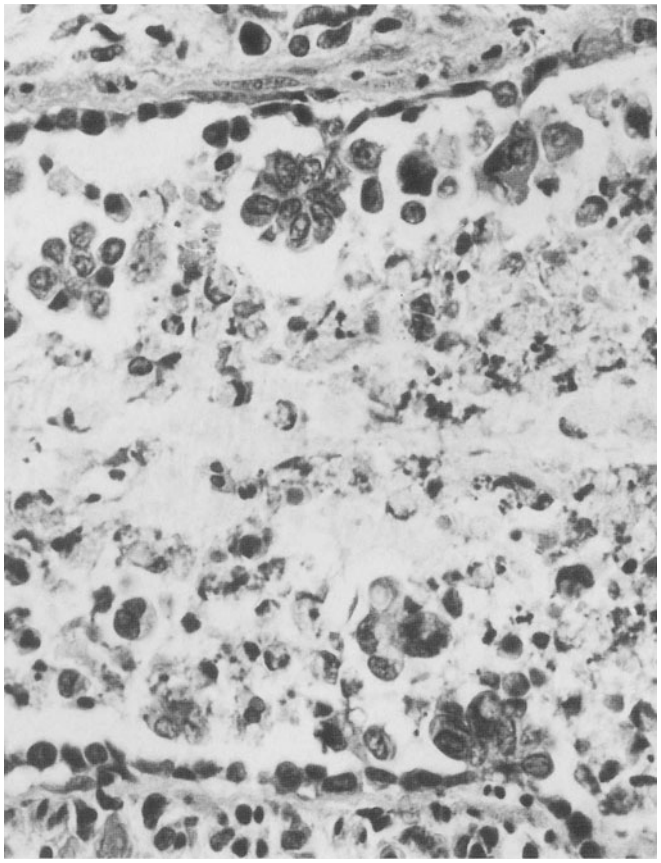


Fig. 9–16. Respiratory syncytial virus bronchiolitis. Epithelium of small bronchiole is extensively damaged, leaving intact basal layer. Several distinctive papillary projections of epithelium are present. Lumen filled with mononuclear cells, desquamated epithelial cells, and debris. H and E, ×450.

to the pharynx, larynx, trachea, bronchi, bronchioles, and alveoli. Clinical, epidemiologic, and pathogenetic similarities with RSV infection are remarkable, except for the maximal occurrence of parainfluenzavirus bronchiolitis in 6- to 12-month-old children instead of at 1–3 months of age for RSV infection.[150]

Immunity to parainfluenza viruses is incomplete. Reinfections with both heterotypic and homotypic strains are frequent.[148] Definitive diagnosis of parainfluenzavirus infections requires cultivation of the virus or specific detection of viral antigens, for example, by demonstration of viral antigen in nasopharyngeal secretions, using immunofluorescence.[147,150,151] Serologic diagnosis is achieved by demonstration of a fourfold or greater rise in serum antibodies in hemagglutination inhibition, complement fixation, or neutralization tests. Heterotypic antibody responses occur often, however, and confound type-specific serologic diagnosis.

The morphology of virus-infected cells in vitro depends on the type of virus. Types 2 and 3 may cause cell fusion, multinucleated syncytial giant cells, and cell death. Some parainfluenzavirus strains cause no perceptible cytopathic effect; these strains are detected by demonstration that guinea pig erythrocytes adsorb to infected cells (hemadsorption). Neuraminic acid-containing receptors in the red blood cell membrane bind to the viral attachment protein that is inserted into the cell membrane of the infected cell. Fusion to multinucleated giant cells is mediated by the viral F protein that is also inserted into the cell membrane and causes cell-to-cell spread of virus. Virus-infected cells contain cytoplasmic aggregates of viral nucleocapsids that may occur as inclusion bodies.

Human infection with parainfluenzavirus rarely results in the patient's death. The respiratory epithelium, particularly ciliated cells, is the target of the virus.[139,147,152] Viral antigen, which can be demonstrated by immunofluorescence in infected nasopharyngeal, tracheal, and bronchial epithelial cells, varies from cytoplasmic particles and inclusions to poorly formed strains.[153]

Immunologically competent patients may manifest acute laryngotracheobronchitis without pulmonary parenchymal lesions (Fig. 9–17) or as bronchitis with epithelial hyperplasia, moderate hyperplasia of alveolar lining cells, and interstitial pneumonia.[139,147] Patients with T-lymphocyte deficiency who die after prolonged, progressive parainfluenzavirus infection have a different constellation of lesions. There is interstitial pneumonia, an exudative alveolar exudate, alveolar cell hyperplasia and hypertrophy, and interstitial fibrosis. The alveoli are lined by multinucleated giant cells, which may have cytoplasmic inclusions (Fig. 9–18).[152,154–156] This lesion can be differentiated from RSV infection only by immunologic or virologic identification of the etiologic agent.

Parainfluenza viruses do not shut off host cell protein synthesis[157] and are variably cytopathic. As in RSV bronchiolitis, the airways are hyperactive, and immunopathologic mechanisms appear to be important.[150,158] Virus-specific IgE appears in respiratory secretions earlier and in greater quantity in those patients who have parainfluenzavirus croup and bronchiolitis than in their counterparts who have infection of the upper airways. The amount of histamine in secretions is also greater in croup than in upper respiratory infection. There is a correlation between the severity of illness and the maximum quantity of virus-specific IgE and histamine in nasopharyngeal secretions. It is possible that the persistent abnormalities after parainfluenzavirus-associated croup and bronchiolitis result from congenitally hyperreactive airways rather than from viral damage to the airways.

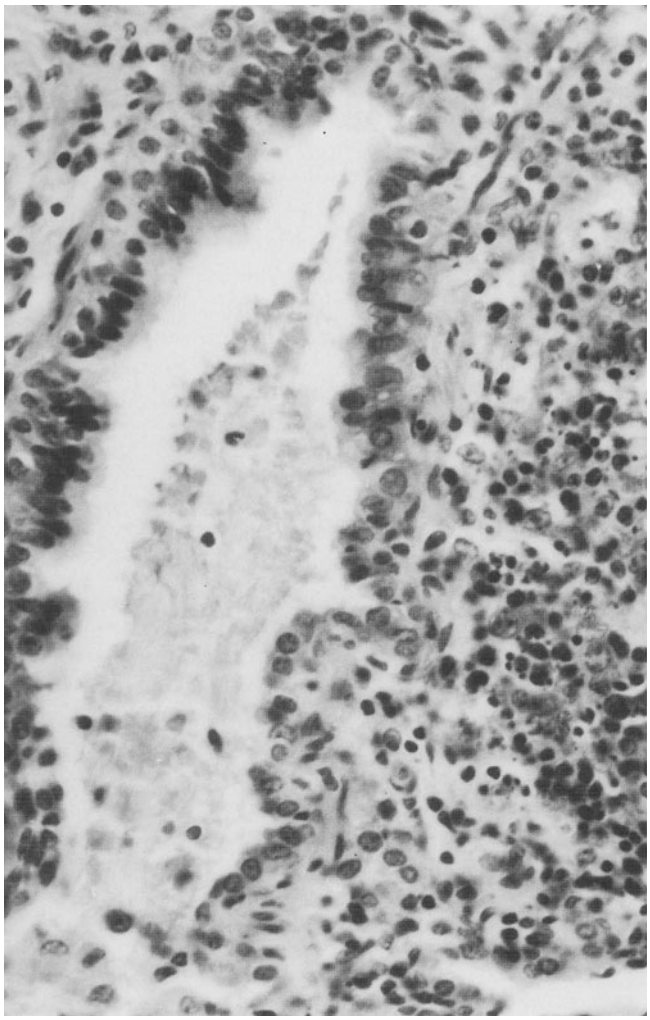


Fig. 9–17. Parainfluenzavirus 1 pneumonitis. Small bronchiole contains proteinaceous material and scant cells. Adjacent airspaces, best seen at right, are filled with mononuclear exudate with extensive nuclear fragmentation. No multinucleated giant cells present. Parainfluenzavirus 1 isolated from lung. Hematoxylin-eosin and stain. H and E, ×350.

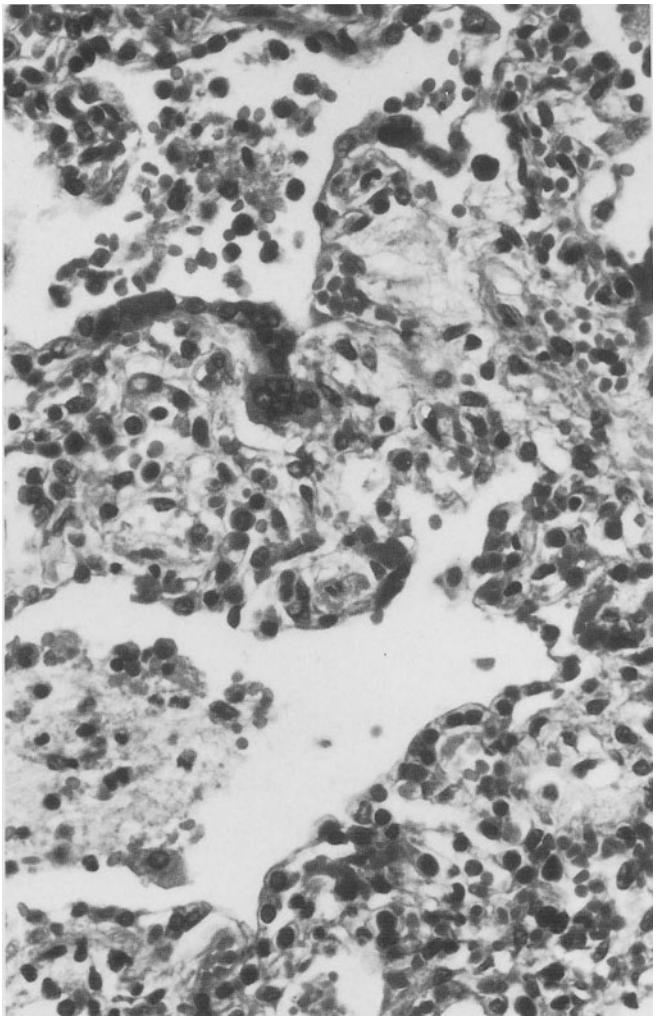


Fig. 9–18. Parainfluenzavirus 3 pneumonitis. Interstitium is edematous and heavily infiltrated by mononuclear cells. Alveoli contain fibrin, red cells, and mononuclear inflammatory cells. Several large multinucleated (syncytial) giant cells line alveolar membrane. Parainfluenzavirus 3 isolated from lung. H and E, ×300.

Varicella-Zoster Virus

Varicella pneumonia is a rare complication of a common disease. The annual incidence of chickenpox in the United States is 1,500 cases per 100,000 people. A history of the illness can be obtained from 70–80% of young adults; 60–90% of adults who do not remember the infection have antibodies to varicella-zoster virus.

In contrast, varicella pneumonia is a disease of adults and immunocompromised or newborn children. Pneumonia was diagnosed by chest radiograph in 18 (16%) of 110 previously healthy young men with chickenpox in one study and in 14% of adults in another series.[159–161] Patients who are over 19 years of age account for 90% of cases of varicella pneumonia. The severity of varicella in adults, including the frequent occurrence of pneumonia, may result from the large inoculum received from infected children. Children who have malignancies, primary or acquired immunodeficiency, or have been treated with corticosteroids or cytotoxic drugs have severe, widespread dissemination of the infection because cell-mediated immune mechanisms fail to restrict viral replication.

Although gestational chickenpox occurs in only 1 to 7 pregnancies per 10,000 pregnancies, the disease is often severe in infants who are infected in utero after their mothers develop chickenpox 5 days or less

before delivery.[162,163] These babies acquire the viral infection, but do not have passively transferred antibodies from their mothers. The rash, which develops on the fifth to tenth day of life, is followed by cutaneous purpura and pneumonia. The mortality is 30%. When the vesicular rash appears in the mothers more than 5 days before delivery and antibodies to varicella-zoster virus (VZV) are presumably transferred to the infants, the rash develops in the infants before the fourth day of life and there are very few deaths.

Pregnancy itself is also a predisposing factor for varicella pneumonia,[164] and the mortality rate is 42%. A large proportion of immunocompromised patients also succumb to this infection. Even among immunocompetent patients with varicella pneumonia, however, the fatality rate is 10%.

After a mean incubation period of 14 days, a vesicular rash and mucosal lesions appear. Symptoms of pneumonia—cough, dyspnea, tachypnea, hemoptysis, and pleuritic chest pain—often begin abruptly 1–6 days later. The severity of pneumonia is usually out of proportion to the physical signs. Rhonchi and rales are detected in only 50–60% of patients. Chest radiographs reveal a bilateral, nodular infiltrate that is often peribronchial and is more dense near the hilum. Cyanosis may occur, and death results from respiratory insufficiency.

It is unlikely that lung biopsy would be performed for varicella pneumonia because the diagnosis can usually be established on the epidemiologic basis of exposure to VZV and on the clinical appearance of the enanthem and the vesicular exanthem, which may appear in successive crops. When Tzanck preparations (scrapings of cells obtained from the base of a vesicle) are stained with H and E or by Giemsa or Papanicolau methods, multinucleated giant cells are diagnostic of either VZV or HSV infection. The nuclei appear glassy, and inclusions may be visible, especially when H and E or PAP stain have been used. The Tzanck smear from skin lesions of chickenpox and shingles is more sensitive than culture, whereas the reverse is true for herpes simplex vesicles.[165,166]

Virologic and microbiologic diagnostic methods have also been developed. A definitive diagnosis may be established by viral culture of vesicle fluid collected during the first 3 days of illness. Electron microscopy may demonstrate virions compatible with VZV and HSV, and antigen detection methods including gel diffusion, countercurrent immunoelectrophoresis, and direct immunofluorescence have been developed. Varicella-zoster virus is difficult to culture. Detection of viral antigen is a more sensitive diagnostic method than inoculation of cell cultures.[167]

A variety of serological tests are available for documentation of VZV infection.[168] They are useful for determining the susceptibility of individuals who do not have a history of chickenpox in childhood. For the acute infection, they are all retrospective.

Cells that have been infected with VZV in vitro become refractile, then round, swell, and separate from one another before detaching from the surface of the flask. Multinucleate giant cells form. Eosinophilic intranuclear inclusions surrounded by a clear zone are observed if samples are exposed to a fixative that precipitates protein.[31] Ultrastructural examination of the nucleus reveals viral particles with a dense central DNA core and a single membrane; the envelope is formed by budding through the nuclear membrane. Cytoplasmic virions are present in large membrane-bound vacuoles.

Cytopathology is similar in vivo. Cells swell and develop "balloon degeneration" necrosis with intranuclear inclusions. Giant cells with up to 30 nuclei often contain inclusion bodies.

The portal of entry of VZV is probably the upper respiratory tract, oropharynx, or conjunctiva, where initial replication precedes primary viremia. A second phase of viral replication then follows, possibly in the mononuclear phagocytic system, before a second viremia and clinical symptoms develop. Direct viral injury of capillary endothelium leads to thrombosis (Fig. 9–19), hemorrhage, and the spread of viral infection to cells in the adjacent tissue.

The pulmonary lesions of varicella pneumonia are multiple foci of hemorrhage and/or necrosis. They may be peribronchial, but more often appear as miliary parenchymal foci, suggesting hematogenous dissemination of virus (Fig. 9–18). Interstitial pneumonia, including alveolar septal edema, mononuclear cell infiltration, and hyaline membranes may be present, especially adjacent to the necrotic foci. Bronchiolar exudates and even bronchial or pleural vesicles have been described. In the lungs, virally infected cells with intranuclear inclusions may be found in capillary endothelium, interstitial connective tissue, tracheobronchial mucosal epithelium, and alveolar epithelium. The intranuclear inclusions of varicella-zoster virus should be sought in viable cells at the edges of necrotic lesions. They are considerably more difficult to find than are the inclusions of herpes simplex infection.

Complications and sequelae of varicella pneumonia include secondary bacterial pneumonia, pulmonary fibrosis, diffusion abnormalities, and diffuse nodular pulmonary calcifications.[169]

Because VZV is narrowly species specific and because simian varicella-like viruses produce infections quite different from human chickenpox, experimental elucidation of VZV pathogenesis has been difficult.

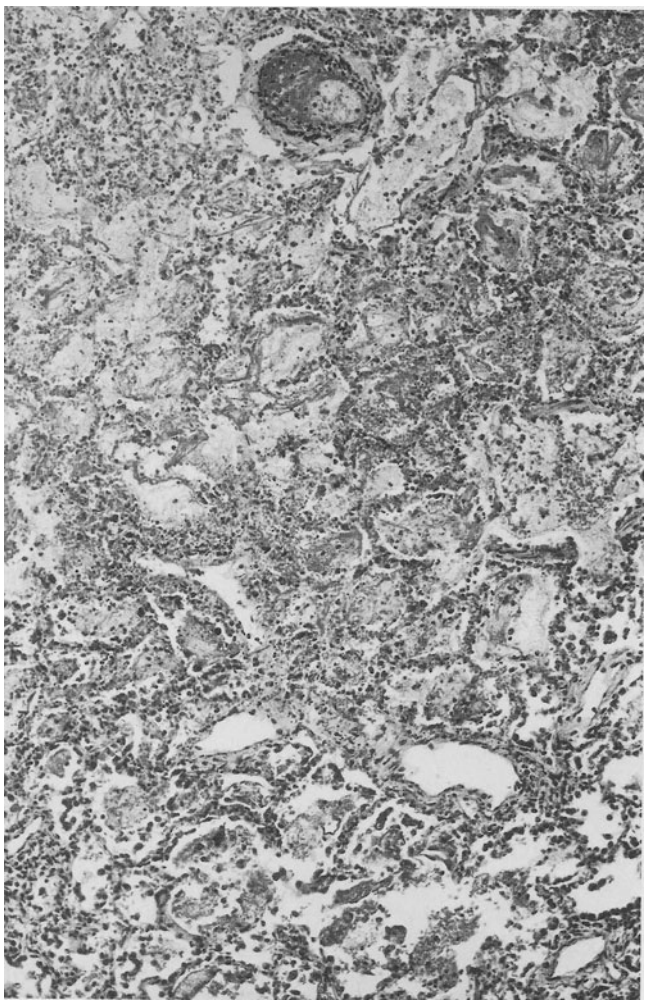


Fig. 9–19. Varicella-zoster pneumonitis. Edge of miliary, necrotic focus. At top, pulmonary arteriole contains fibrin thrombus; airspaces are necrotic. In center, alveoli contain red blood cells, abundant fibrin, and scattered inflammatory cells. At bottom, inflammation becomes less intense at edge of lesion, although no alveolus in this photomicrograph is normal. H and E, ×150.

Cytomegalovirus

Human cytomegalovirus (CMV), probably the virus with the most spectacular cytopathology in the human lung, provides some of the thorniest of virologic problems in the immunocompromised host. The prevalence of infection by CMV is very high. It is difficult to establish CMV as the cause of pneumonia in the individual living patient because many CMV infections are asymptomatic. Isolation of the virus or even demonstration of viral inclusions in pulmonary cells does not establish unequivocally that CMV is responsible for clinical symptoms, because these same observations may also be made in asymptomatic infections.

Cytomegalovirus infection can be serious and even fatal. Unfortunately, there is as yet no effective antiviral chemotherapy. The severity of CMV infection depends upon the maturity and integrity of defense mechanisms, including the immune system of the host. There is increased risk of severe disease in congenitally and neonatally infected infants and in patients of all ages who are immunosuppressed or are undergoing a primary infection. CMV may be transmitted transplacentally, by cervical secretions intrapartum, by aerosol droplets, by transfusion of blood or transplantation of kidney, bone marrow, or heart, and by contact with semen, cervical secretions, and breast milk.[170] Moreover, endogenous virus may be the source of recurrent infection, because CMV causes latent infections that may become reactivated. Reinfection with a second strain of CMV has also been observed.[171]

Congenital CMV infection occurs in 1% of infants, of whom only 5% are symptomatic.[172] Severe disease is usually characterized by jaundice, hepatosplenomegaly, thrombocytopenia, purpura, growth retardation, and less frequently by microcephaly, periventricular cerebral calcifications, chorioretinitis, optic atrophy, psychomotor retardation, and sensorineural hearing loss. Congenital CMV infection is more often severe when it is associated with primary maternal infection.[163]

Fewer than 1% of patients who are congenitally infected with CMV have pneumonia, which is incidental to widespread dissemination of virus.[173] Neonatal CMV infection is acquired from maternal cervical secretions, blood transfusion, or breast milk; although neonatal infection is usually asymptomatic, protracted pneumonia may develop.[174,175] The clinical signs—paroxysmal cough, tachypnea, intercostal retractions, rales, and bilateral pulmonary infiltrates with airtrapping—are indistinguishable from pneumonia caused by *Chlamydia trachomatis* or *Pneumocystis carinii*.[176] In fact, mixed infections of CMV and these agents occur often and are more severe than infections caused by CMV alone. Neonatal CMV pneumonia usually resolves, but virus may replicate to high titer (10^5–10^7 plaque-forming units/g) and fatal disease may result.[177,178]

CMV infection is virtually universal early in life in developing countries. In the United States 50–90% of adults have antibodies to this virus. Infection in older children and adults is usually asymptomatic, but it is associated with an infectious mononucleosis syndrome without heterophil antibodies, particularly in patients older than 30 years of age who have received moderate-to-massive amounts of transfused blood.[179,180] Fresh donor blood may contain CMV in leukocytes. The virus may be reactivated by allogeneic interaction with histoincompatible recipient cells. Pneumonia has been observed in 6% of patients with the CMV infectious mononucleosis syndrome.

Immunocompromised patients are at greater risk for CMV pneumonia, particularly those who have the acquired immunodeficiency syndrome (AIDS) or who have received transplanted allogeneic organs or bone marrow. Nearly one-third of renal transplant patients who are treated with prednisone, cytotoxic drugs, and/or antithymocyte serum develop fever 1–3 months after transplantation. An average of 15 days later respiratory symptoms develop in 42% of these patients; these are nonproductive cough, tachypnea, dyspnea, and hypoxemia. Radiographic infiltrates usually begin as bilateral, peripheral, and basilar shadows, then spread centrally and superiorly.[181–184]

Fatalities supervene in 20% of renal transplant patients who have CMV infection, usually in those with diffuse pulmonary infiltrates and superinfection by bacteria, fungi, or *P. carinii.* Cell-mediated immunity, particularly cytotoxic T lymphocytes and natural killer cells, is an important host defense against CMV.[184] CMV-infected patients who are treated with high-dosage, intravenous methylprednisolone often have reduced cytotoxic T-cell activity, prolonged viremia, and a fatal outcome despite the presence of antibody to CMV before transplantation. Risk factors for overt disease in transplant patients include male sex, diabetes mellitus, transplanted kidney from parent, cadaver, nonsibling, or non-HLA identical sibling, and a donor seropositive for CMV antibodies.

The major complication of allogeneic bone marrow transplantation is pneumonia, which develops in 41% of patients.[185] The most frequently identified etiologic agent is CMV, which causes pneumonia in 16% of bone marrow recipients; the mortality rate in this population is 91%. Graft-versus-host disease, an important complication of bone marrow transplantation, is associated with an increased incidence of CMV pneumonia.

Patients with human immunodeficiency virus (HIV) infection who have AIDS are among the most immunosuppressed of patients. Almost all of these individuals develop CMV disease, often including pneumonia, at some time during their course.[186]

Lung biopsies in which CMV might be found come from immunocompromised patients with progressive pneumonia in whom the diagnosis has not been established by microbiologic and cytologic examination of respiratory specimens or by serology and in whom there has no response to empiric therapy directed against *P. carinii* and common bacterial pathogens.

Virologic and immunologic methods may establish a diagnosis of CMV infection; however, they are less effective in establishing the diagnosis of CMV pneumonia. CMV infection is diagnosed by isolation of virus from urine and, less frequently, from saliva, vaginal and cervical secretions, blood, milk, semen, tears, stool, respiratory secretions, and tissue. As has been mentioned above, however, viral excretion occurs in many asymptomatic subjects so that association of an isolated virus with a pathophysiologic process may be problematic. Isolation of CMV from blood leukocytes is less sensitive than from urine, but it indicates that there is active CMV disease.[187]

There are numerous pitfalls in the interpretation of CMV serologic data. The presence of antibodies to CMV indicates only past and possibly latent infection. Transplacental transfer of IgG assures that the majority of infants have antibodies to CMV at birth even if they are not infected. Immunosuppressed patients may not mount a detectable antibody response to CMV, even in fatal cases. Transfusion with seropositive blood can lead to the appearance of passive antibodies.

A variety of serologic assays have been developed. The complement fixation test, which is not useful in the 10% of sera that are anticomplementary, is relatively insensitive. The sensitive indirect fluorescent antibody assay and even more sensitive anticomplement immunofluorescence technique are cumbersome and require a fluorescence microscope. Passive hemagglutination, enzyme immunoassay, latex agglutination, and automated fluorescence (FIAX), which are sensitive, are suitable for screening purposes. Immune adherence hemagglutination has also been used.[172,188–190]

The appearance of Fc receptors for IgG in infected cells that are employed as CMV antigens can cause problems in the indirect immunofluorescence test unless isolated nuclei are used. Assay of IgM by the indirect fluorescent antibody test is only 50% sensitive and yields false-positive results when rheumatoid factor is present. Griffiths and colleagues[191] developed a radioimmunoassay that was able to distinguish accurately primary from secondary infections in pregnant women; false-positives were found in 19 of 104 (18%) of the women when the sera were tested by indirect immunofluorescence. However, radioimmunoassay is not well suited to routine laboratory use. Enzyme immunoassays for antibody to IgM have performed in a manner similar to radioimmunoassay.[192] With the herpes group of viruses, however, even the demonstration of IgM is not absolute assurance that the infection is primary.[193]

The diagnosis of CMV pneumonia is usually made on clinical grounds, as when there is prolonged unexplained fever followed by radiographic pulmonary infiltrates in the proper clinical setting. If there is a fourfold or greater increase in antibody titer or if CMV is isolated from secretions, the likelihood of an active infection is increased.

When a lung biopsy is obtained, viral cultures are more sensitive than histologic examination of tissue.[38,194–196] Likewise, cytologic diagnosis of CMV

infection was established in only 3 of 13 cases with positive viral cultures. Two newer methods that will likely be used in the future are immunofluorescent detection of CMV antigens and detection of CMV DNA by in situ hybridization, both of which appear comparable in sensitivity to viral culture.[48,195,197,198] When reagents of good quality are available and methods are established more widely, the timeliness of immunofluorescence (2–4 hr) and DNA hybridization (2 days) will offer advantages over viral cultures, which may not yield CMV for 2–3 weeks. The virologists have also made progress in the diagnosis of this infection, however. Most isolates of CMV can be detected in less than 48 hr when the inoculum is centrifuged onto a monolayer of susceptible fibroblasts, which are incubated for 16 hr before testing for the presence of CMV antigen in the monolayer by immunofluorescence.[29]

CMV-infected cells were described first in 1904 by Jesionek and Kiolemenoglou[199] as large protozoan-like cells. In 1921 Goodpasture and Talbot[200] reported a 2-month-old child with the same large cells, which they recognized as altered host cells. They described enlarged cells (10- to 30-μm diameter) that had large nuclei, large oval intranuclear inclusion bodies, and basophilic cytoplasmic inclusions. They suggested that these cells were spread hematogenously, and proposed the term cytomegalia for the cellular enlargement.

In 1932 Farber and Wolbach[201] described cytomegalic intranuclear and cytoplasmic inclusions in the submaxillary salivary glands of 12% of 183 infants. They pointed out the similarity of the inclusions to those found in diseases caused by filterable viruses. Cole and Kuttner[202] had already demonstrated that the cytomegalic lesions found in guinea pig salivary glands were caused by a filterable virus. Smith first successfully propagated a cytomegalovirus by serially transmitting murine CMV in mouse fibroblasts. Shortly thereafter, human CMV was isolated concurrently and independently in three laboratories.[203–205]

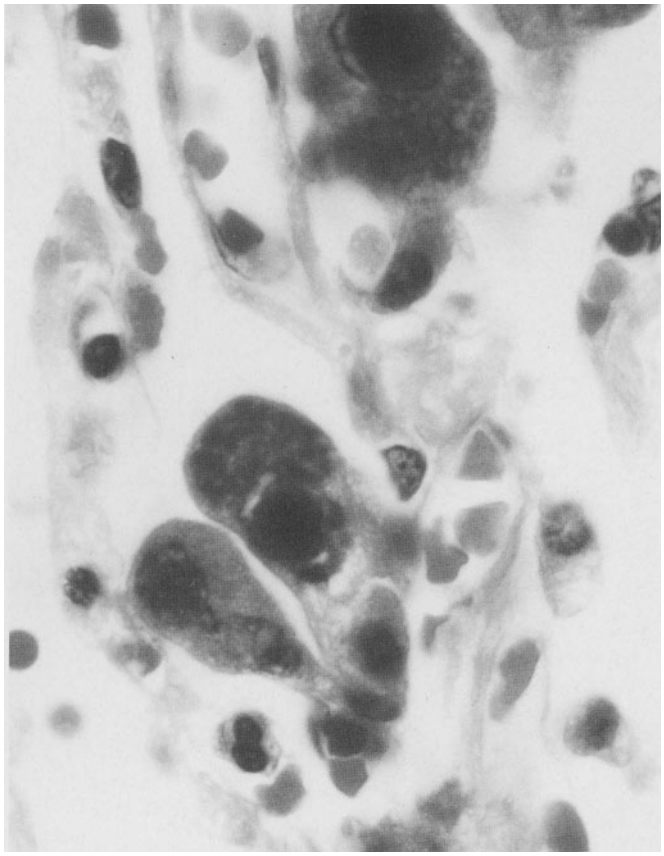
CMV replicates in the nucleus, where assembly into typical herpesvirus nucleocapsids occurs, corresponding to the Feulgen-stained polymerized DNA in the intranuclear inclusion.[201] The viral envelope is acquired by budding through the nuclear envelope (Fig. 9–20). Noninfectious enveloped particles contain the capsid assembly protein without viral DNA.[206] In Giemsa-stained touch preparations of lung biopsies, the cytomegalic cells measure up to 40 μm in diameter. Cellular enlargement may be related to virus-induced depolymerization of microtubules, secondary to the influx of calcium ions and breakdown of intermediate filaments.[207]

Viral inclusions are present both in the nucleus and in the cytoplasm. Intranuclear inclusions measure up to 20 μm in diameter and cytoplasmic inclusions are 1–3 μm in size.[186] In sections that have been stained with hematoxylin and eosin, the early forms of the intranuclear inclusions resemble those of herpes simplex and varicella-zoster viruses. The mature inclusions are densely stained and vary from eosinophilic to deeply basophilic (Fig. 9–20; Color Plate 9–VI, front). The inclusions are usually surrounded by an artefactual halo in formalin-fixed tissue. The marginated chromatin is uniformly compressed but not beaded. A prominent, single rounded clump of peripheral chromatin is often seen protruding into the otherwise clear halo zone. This probably represents a remnant nucleolus and is characteristic of CMV.[31] Cytoplasmic inclusions appear after the intranuclear inclusions are well developed; they are not found in all cells that have intranuclear inclusions.

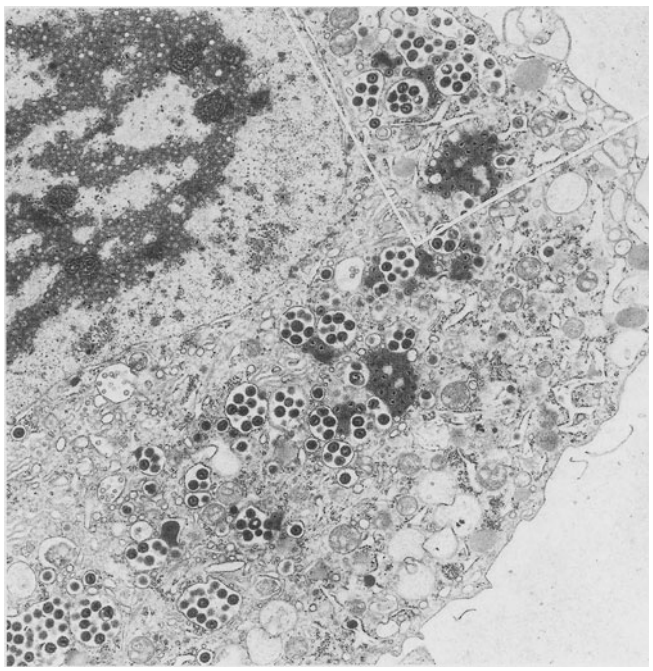
The cytoplasmic inclusions, which are rounded, granular, and slightly basophilic, are stained by the periodic acid–Schiff and Gomori methenamine silver procedures, but the intranuclear inclusions are not.[208] Whereas the Feulgen-positive intranuclear inclusions contain viral nucleoprotein and assembled capsids, the cytoplasmic inclusions appear to be of varied nature.[209] Some are composed of cellular elements, such as accumulations of endoplasmic reticulum, vesicles, dense bodies, mitochondria, and lysosomes; some are composed almost entirely of virions;[208] and others are a mixture of virions and cellular elements.

Hybridization with three cloned, biotinylated DNA segments that represented 56% of the CMV genomic DNA demonstrated that CMV-infected cells were not necessarily cytomegalic, nor did they always contain inclusions.[197] In fact, morphologically normal cells including fibroblasts were also virally infected. By immunofluorescence, CMV antigens are present in cells of the alveolar lining and lumen and in the endothelium. Both intranuclear and cytoplasmic inclusions contain viral antigens.[197,210]

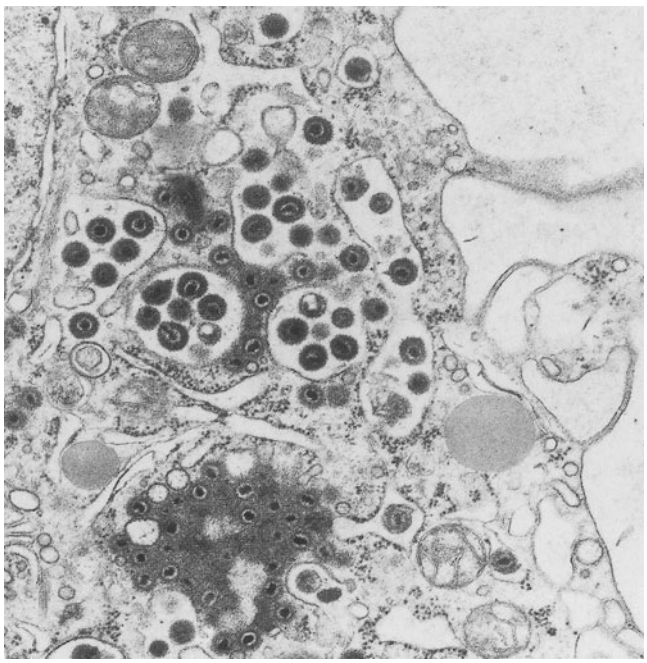
Three different histopathological patterns have been described in the lungs of patients with CMV.[17,197,211] Cytomegalic cells may be found in the alveolar epithelium with minimal evidence of inflammation and injury (Fig. 9–21). Alternatively, there may be miliary, multifocal lesions that contain cytomegalic inclusion cells. In these focal lesions the pulmonary architecture is obliterated by an exudative inflammatory response in the interstitium and in the airspaces. There may be central necrosis, hemorrhage, and an accumulation of fibrin, mononuclear cells, and a small number of neutrophils (Fig. 9–22). The third pattern is a diffuse interstitial pneumonia with lymphocytes, macrophages, and plasma cells. An exudative reaction is characterized by edema in the interstitium, serofibrinous exudates in



A



B



C

Fig. 9–20 A–C. Cytomegalovirus pneumonitis. **A.** High power: three inclusion-bearing cells are typically enlarged, containing basophilic intranuclear inclusions separated from nuclear membrane by clear space. **B,C.** Irregular, aggregated cytoplasmic inclusions are most discrete in center. Such characteristic cells are pathognomonic of cytomegalovirus infection. Cytomegalovirus isolated from lung on culture. (See Color Plate 9–If.) H and E, ×750. **B.** Cytomegalovirus is seen replicating in nucleus (naked virions) and receiving coat (nucleocapsid) in cytoplasm. TEM, ×6,300. **C.** Higher power of marked area from Fig. 9–20B TEM, 16,000. (Figs. 9–20B and C provided courtesy of D Bockus, F Remington, and S Friedman, Mason Clinic, Electron Microscopy Laboratory, Seattle, Washington.)

the alveolar spaces, and alveolar cell hyperplasia (Fig. 9–23). Hyaline membranes may be present, but are not frequent or widespread. Cytomegalic cells are scattered diffusely through the parenchyma of the lung. The miliary pattern is believed to represent hematogenous spread of virus infection to the lungs, after which slow centrifugal spread of this highly cell-associated virus produces focal lesions, just as occurs in cell monolayers.

CMV is a relatively nonvirulent virus, and infection depends on defective defenses of the immunocompromised or immature host.[212–214] In vitro the virus has a relatively slow replicative cycle that produces slowly progressive lytic foci. Defective interfering particles are formed, and latent infection is established readily. In addition to direct virus-induced cytopathic effect, CMV-infected cells may be lysed by cytotoxic T lymphocytes and natural killer cells. Other possible immunopathologic mediators of tissue damage are circulating immune complexes, which are detectable in

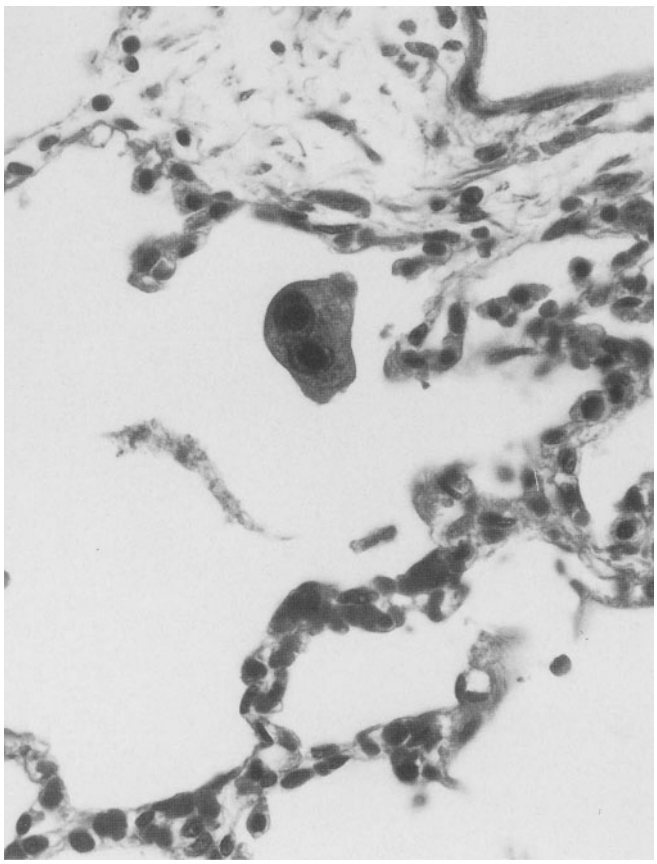


Fig. 9–21. Cytomegalovirus infection. Single inclusion-bearing cell present in airspace is binucleate, an unusual finding in cytomegalovirus infection. Intranuclear inclusions present in both nuclei but cytoplasmic inclusions are absent. Minimal evidence of host reactivity: a wisp of proteinaceous material in airspace and slight increase in prominence of respiratory epithelial cells. Cytomegalovirus isolated from lung. H and E, ×300.

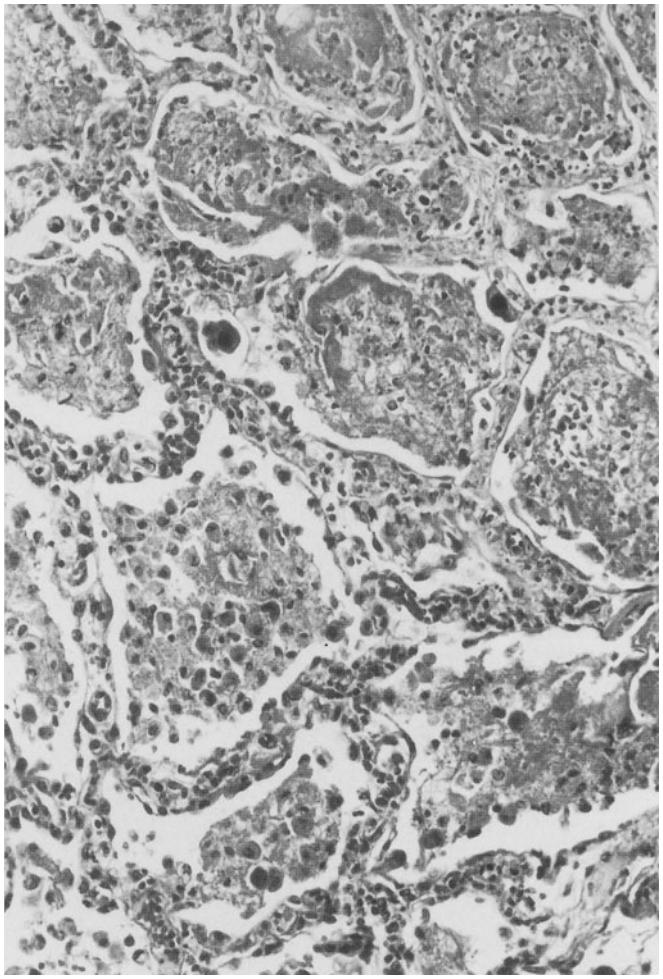


Fig. 9–22. Cytomegalovirus pneumonitis. Edge of nodular lesion depicted. Necrotic center of nodule, surrounded by damaged airspaces, is at top where alveoli are filled with coagulum of fibrin, inflammatory cells, and nuclear debris. Interstitium is edematous and contains sparse mononuclear exudate. Two large cells have dense intranuclear inclusions. Toward edge of nodule of inflammation (bottom), airspaces contain less dense collections of fibrin and mononuclear cells. Cytomegalovirus isolated from lung. H and E, ×150.

45% of neonatal and congenital CMV cases. In some instances, deposits of IgG without CMV antigens have been observed in glomerular basement membrane.[215] Animal models for CMV must be interpreted cautiously, because cytomegaloviruses, including human CMV, have a narrow host range. In fact, human CMV shares less than 5% of its DNA with murine CMV and simian CMV. Thus, human CMV is no more closely related to these agents than to the two serotypes of herpes simplex virus.

Primary CMV pneumonia may be fatal in its own right. In addition, this infection may be complicated by fatal bacterial, fungal, and protozoal infection. There is a close association of infection by CMV and the protozoan parasite *Pneumocystis carinii*. CMV pneumonia may resolve completely or may organize, leaving multifocal pulmonary scars.[216]

Other Viruses

Many other viruses replicate and cause disease in the upper respiratory tract. On rare occasions they may also infect the lower respiratory system, particularly in infants and children. The details of the pathologic changes are scant; for the most part the brief descriptions suggest nonspecific pulmonary damage or interstitial pneumonitis without any unique features. When the viruses produce latent infections, such as Epstein-Barr virus, or when they are frequently isolated from respiratory secretions in the absence of disease, as are enteroviruses, it may be difficult even to document the

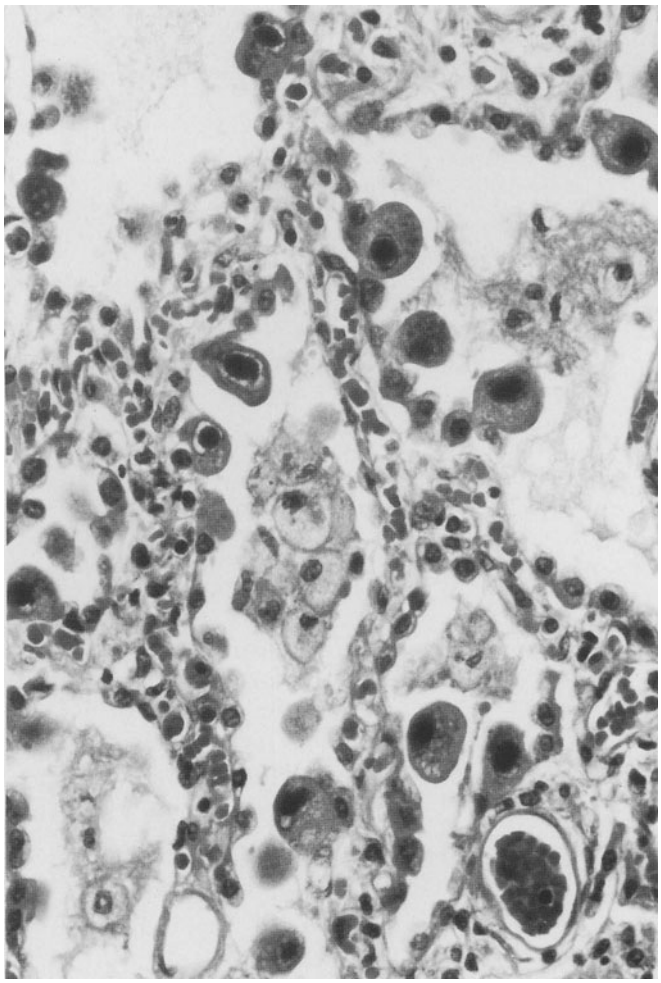


Fig. 9–23. Cytomegalovirus pneumonitis. Interstitium is edematous and contains sparse mononuclear cells. Alveolar epithelium is hyperplastic and airspaces contain macrophages and fibrin. Multiple enlarged cells contain both intranuclear and cytoplastic inclusions. Cytomegalovirus isolated from lung. H and E, ×300.

association with absolute certainty. Such viruses include rhinovirus,[217,218] an important etiologic agent of the common cold, Epstein-Barr virus,[219] a major cause of infectious mononucleosis, and enteroviruses,[220] which produce systemic disease, especially of the cardiac and central nervous systems.

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