CLINICAL MICROBIOLOGY REVIEWS, Jan. 1988, p. 27–39
0893-8512/88/010027-13$02.00/0

Vol. 1, No. 1

Detection, Pathogenesis, and Therapy of Respiratory Syncytial Virus Infections

ROBERT C. WELLIVER

Department of Pediatrics, State University of New York at Buffalo, and Division of Infectious Diseases and Microbiology Laboratories, The Children's Hospital of Buffalo, Buffalo, New York 14222

INTRODUCTION

Respiratory syncytial virus (RSV) is the major cause of acute lower respiratory illness among infants and young children (25, 50). An understanding of the spectrum and frequency of illnesses caused by this agent was initially limited by difficulties in recovering the virus from infected individuals, presumably because of its lability in nature. Although culturing techniques have been improved, more recently it has become possible to detect RSV infection by using rapid diagnostic methods such as immunofluorescence assays and enzyme-linked immunosorbent assays (ELISA). The use of these techniques has also provided an appreciation of how RSV spreads among hospitalized patients and has afforded an opportunity for early therapeutic intervention.

It has long been assumed that immunologic mechanisms may play a role in the pathogenesis of RSV infection, and some advances have been made in delineating the immunopathogenetic mechanisms involved in RSV infection. These discoveries also provide clues to the development of new therapeutic modalities in RSV infection.

This paper reviews critically the array of techniques available for diagnosis of RSV infection, summarizes recent developments in understanding of disease mechanisms, and comments on the usefulness of the various therapeutic modalities which have been evaluated in RSV infection up to now.

DIAGNOSTIC METHODS

Cell Culture and Rapid Diagnostic Techniques

Cell culture. Standard cell culture infectivity is a useful method for recovering RSV from clinical specimens. Nasal swabs, throat swabs, tracheal aspirates, nasal washes, and nasopharyngeal aspirates have all been tested for their efficiency of recovery of virus in cell culture. Nasal washes have been shown to be superior to swabs or aspirates of tracheal secretions (84). Direct aspiration of secretions into polyethylene catheters is also an effective method of obtaining secretions (23). Human epithelial (HEp-2), human lung fibroblast (WI-38 or MRC-5), or rhesus monkey kidney cells are all appropriate for recovery of RSV. In a recent study (6), 90% of isolates were detectable by day 7 after inoculation in rhesus monkey kidney cells, by day 8 to 10 in HEp-2 cells, and by day 14 in human fibroblast cells. Growth at 36°C was equivalent to growth at 33°C. It should be noted

that the efficacy of individual cell lines varies from year to year and with the age of the particular cell line. It has been stated in the past that recovery of RSV in cell culture is limited by the lability of the virus outside of culture systems. On the one hand, it has been estimated that perhaps 50% of active virus is lost during transport of specimens from inpatient areas to the laboratory. In contrast, the virus is reasonably stable in nasal secretions on flat surfaces for several hours (28). McIntosh and colleagues (55) recently demonstrated that virus could be recovered from four of eight specimens stored in transport medium on a bench top for 3 to 5 days and from two of six specimens similarly stored for 7 days. Similar samples were initially received in the laboratory and then sent through the mail to the same laboratory. RSV could still be recovered in culture in two of eight specimens handled in this fashion. Cell culture remains the standard against which all other methods for diagnosis of RSV infection must be measured. Nevertheless, the advent of specific antiviral therapy for RSV requires that a diagnosis of RSV infection be made at an earlier time than is currently possible through cell culture.

Immunofluorescence. In the late 1960s, Gardner and McQuillin (23) were among the first to use the fluorescent-antibody assays for detection of RSV antigen in nasopharyngeal epithelial cells. These investigators applied the immunofluorescence technique to cells which had been inoculated a few days earlier with clinical specimens believed to contain RSV. The cytoplasmic, dustlike, granular fluorescence characteristic of RSV infection frequently was observed as early as day 2 following inoculation of cell cultures. Numerous investigators have subsequently reported the utility of both direct (22, 27, 86) and indirect (45) immunofluorescence techniques for the detection of RSV antigen in exfoliated nasopharyngeal epithelial cells. In our laboratory, results of immunofluorescence testing were in agreement in 92% of cases with cell culture infectivity for positive specimens and in 94% of cases for negative specimens (Table 1). In comparison to the study by Fulton and Middleton (22), who found that >30% of specimens sent to the laboratory had either an insufficient number or inappropriate types of cells for immunofluorescence assay, results in our laboratory over several years have indicated that only 2 to 3% of submitted specimens have an insufficient number of cells for adequate examination by immunofluorescence.

Several kits for identification of RSV infection by detection of antigen in nasopharyngeal secretions with direct immunofluorescence methods are now commercially available. In one study (15), direct immunofluorescence methods had sensitivity essentially similar to that of indirect immunofluorescence methods. In this study, specificity was apparently unacceptably low, although this difference may have been due to the use of cells which were relatively insensitive for recovery of virus in culture. This study, and ones similar to it, has used monoclonal antibodies to individual viral proteins as the detector antibodies in direct or indirect immunofluorescence assays for detection of RSV. Different monoclonal antibodies result in different staining patterns for antigen. In general, individual monoclonal antibodies yield staining which is less bright than that obtained when polyclonal antibodies are used as the detector antibodies. Pools of monoclonal antibodies, including antibodies directed against different viral proteins, give fluorescent signals of equal intensity to those provided by polyclonal antibodies. Monoclonal antibodies exhibit less staining of bacteria present in nasopharyngeal secretion specimens compared with polyclonal antibodies, resulting in less background fluorescence (73). The best results seem to be obtained when antibody pools include antibodies to the fusion protein and nucleoprotein of RSV (7, 52). The indirect immunofluorescence method currently has the advantage of more extensive clinical experience, and the conjugate can be used in assays for a number of viruses other than RSV. The direct fluorescence assay has the advantage of omitting one step, thereby shortening the time required for completion of the assay. By either method, results should be available within a few hours from specimen collection to report of the result, whereas characteristic syncytial formation in cell culture is unusual before 4 days after inoculation.

ELISA. ELISA methods have been used effectively to detect RSV antigen in nasopharyngeal secretions of infants and older children with RSV infection. Chao and colleagues (14) tested an ELISA system by using commercially available reagents. The ELISA method identified only 79% of specimens that were positive by cell culture infectivity, a result that was approximately equal to the efficacy of immunofluorescence assays in their hands. Hornsleth and colleagues (40) used an ELISA technique to identify 79% of specimens that were positive by cell culture infectivity, results which were inferior to the 91% sensitivity of immunofluorescence assays tested with the same specimens. The ELISA method could detect as little as 20 to 30 ng of RSV protein. The same authors (39) also studied a competitive inhibition ELISA technique to detect RSV in nasopharyngeal secretions. The reactivity of a standard antiserum with RSV antigen was competitively inhibited by incubating the serum with nasopharyngeal secretion samples from patients with RSV infection. With this technique, the ELISA method detected RSV in 61% of 41 specimens found positive by cell culture, while immunofluorescence techniques gave positive results for 65% of the specimens.

ELISA kits for the detection of RSV antigen in nasopharyngeal secretions are now commercially available, and several studies that evaluate their utility have appeared (9, 53, 82). Assays performed with the kits from Ortho Diagnostics and Abbott Laboratories have demonstrated a sensitivity (versus cell culture isolation) of 87 to 94% and specificity ranging from 72 to 95%. The apparently low specificity is because ELISA techniques identify many specimens which are negative by cell culture infectivity. Blocking experiments indicate that many of these apparently false-positive ELISA results are in fact true positives. In one study (9), 37 specimens were positive by the ELISA but negative by cell culture. Blocking assays confirmed that 29 of these 37 samples which were positive by ELISA probably represented true positives. False-positive results with ELISAs occur in a very low percentage of cases, especially when the

TABLE 1. Comparison of indirect immunofluorescence and standard cell culture techniques for detection of RSV infection[a]

Cell culture infectivity results	Indirect immunofluorescence results			
	RSV positive		RSV negative	
	No.	%	No.	%
No. tested (n = 387)	115	30	272	70
RSV positive (n = 106)	98	92	5	5[b]
RSV negative (n = 281)	17[c]	6	264	94

[a] Data from reference 45 used by permission.
[b] Three other samples (3%) had insufficient cells for suitable analysis.
[c] All 17 specimens represent subsequent samples obtained from patients who were initially RSV positive by both cell culture and immunofluorescence techniques.

absorbance readings are fairly low (82). Repeat testing of the same specimens frequently gives a negative result, so routine repeated testing of specimens which are only weakly positive by ELISA techniques may be prudent.

Specimens handled improperly with regard to recovering RSV in cell culture may still yield positive results when tested by ELISAs. In the study by McIntosh and colleagues (55), in which samples received in the laboratory were then mailed to the same laboratory, five of five samples were positive by ELISA after mailing, while only two of eight samples mailed to the laboratory grew RSV in cell culture. In several of these studies, the sensitivity of cell culture for recovery of RSV could be compared with the frequency of confirmed positive ELISAs on the same specimens. In various studies, the sensitivity of cell culture ranged from 69% (9) to 83% (82).

Nasopharyngeal aspirates appear to be preferable to nasal swabs for obtaining suitable specimens for ELISAs. Centrifugation of the specimen before testing seems to increase the sensitivity somewhat, but at a drastic reduction in specificity in comparison to uncentrifuged specimens (53). The use of whole samples of secretions also seems to result in significantly greater sensitivities of the ELISA, in comparison to assays with washed cells as the target (34). Sonication of the sample to release cell-bound antigen did not seem to result in an increased number of positive specimens, although marginally higher absorbance values were observed.

All ELISAs have been directly compared with immunofluorescence assays for detection of RSV antigen in samples of nasopharyngeal secretions. McIntosh and colleagues (55) found similar sensitivities and specificities with the two rapid diagnostic techniques. Hendry and colleagues (34) found that immunofluorescence was superior to ELISA when intact cells were used as the source of target antigen, but minimal differences were seen when whole secretions were used. In contrast, Lauer and colleagues (53) found sensitivities for ELISAs to be between 49 and 57% with uncentrifuged and centrifuged specimens, respectively. Sensitivities with immunofluorescence assays on the same specimens ranged from 32 to 47% in uncentrifuged and centrifuged specimens. Swenson and Kaplan (82) presented evidence suggesting that testing by immunofluorescence assays may result in positive results in samples taken up to 1 week after the onset of illness, when specimens obtained at the same time have become negative for RSV by cell culture infectivity assay or ELISAs.

Immunofluorescence assays may have greater sensitivity for rapid diagnosis of RSV infection than other methods. Use of indirect immunofluorescence requires a highly trained technician and a fluorescence microscope, and the amount of time consumed performing the assay is appreciable. ELISAs require no special equipment and only minimal training. When the accumulated experience with ELISA methods is greater, such methods may replace immunofluorescence as the optimal methods for rapid diagnosis of RSV infection.

It has recently been demonstrated that two antigenic subgroups of RSV exist (36) and that strains from each of these subgroups may circulate concurrently in the community (2, 35). Strain differences have been detected with monoclonal antibodies against different viral proteins. The principal difference in strains seems to be in epitopes on the G (attachment) protein of the virus. Theoretically, this could lead to problems with rapid diagnostic tests in that monoclonal antibodies reacting with strains from one antigenic subgroup might not necessarily detect strains from a second subgroup. These concerns are largely theoretical, since pools of monoclonal antibodies to RSV which contain antibodies to the fusion (F) protein and nucleoprotein (NP) of the virus react quite well with strains from either subgroup (5).

Radioimmunoassay. Two other techniques have been used for the detection of RSV antigen with initially positive results. By using a radioimmunoassay, Meurman and colleagues (58) diagnosed RSV infection in 79% of children in whom infection was established by serologic methods. As with all antigen detection techniques, radioimmunoassay was more likely to be positive if specimens were collected early in the course of illness. In this regard, 88% of specimens taken during the first 5 days and 50% of specimens taken 6 to 10 days after the onset of illness were positive. Performance of the test was dependent also on the age of the patients, the diagnostic efficacy being 88% in children under 6 months of age and 76% in children over 6 months of age at the time of infection.

Reverse passive hemagglutination. Cranage and colleagues (17) used a reverse passive hemagglutination test for detection of RSV in nasal secretions of infants. This assay detected RSV in 24 of 25 (96%) samples positive for RSV by either tissue culture or fluorescent-antibody staining and in an additional two samples which were negative for RSV by these techniques. Considering all of the above information, ELISA and immunofluorescence techniques have received the greatest amount of attention and must be considered the techniques of choice for detection of RSV antigen. Based on the limited studies available, it seems that immunofluorescence might have a slight advantage in sensitivity over ELISA techniques, while both techniques exhibit high specificity. Borderline positive ELISA results may sometimes be found to be truly negative upon retesting (82). ELISA techniques have the advantage of not requiring a fluorescence microscope or a trained technician and are more adaptable to office practice or to community laboratories where fluorescence microscopes might not be available.

Serology

CF. Standard complement fixation (CF) techniques have long been available for serologic diagnosis of RSV infection. Nevertheless, CF techniques are quite insensitive for diagnosis in young infants, the group most prone to serious illness due to RSV. The data from Richardson et al. (71) are typical. In this study, only 1 of 49 infants 1 to 3 months of age at the time of RSV infection had a diagnostic increase in antibody titer at the time of RSV infection. Although older infants more regularly demonstrate seroconversion with CF techniques upon RSV infection, nevertheless the overall results are unsatisfactory, and CF now is rarely used for diagnosis of RSV infection at any age.

Immunofluorescence. Serologic diagnosis of RSV infection was enhanced by the development of immunofluorescent-antibody techniques. Scott and colleagues (76) showed that RSV-specific immunoglobulin G (IgG) and IgM antibody could be detected in sera of patients with respiratory infections, using indirect membrane fluorescence on infected cultures. The immunofluorescence technique was adapted to detect RSV-specific antibody in IgG, IgM, and IgA isotypes in sera of patients with RSV infection, using virus-infected substrate cells fixed to glass microscope slides (89). With the immunofluorescence technique, diagnostic increases in titer were observed in 75% of infants aged 1 to 3 or 4 to 6 months at the time of RSV infection and in 100% of infants older than

6 months (Table 2). RSV-specific IgM responses, which also would be indicative of acute infection, were observed in 13% of infants between 1 and 3 months of age and in >50% of infants who were more than 3 months of age at the time of primary RSV infection. Kaul and colleagues (46) compared fluorescent-antibody, neutralizing-antibody, and complement-enhanced neutralizing-antibody assays for detection of antibody to RSV in serum specimens. In this study, titers detected by complement-enhanced neutralization assays were significantly greater at all intervals after the onset of illness than titers obtained by the other methods. Fluorescent-antibody titers in the IgG isotype assay correlated well with neutralization titers detected in either the presence or the absence of complement, although titers were somewhat lower with immunofluorescence techniques. Immunofluorescence assays for detection of RSV antibody in serum are therefore less sensitive than neutralization assays but also much less laborious to perform and more rapid. The immunofluorescence technique also appears to be superior to CF techniques in terms of sensitivity.

ELISA. ELISAs also have been used for the detection of serum antibody to RSV. Richardson et al. (71) compared an ELISA technique that used crude RSV antigen with standard CF assays as well as a plaque reduction neutralization assay. The results (Table 2) indicate the superiority of the ELISA method to the other two techniques for detection of RSV infection in infants of <6 months in age. Results were similar to those obtained with indirect immunofluorescence assays. These authors used relatively crude preparations of RSV grown in tissue culture as the target antigen, and background absorbance values were high. Lower background absorbance can be obtained if the RSV antigen is first partially purified by sucrose gradient centrifugation.

Other investigators have evaluated ELISA techniques, using purified or partially purified RSV antigen. Bruckova and associates (11) found that titers obtained by an ELISA were 10 to 15 times higher than those determined by CF. However, the ELISA method was superior to the CF assay for detection of RSV infection only in individuals of <1 year of age at the time of infection. In tests of sera obtained from a normal population, the ELISA method revealed twice as many antibody-positive sera as the CF test. Meurman et al. (57) studied 26 patients with RSV infection and, using an ELISA, found significant IgG antibody increases in 24 (92%). The only two patients who failed to develop a positive response were <4 months of age at the time of infection. ELISA techniques, like immunofluorescent assays, may be adapted for detection of virus-specific IgM antibody responses. Meurman et al. (57) found significant IgM antibody responses in 19 of 26 (73%) patients. Only five of eight (63%) patients aged 6 months or less at the time of infection produced detectable IgM antibodies, whereas all patients older than 12 months had a detectable IgM response. IgM antibodies appeared within 1 week after the onset of illness and persisted for up to 3 months. Hornsleth and colleagues (37, 38) studied IgG subclass-specific antibody responses to RSV in serum specimens from infants with RSV infection. The bulk of the RSV antibody response was in the IgG1 and IgG3 subclasses, while the IgG3 response was more transient.

TABLE 2. Comparison of various serologic techniques in the diagnosis of RSV infection in infants

Patient age (mos)	No. with diagnostic response/no. tested (%)			
	CF[a]	Indirect immunofluorescence[b]	Plaque reduction[a]	ELISA[a]
1–3	1/49 (2)	6/8 (75)	1/47 (42)	25/49 (51)
4–6	9/19 (47)	9/12 (75)	11/19 (58)	18/19 (95)
7–12	9/10 (90)	7/7 (100)	9/10 (90)	9/10 (90)
≥12	9/12 (69)		11/13 (85)	11/13 (85)

[a] Data from reference 71 used by permission.
[b] Data from reference 89; IgG isotype-specific response only.

In addition to responses in the IgG and IgM isotypes, RSV-specific antibody responses in the IgA isotype also have been observed with either immunofluorescence or ELISA methods in both serum (57, 89) and secretions (49, 91).

ELISA and hemadsorption neutralization. The basic ELISA technique has been adapted to standard neutralization assays. After incubation of virus with serum specimens thought to contain RSV antibody, the resulting fluid mixture is added to microtiter wells containing tissue culture cells. After a sufficient period of time for infection is allowed, the wells are drained and fixed with acetone. Virus-infected cells in the microtiter wells are detected by the ELISA technique (4, 24). This method appears to have the same sensitivity for diagnosis of RSV infection as standard neutralization tests but has the advantage of requiring considerably less time and equipment and provides results which can be read and recorded automatically. Finally, a reverse passive hemadsorption technique for detection of antibodies to RSV has been described, but has not been evaluated in a clinical setting (16). Considering cost, need for special equipment, ease of performance of the assay, rapidity with which results are available, and sensitivity, the ELISA method seems to be the most useful of those described for the serologic detection of RSV infection. The ability to adapt this assay to microneutralization assays, as well as its use in detecting isotype-specific or isotype subclass-specific responses, is a considerable additional benefit.

As noted above, more than two antigenic subgroups of RSV have been identified (36), raising the theoretical possibility that use of one antigenic subtype of the virus as the target for antibody detection tests might give falsely negative results in individuals infected with a strain of virus from the second antigenic subgroup. Nevertheless, when RSV strains isolated over a period of 23 years, including strains of the two hypothetically distinct types, were tested by either in vitro neutralization with specific antiserum or in vivo resistance to infection in cotton rats previously exposed to a strain of RSV from one subgroup, the two subgroup strains did not appear to be antigenically distinct. Therefore, although antigenic differences can be detected by using panels of monoclonal antibodies to individual RSV proteins, these differences do not seem to be important in the protective neutralization response (68). Furthermore, as long as samples of serum or secretions are being tested for polyclonal antibody responses or for responses to any RSV protein other than the attachment (G) protein, there should be no difficulty in detecting maximal antibody responses.

THEORIES OF PATHOGENESIS

Over the past 3 decades, there has been a tremendous amount of interest in the mechanisms of pathogenesis of RSV infection. At least part of the virulence of RSV is attributable to its ability to infect the terminal airways of the lung in infancy, when the diameter of these airways is quite small. Whether or not RSV has a proclivity for infection of

the bronchiolar epithelium in preference to the rest of the respiratory tract is unknown, but pathologic studies indicate that the inflammatory response is much greater in the area of the terminal airways than in the upper portions of the respiratory tract (93). Also, in the ferret model of RSV infection, organ cultures obtained from 3-day-old ferrets replicated RSV to a 100-fold-higher titer than did organ cultures from adult ferrets. A similar age dependence for viral replication has not been shown in humans, but the ferret data certainly mimic the age dependence of severe RSV infection in humans (64).

Nevertheless, the pathogenesis of RSV infection does not seem to be simply attributable to enhanced susceptibility to infection at a time when the airway diameter is small. The suspicion that other factors, particularly immunologic mechanisms, might be involved in RSV pathogenesis first arose from studies of a Formalin-inactivated RSV vaccine (44). In these studies, infants who received the vaccine subsequently developed more severe RSV disease at a later age than did an unvaccinated control group. These results were the basis for numerous investigations of which segments of the immune system might play the most important role in pathogenesis and protection.

Antibody-Mediated Immunity

IgG, IgM, and IgA. The contribution of antibody to RSV to eradication of infection is unknown presently. McIntosh and colleagues (56) found that RSV IgA antibody first appeared in nasopharyngeal secretions at the same time that virus disappeared from the respiratory tract. These authors thus concluded that the development of an RSV-specific antibody response in the respiratory tract was a principal event in termination of virus shedding. In contrast, Kaul and colleagues (49) found that antibody was present in nasopharyngeal secretions as early as 1 to 3 days after the onset of symptoms, at a time when high quantities of RSV are known to be shed in secretions. These investigators demonstrated RSV in the form of immune complexes in secretions and conclude that antibody played a minor role in the eradication of RSV infection. Studies of neutralizing antibody responses in nasopharyngeal secretions are complicated by the fact that neutralizing activity may often be found in the absence of detectable antibody and that IgA antibody to RSV may be non-neutralizing (55). Hemming and colleagues (33) have shown that infusion of intravenous immunoglobulin preparations with extremely high titers of antibody to respiratory syncytial virus (>1:10,000) terminates virus shedding abruptly in owl monkeys and humans. This study suggests that antibody plays an important role in termination of shedding of RSV, although it is not clear how this rather unique experiment applies to natural infection.

The antibody response to primary infection with RSV in both serum and respiratory secretions is transient. No antibody is detectable 6 months after the onset of primary infection. With secondary infections, the antibody response in both serum and respiratory secretions is enhanced, being higher in the first few days after the onset of illness and more persistent in comparison to the response following primary infection (49, 89). The transient nature of the antibody response following primary infection affords a possible explanation for the frequency with which recurrent RSV infections occur.

The possibility that the antibody response to RSV might be important in the pathogenesis of illness has received considerable investigation. It does not appear that serious illness due to RSV infection occurs as a result of a deficient antibody response in that the magnitude of the antibody response in the IgG, IgM, and IgA isotypes is equivalent, both in serum and in respiratory secretions, among individuals with mild upper respiratory illness or mild to severe lower respiratory illness at the time of RSV infection (49, 56, 89). However, since RSV infection in infancy occurs at a time when high levels of transplacentally acquired maternal antibody are present in infant serum, it was at first suspected that severe disease is a result of an Arthus-like reaction occurring in the lung. However, it was soon recognized that severe bronchiolitis could occur in the absence of any detectable antibody in serum, and it has since been recognized that infants with the highest titers of transplacentally acquired antibody develop less severe disease at a later age than do infants with lower titers of such antibody (26). Further studies of the cotton rat model of RSV infection have demonstrated that passively acquired serum antibody protects cotton rats from pulmonary infection with RSV and, to a lesser extent, infection of the upper respiratory tract (66, 67). In summary, antibody to RSV in the IgG, IgM, and IgA isotypes provides incomplete protection against reinfection and potentially has some role in the eradication of infection, but such antibody does not appear to contribute to the pathogenesis of disease.

IgE. In contrast to the absence of a role for serum or secretory antibody in the IgG, IgM, or IgA isotype in the pathogenesis of infection due to RSV, the development of virus-specific IgE antibody in the respiratory tract appears to be associated with more severe disease at the time of RSV infection (12, 88, 92). In initial studies with immunofluorescence techniques to detect IgE antibody bound to the surface of RSV-infected nasopharyngeal epithelial cells, IgE antibody was found in week 1 after the onset of illness in 75% of individuals with RSV infection, regardless of the type of illness (84). After week 1 of illness, IgE antibody was found bound to exfoliated cells more commonly in individuals with wheezing than in those infected individuals without wheezing. These observations suggest that the production of a small amount of virus-specific IgE at the time of infection is a normal phenomenon and that the IgE may play some role in recovery from infection. On the other hand, production of larger amounts of virus-specific IgE might have a negative effect on the outcome of illness. These hypotheses could not be tested due to the nonquantitative nature of the techniques used to detect antibody. In subsequent studies, ELISA methods (92) and radioimmunoassays (12) have been utilized to detect free virus-specific IgE antibody in secretions of individuals with different forms of illness due to RSV infection. Titers of RSV-specific IgE were significantly greater in individuals with wheezing at the time of RSV infection than in those with upper respiratory illness alone or RSV pneumonia without wheezing (Table 3) (12, 92).

The concentration of histamine in secretions of individuals with various forms of RSV infections has been determined (92) (Table 4). Histamine was detectable in secretions of some patients with all forms of illness but was detected significantly more often and in higher concentration in patients with wheezing. Simultaneous testing of secretions, both for RSV IgE content and histamine concentrations, was not performed. In this study, the severity of illness was determined by obtaining arterial blood gases at the time of hospitalization for RSV infection. Peak concentrations of either RSV-specific IgE or histamine were determined in secretions of these patients. A statistically significant correlation of the magnitude of the RSV IgE response and of the

TABLE 3. RSV-specific IgE responses in nasopharyngeal secretions analyzed by form of illness[a]

Disease category	RSV IgE response (no. positive/no. tested; mean titer) at days after onset of illness	
	0–7	14–90
Upper respiratory infection alone	0/9; 0	0/4; 0
Pneumonia without wheezing	0/9; 0	1/7; 8
Pneumonia with wheezing	3/10; 22	6/10; 45
Bronchiolitis without pneumonia	21/43; 0	17/25; 60

[a] Data from reference 92 used by permission.

concentration of histamine to the severity of illness was obtained. Interestingly, there was a stronger relationship of severity of illness to the magnitude of the RSV IgE response than to the quantity of histamine released. These findings might suggest that other mediators of airway obstruction, which are released by IgE-dependent mechanisms, play a more important role than histamine in determination of the severity of illness. These other mediators might include leukotrienes, prostaglandins, or thrombaxanes.

Cell-Mediated Immunity

Cell-mediated immune function is generally believed to be critical to the containment of viral infection within the respiratory tract. In the specific case of RSV, when cotton rats are infected with this agent, leukocytes that can cause significant chromium release from RSV-infected cells in cytotoxicity assays appear in their lungs. Cytotoxic activity peaks 5 days after inoculation and shows a close temporal relationship to termination of virus replication in the lung (81). Similar studies in humans are not currently available, although there is appreciable information that cell-mediated immune responses are also critical in eradication and limitation of human RSV infection. Shedding of RSV and other respiratory viruses is strikingly prolonged in immunodeficient children in comparison to normal children (21) (Table 5). In addition, fatal RSV infection has been reported in two children with severe combined immunodeficiency syndrome (59), and the frequent development of RSV pneumonia at an age at which lower respiratory disease due to RSV is distinctly uncommon has been reported in children who are immunocompromised by chemotherapy or by primary immunodeficiency disorders (31). Infants with acquired immunodeficiency syndrome who develop RSV infection may have extremely severe manifestations and may shed the virus for prolonged periods (W. Borkowski, personal communication). Children receiving long-term corticosteroid therapy do not appear to have more severe clinical manifestations than normal children when infected with RSV, but viral shedding may be significantly greater in quantity and more prolonged (31). These results, showing that lower respiratory illness at the time of RSV infection may be more frequent or more severe in individuals with compromised immune systems, suggest that lower respiratory illness at the time of RSV infection might result from deficits in cell-mediated immune function. However, in the two studies of cell-mediated immune responsiveness to RSV antigen in infants with acute RSV infection which have been published (78, 87), cell-mediated immune responses were greater in infants of <6 months of age at the time of RSV infection (the peak age at which severe illness due to RSV occurs) and cell-mediated immune responses were in fact greater in individuals with bronchiolitis than in infants with other forms of lower respiratory disease or upper respiratory illness alone due to RSV.

Considerable speculation exists as to the possibility that cell-mediated immune hyperresponsiveness may contribute to the pathogenesis of RSV infection. As mentioned previously, recipients of a Formalin-inactivated RSV vaccine developed more severe disease upon subsequent infection than an unvaccinated control group did (44). Subsequent studies indicated that lymphocytes from recipients of the vaccine exhibited greater proliferative responses to RSV antigen than lymphocytes from unvaccinated individuals who had undergone natural RSV infection. Cell-mediated immune responses in vaccinees were equivalent whether or not they subsequently experienced RSV infection. This study suggests that Formalin-inactivated vaccine induced a persistent cell-mediated immune sensitization, which may have resulted in more severe illness at the time of natural RSV infection (51).

A similar vaccine was administered to cotton rats that were then infected with RSV. Unimmunized cotton rats experimentally infected with RSV develop only minimal histologic changes consistent with a very mild pneumonitis. In contrast, recipients of the Formalin-inactivated vaccine developed an extensive neutrophilic infiltration and an increased infiltration with lymphocytes (69). Unpublished results from our laboratory also showed enhanced pulmonary pathology in RSV-infected cotton rats which had been previously immunized with a vaccine consisting of RSV and one of several types of adjuvants. In our studies, infiltration was primarily perivascular with a somewhat lesser extent of peribronchiolar infiltration.

Several studies of the cell-mediated immune response to RSV infection in human infants have been performed. Scott and colleagues (78) found that peak cell-mediated immune responses (lymphoproliferative responses to RSV antigen) were observed approximately 8 weeks after the onset of illness due to RSV infection. Peak cell-mediated immune

TABLE 4. Histamine content of nasopharyngeal secretions from infants with various forms of illness due to RSV infection[a]

Illness group	No. positive/no. tested	Histamine concn (ng/mg of protein)
Upper respiratory infection alone	1/2	1.1 ± 1.0
Pneumonia, no wheezing	2/10	0.6 ± 0.01
Pneumonia and wheezing	7/8	2.7 ± 0.3
Bronchiolitis, no pneumonia	20/29	2.8 ± 0.2

[a] Data from reference 92 used by permission.

TABLE 5. Duration of shedding of certain respiratory viruses in normal and immunodeficient children[a]

Virus	Duration of shedding (days)	
	Normal children (mean)	Immunodeficient children (range)
Respiratory syncytial	6.7	40–112
Parainfluenza		
Type 1	4.4–7.0	
Type 3	8.2–8.9	20–235
Influenza A	6.0–8.0	15

[a] Data from reference 21 used by permission.

TABLE 6. Histamine-induced suppresion of cell-mediated responses to phytohemagglutinin (PHA) and FSV antigen after primary RSV infection[a]

Form of illness	Lymphocyte stimulant	% Suppression of response by addition of histamine at:		
		10^{-9} M	10^{-7} M	10^{-5} M
Upper respiratory infection alone (n = 6)	PHA	37.4 ± 11	54.6 ± 4	60.2 ± 6
	RSV	37.0 ± 21	54.5 ± 7	55.2 ± 9
		$P < 0.05$	$P < 0.05$	
Bronchiolitis (n = 18)	PHA	37.2 ± 9	59.1 ± 7	66.8 ± 6
		$P > 0.025$	$P < 0.025$	$P < 0.025$
	RSV	8.4 ± 10	34.8 ± 7	42.3 ± 9

[a] Data from reference 90 used by permission.

responses were greater in infants 6 months of age or less at the time of infection than in those 7 months to 10 years of age at the time of infection. In contrast, antibody titers were slightly higher in older infants and children than in infants 6 months of age or less at the time of RSV infection. No differences in cell-mediated immune responsiveness were observed when patients with bronchiolitis, asthma, or pneumonia were compared, although patients in the study were not examined directly by the authors, and the accuracy of the clinical diagnoses was not confirmed. In a later study with the same lymphoproliferative assay (87), cell-mediated immune responses among infants with bronchiolitis or asthma at the time of RSV infection were significantly greater than corresponding responses in infants with upper respiratory illness alone or pneumonia without wheezing due to RSV. These differences were observed in samples taken both during the acute phase and in the convalescent phase following infection. In this study, all patients were examined by a single physician member of the study team to establish an accurate diagnosis. When patients were classified by the degree of hypoxemia as measured by initial arterial blood gas values, it was observed that patients with more severe illness (lower arterial partial pressures of oxygen [pO_2]) had greater convalescent cell-mediated immune responses to RSV antigen. Results of this study suggest therefore that both the form and severity of illness at the time of RSV infection are related to the magnitude of the cell-mediated immune response. Cell-mediated immune responsiveness to RSV persisted for only 3 to 6 months following primary infection.

Several others have studied cell-mediated immune responsiveness to RSV antigen. Schauf and colleagues (75) studied infants with and without respiratory illness but documented RSV infection in only two patients. Among those individuals who had positive cell-mediated immune responses, the magnitude of the response was greater in individuals with lower respiratory disease than in individuals who had upper respiratory illness alone. Mito et al. (60) studied 78 infants with various forms of illness due to RSV. These investigators found that patients with lower respiratory disease generally had increased cell-mediated immune responses in comparison with individuals with upper respiratory tract infection alone, but no distinction could be made between individuals with tracheal bronchitis, pneumonia, or bronchiolitis on the basis of their cell-mediated immune responses. Fernald and colleagues (20) studied cell-mediated immune responsiveness to RSV in infants and children over several years. Cell-mediated immune responsiveness increased transiently following the first two RSV infections in a given individual. Eventually, stable degrees of cell-mediated immune responsiveness were observed. No correlation between the magnitude of the cell-mediated immune response and the type of illness at the time of RSV infection was observed, although infants were not studied during the acute illness. Since peak responses following an infection are not maintained, studying all patients several months after RSV infection would tend to obscure any differences which might have existed at the time of acute infection. In summary, studies of cell-mediated immune responses to RSV suggest that greater responses are observed in individuals with lower respiratory illness at the time of RSV infection. Antibody responses are equivalent in patients with upper and lower respiratory illness due to RSV, suggesting that the greater cell-mediated responses observed in patients with lower respiratory tract disease are not simply due to a greater severity of illness. However, further studies of patients acutely infected with RSV are required to determine the role of cell-mediated hyperresponsiveness in pathogenesis of illness.

Suppressor Cell Dysfunction

On the basis of the data suggesting that both cell-mediated immune responsiveness and production of virus-specific IgE were greater in patients with wheezing at the time of RSV infection than in patients without wheezing, an investigation was undertaken to determine whether defects in lymphocyte suppressor cell number or function were present in patients with bronchiolitis due to RSV (90). Such a defect could account for both the exaggerated cell-mediated immune responses to RSV antigen and the overproduction of virus-specific IgE in infants with bronchiolitis. By using histamine-induced suppression of lymphocyte transformation responses to phytohemagglutinin and RSV antigen as indicators of nonspecific and specific suppressor cell function, the results shown in Table 6 were obtained. While suppression of lymphocyte responses to phytohemagglutinin was equivalent in patients with bronchiolitis and in patients with upper respiratory tract infection alone at all concentrations of histamine tested, the degree of suppression of cell-mediated immune responses to RSV antigen was significantly less in individuals with bronchiolitis at 10^{-9} and 10^{-7} M concentrations of histamine. These findings suggest that the immunologic abnormalities which have been demonstrated among individuals with bronchiolitis due to RSV may occur as a result of a deficiency in antigen-specific suppressor cell function. These findings also suggest that attempts to induce adequate suppressor cell function in these individuals might represent an effective new approach to prophylaxis or therapy of RSV infection.

While it is currently unclear how cell-mediated immune hyperresponsiveness might result in more severe forms of respiratory illness at the time of RSV infection, several potential mechanisms are suggested by the results of previous investigations. The degree of the inflammatory response, or overresponse, in the course of limitation and cure of viral infection may determine the extent of tissue injury during the infection (63). In animal models of influenza virus infection, mice with T-cell deficiencies develop almost no pulmonary pathology at the time of influenza A infection, although the virus disseminates widely throughout the body (80). In contrast, normal mice with influenza virus infection develop symptomatic pneumonia, but the virus and the pathology are restricted to the lung (94). Results of other studies show that exposure of leukocytes to live or inactivated virus enhances basophil histamine release (13, 42). In one study (42), histamine release was believed to be mediated by a lymphokine (probably interferon) produced by lymphocytes proliferating in response to viral antigen. In the second study (13), histamine release was enhanced both in response to IgE-mediated mechanisms, which would depend on interferon, and by calcium ionophore-mediated mechanisms, which would be independent of the effects of interferon. Viral infections may result directly or indirectly in enhanced histamine release from basophils and perhaps from airway mast cells.

Under the appropriate conditions, arachidonic acid metabolism of neutrophils can be stimulated, with the subsequent release of several chemical mediators of airway obstruction. In a recent experiment, phagocytosis of RSV-antibody immune complexes stimulated oxidative and arachidonic acid metabolism in neutrophils. A similar production of inflammatory mediators and mediators of airway obstruction in the respiratory tract of infants could contribute to the development of more serious forms of disease at the time of RSV infection (19).

Complement and Antibody-Directed Cytotoxicity

In addition to direct cell-mediated cytotoxic mechanisms, two studies have demonstrated the development of antibody-dependent, cell-mediated cytotoxicity. In these studies, antibody, colostral antibody, and antibody in respiratory secretions were used as mediators of the cytotoxic response (47, 77). While such responses may play a role in eradication of infection, no difference in the magnitude of these responses was observed among patients with different forms of illness due to RSV infection. Therefore, antibody-dependent, cell-mediated cytotoxicity does not seem to play a role in the pathogenesis of RSV disease. Finally, the initial studies in which Arthus reactions were hypothesized as underlying factors in the development of severe forms of pulmonary disease due to RSV infection did not include measurements of complement in the respiratory tract. A recent study has demonstrated that the third component of complement binds to the surface of exfoliated airway epithelial cells in RSV infection, although no differences in the appearance of complement were observed in patients with different forms of respiratory illness. Again, it can be hypothesized that fixation of complement to virus-infected cells may play a role in eradication of infection, but there is no current evidence that fixation of complement plays any role in the pathogenesis of RSV infection in humans (48).

Another mechanism by which proliferating lymphocytes might cause pulmonary pathology is suggested by experiments in rats sensitized with aerosolized ovalbumin (1). In these experiments, immunization seemed to induce epithelial cells to differentiate into mucous cells. The induction of mucous cell differentiation by lymphokines produced by lymphocytes proliferating in response to RSV antigen certainly could contribute to airway obstruction at the time of infection, although direct proof for this phenomenon is lacking.

THERAPY

Bronchiolitis due to RSV is an extremely common illness, and the vast majority of cases have been managed effectively for decades with supplemental oxygen and fluid replacement alone. Mortality from the disease is quite low, and whether any long-term morbidity results from this disease entity is arguable. It is important to maintain this perspective when evaluating any new potential therapeutic modalities. Therefore, any mode of treatment for bronchiolitis should be utilized with the goal of first doing no harm kept clearly in mind.

Bronchodilator Therapy

In spite of the fact that bronchiolitis is a disease mediated by airway obstruction, possibly through IgE-mediated hypersensitivity mechanisms, it has generally been appreciated that bronchodilator therapy is not of any benefit in the vast majority of cases. While truly definitive studies are lacking, studies of both intravenous theophylline (10) and aerosolized bronchodilator agents (54, 74) have failed to demonstrate conclusive benefit in infants under the age of 18 months with viral bronchiolitis.

Corticosteroids

In a similar fashion, double-blind studies of the effects of corticosteroids in the treatment of bronchiolitis have not convincingly demonstrated a beneficial effect. The study by Dabbous and colleagues (18) is typical of the data.

Ribavirin

Efficacy. Ribavirin is a synthetic nucleoside that has antiviral activity against a large number of both ribonucleic and deoxyribonucleic acid viruses in vitro. Based on preliminary studies showing an inhibitory effect of ribavirin against RSV in vitro, Hruska and colleagues (41) demonstrated that ribavirin could reduce the amount of RSV shed in nasal turbinates and lungs of experimentally infected cotton rats. Equivalent effects were observed when the drug was given intraperitoneally or by aerosol. On the basis of these studies, ribavirin has been subsequently evaluated in humans with various forms of RSV infection. In initial studies, healthy volunteers with experimental RSV infection received ribavirin by aerosol in a double-blind fashion (32). In this study, shedding of RSV was diminished in the ribavirin-treated group. Ribavirin did not appear to have any effect on minor upper respiratory tract symptoms, but systemic complaints and fever were noted significantly less often in the ribavirin-treated group. In particular, no significant changes in pulmonary function test results, clinical findings, laboratory tests, or other evidence of toxicity were noted.

Studies of ribavirin were next carried out in infants with lower respiratory disease due to RSV infection (30). Since most infants with RSV infection will stabilize and improve rapidly upon hospitalization and after being given supple-

TABLE 7. Efficacy of ribavirin aerosol in lower respiratory disease due to RSV[a]

Treatment group	Time studied during therapy	Virus shedding (log_{10} $TCID_{50}$)	Arterial pO_2 (mmHg)	Illness score (approx)
Ribavirin	Onset	2.1	49.4	3.0
	End	0.3[b]	62.4[b]	0.9[d]
Placebo	Onset	3.0	52	2.9
	End	1.3[b]	56[c]	1.8[d]

[a] Data from reference 30 used by permission. $TCID_{50}$, 50% tissue culture infective dose.
[b] $P < 0.03$.
[c] Increment change, $P < 0.001$.
[d] Increment change, $P < 0.01$.

mental oxygen and fluid replacement, the investigators in this study appropriately chose to study only infants with relatively severe forms of lower respiratory illness due to RSV. In particular, infants all had pO_2s of <62 mm Hg (ca. 8.3 kPa) breathing room air. To determine the effects of therapy, illness scores were compiled at the beginning and end of therapy and pO_2s were determined at the same intervals. The results of this study are shown in Table 7. The quantity of virus shed was slightly but not significantly less in the ribavirin group at the onset of therapy. However, at the end of therapy, the concentration of the virus shed was significantly lower in the ribavirin-treated group than in the placebo group. More importantly, arterial oxygenation had been improved by 13 mm Hg (ca. 1.7 kPa) in the ribavirin-treated group and by only 4 mm Hg (533.2 Pa) in placebo recipients. The change in arterial pO_2 was significantly greater for the ribavirin-treated group than for the placebo group. In a similar fashion, illness scores were not significantly different for the two groups at the onset of therapy, but the change in illness score was significantly greater in the ribavirin group than in the placebo group.

Participants in this study were not completely matched, since 13 of 16 ribavirin recipients were males and only 8 of 17 placebo recipients were males. Nevertheless, it is generally accepted that males usually encounter more severe illness with RSV infection than females do. If any bias were present, it would presumably be in the direction of having more seriously ill patients in the ribavirin group. It also should be noted that double-blind studies of ribavirin therapy are difficult to do because the drug is somewhat insoluble in water and tends to precipitate on the surfaces of mist tents and in respiratory tubing. Therefore, breaks in the double-blind nature of the study conceivably could have been present. Nevertheless, the study does seem to show a convincing effect of ribavirin therapy in the most seriously ill patients with lower respiratory disease due to RSV.

A similar study of ribavirin aerosol treatment of bronchiolitis was carried out by Taber and colleagues (83). Again, more rapid clinical improvement appeared to result from use of ribavirin aerosol, and no local or systemic toxicity was encountered. Interestingly, in this study there was no effect of ribavirin on the frequency of individuals shedding virus at any time during the study or in the quantity of virus shed at any point. This finding is difficult to explain, but it suggests that ribavirin therapy has beneficial effects other than simply that of reducing the quantity of virus being shed.

Infants with underlying cardiopulmonary disease are particularly at risk for mortality and increased morbidity from RSV infection. Therefore, studies of the efficacy of ribavirin therapy in these individuals would be particularly helpful. Hall and colleagues (29) found that the quantity of virus shed in infants was reduced in a statistically significant fashion in ribavirin recipients in comparison to recipients of a placebo. By the end of therapy, the group of infants with underlying cardiopulmonary disease who received ribavirin showed a 45% improvement in their illness scores, which was statistically significantly greater than the 21% improvement shown by placebo recipients, with underlying cardiopulmonary disease. Arterial oxygenation improved from 50.6 to 72.5 mm Hg (6.75 to 9.66 kPa) in ribavirin recipients, while for placebo recipients the initial arterial pO_2 was 56.8 mm Hg (7.6 kPa) and the final arterial pO_2 was 58.2 mm Hg (7.8 kPa).

Ribavirin is a drug that is somewhat difficult to use. An appropriate particle size (1 to 2 μm) must be generated to deliver the drug to the terminal airways. Because of its relative insolubility, conventional nebulizers tend to become plugged with the drug. In all studies, ribavirin has been administered by Collison generators which are provided free of charge by the manufacturer (ICN Pharmaceuticals, Costa Mesa, Calif.). A concentration of 20 mg of ribavirin per ml of water is maintained in the reservoir, and a flow rate of 12.5 liters of air-oxygen mixture per min is delivered to the mist tent. According to the package insert, the drug is contraindicated in individuals who are receiving mechanically assisted ventilation, again because the drug tends to precipitate and obstruct the ventilatory apparatus. Nevertheless, these obstacles can be overcome and the drug can be given safely to the most severely ill individuals, including those on ventilatory support, by inserting appropriate filters in the inspiratory line between the humidifier and the T-valve and in the expiratory line before the expiratory valve. Careful observation and replacement of the filters are necessary. The manufacturer provides free in-service education in the placement of filters and all other aspects of administration of the drug.

Adverse effects. The antiviral mechanism of ribavirin currently remains unknown, although the drug interferes with both viral messenger ribonucleic acid synthesis and, in the case of influenza A virus, the ribonucleic acid polymerase. While acute toxicity from use of the drug appears to be minimal, there are some reasons for caution in the use of ribavirin. In particular, the drug is teratogenic or embryolethal in most species in which it has been tested. This may cause concern among health care personnel who are caring for patients receiving ribavirin, although to this time there is no evidence in humans to support this concern. Apparently, levels of the drug are undetectable in plasma samples of individuals caring for patients receiving ribavirin. Ribavirin induces cardiac lesions in many animal species at extremely high dosages, is mutagenic to mammalian cells, and is tumorigenic in rats (but again when given in high prolonged doses). The drug is also expensive, and the cost of drug use should be weighed against the anticipated reduction in duration of hospitalization. Finally, in all studies to this point, ribavirin has been given by near-continuous aerosol administration. Infants treated have received the drug into mist tents for 15 to 23 h/day, a process that tends to minimize the amount of time that can be devoted to feeding and to standard nursing care.

Immunosuppression. Ribavirin has some interesting immunosuppressive effects in laboratory animals. The drug causes lymphoid atrophy of the thymus, spleen, and lymph nodes in rats. Low concentrations of the drug enhance antibody formation in mice, but higher concentrations (concentrations easily achieved or exceeded in humans) block the induction of primary and secondary (memory) responses in mice (62,

65). This immunosuppressive effect also appears to be present in humans receiving ribavirin. Taber and colleagues (83) measured serum neutralizing-antibody responses to RSV and found that induction of responses was blocked significantly more often in ribavirin recipients than in placebo recipients. Rosner and colleagues (72) demonstrated that the development of RSV-specific IgA and IgE responses in nasopharyngeal secretions is also suppressed. Since serum neutralizing antibody is predominantly in the IgG isotype, it appears that ribavirin therapy is capable of suppressing antibody responses in at least the IgG, IgA, and IgE isotypes.

The effect of this immunosuppression on the natural history of primary and recurrent infections with RSV is currently unclear. Beneficial effects of the drug were observed in recipients despite the fact that their IgG and IgA antibody responses were probably suppressed. This observation again sheds some doubt on the primary role of antibody responses in the eradication of RSV infection, but it shows that the immunosuppressive effect of ribavirin should not be a contraindication of its use in RSV infection. Suppression of a virus-specific IgE response could be an additional beneficial effect of ribavirin. Suppression of this response with reduced release of chemical mediators of airway obstruction might account for the clinical improvement seen by Taber and colleagues (83) in the absence of a significant antiviral effect of ribavirin therapy.

The effect of inhibition of IgA and IgE responses on the nature of illness at the time of secondary RSV infection also needs to be determined. Inhibition of the development of an IgA response might result in more severe forms of illness at the time of secondary infection, whereas inhibition of development of a virus-specific IgE response at the time of primary infection could have beneficial effects on the outcome of repeated infections. Follow-up studies of patients initially treated with ribavirin at the time of primary RSV infection are in progress to determine the nature of illness upon subsequent RSV infection.

It is known that infants developing bronchiolitis frequently have recurrent wheezing in early childhood and may have abnormal pulmonary function test results when studied a decade after the development of bronchiolitis (70, 79). While there are numerous flaws in the design of these studies because of their retrospective nature and imprecise definition of lower respiratory disease in infancy, nevertheless there is a certain uniformity in their results. That is, they typically show airway hyperreactivity and evidence of small-airway dysfunction in infants with a history of bronchiolitis in infancy. These abnormalities seem to be independent of a family history of atopic disease, but most studies do not control for passive exposure to cigarette smoke or to environmental pollution in interpreting their results. The question therefore remains unanswered as to whether RSV infection in infancy creates some persistent damage to the airway which is evident upon pulmonary function testing 10 years later or whether infants with bronchiolitis are born with a hereditary tendency towards small-airway dysfunction. If RSV infection in infancy does damage the airway, the use of ribavirin therapy to limit the initial infection would appear to be particularly attractive. In the absence of firmer evidence, however, the use of ribavirin cannot be advocated simply on the basis of this hypothesis.

The American Academy of Pediatrics recently published guidelines concerning the use of ribavirin (3). Infants who are otherwise in normal health but with severe disease (pO_2 of <65 mm Hg [ca. 8.7 kPa] or rising pCO_2) would be considered candidates. Infants with chronic heart or lung disease, primary or secondary immunodeficiency, or other underlying conditions likely to complicate the course of illness (multiple congenital anomalies, neurologic or metabolic disease, or aged <6 weeks) should also be considered candidates. These guidelines are not absolute, and even those patients who meet these initial criteria should be observed for a short period of time following hospitalization to see if initial improvement occurs. This somewhat conservative approach to ribavirin appears to be indicated because of the still unknown potential for long-term toxicity from the drug.

In summary, ribavirin seems to be a moderately useful drug, particularly for individuals with severe forms of lower respiratory disease due to RSV infection. It should be emphasized that ribavirin therapy is not a substitute for administration of oxygen or replacement fluid deficits. Again, most individuals with mild to moderate bronchiolitis will improve markedly with only these measures, and ribavirin therapy probably is not currently indicated for these individuals. In patients with severe lower respiratory disease due to RSV, ribavirin therapy may prevent the development of respiratory failure or may shorten the course of hospitalization by a sufficiently significant interval to justify the cost of its use. In addition, RSV infection has a small but appreciable mortality in individuals with bronchopulmonary dysplasia or cardiopulmonary disease or those severely immunocompromised. The use of ribavirin should be considered strongly in individuals with these types of underlying disease who develop moderate to severe illness with RSV infection.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants HL-21829-05 from the National Heart, Lung and Blood Institute, AI-15939-01 from the National Institute of Allergy and Infectious Diseases, and HD-15943-02 from the National Institute of Child Health and Human Development.

LITERATURE CITED

1. **Ahlstedt, S., G. Smedegard, H. Nygren, and B. Bjorksten.** 1983. Immune responses in rats sensitized with aerosolized antigen. Int. Arch. Allergy Appl. Immunol. **72**:71–78.
2. **Akerlind, B., and E. Norrby.** 1986. Occurrence of respiratory syncytial virus subtypes A and B strains in Sweden. J. Med. Virol. **19**:241–247.
3. **American Academy of Pediatrics, Committee on Infectious Diseases.** 1983. Policy statement: ribavirin therapy of respiratory syncytial virus. Am. Acad. Pediatr. News **1983**(12):7–8.
4. **Anderson, L. J., J. C. Hierholzer, P. G. Bingham, and Y. O. Stone.** 1985. Microneutralization test for respiratory syncytial virus based on an enzyme immunoassay. J. Clin. Microbiol. **22**:1050–1052.
5. **Anderson, L. J., J. C. Hierholzer, C. Tsou, R. M. Hendry, B. F. Fernie, R. Stone, and K. McIntosh.** 1985. Antigenic characterization of respiratory syncytial virus strains with monoclonal antibodies. J. Infect. Dis. **151**:626–633.
6. **Arens, M. Q., E. M. Swierkosz, R. R. Schmidt, T. Armstrong, and K. A. Rivetna.** 1986. Enhanced isolation of respiratory syncytial virus in cell culture. J. Clin. Microbiol. **23**:800–802.
7. **Bell, D. M., E. E. Walsh, J. F. Hruska, K. C. Schnabel, and C. B. Hall.** 1983. Rapid detection of respiratory syncytial virus with a monoclonal antibody. J. Clin. Microbiol. **17**:1099–1101.
8. **Bromberg, K., G. Tannis, B. Daidone, L. Clarke, and M. F. Sierra.** 1985. Comparison of Ortho respiratory syncytial virus enzyme-linked immunosorbent assay and HEp-2 cell culture. J. Clin. Microbiol. **22**:1071–1072.
9. **Bromberg, K., G. Tannis, B. Daidone, L. Clarke, and M. Sierra.** 1987. Comparison of HEp-2 cell culture and Abbot respiratory

syncytial virus enzyme immunoassay. J. Clin. Microbiol. 25:434–436.
10. **Brooks, L. J., and G. J. A. Cropp.** 1981. Theophylline therapy in bronchiolitis. Am. J. Dis. Child. **135**:934–936.
11. **Bruckova, M., E. Svandova, and L. Syrucek.** 1981. Detection of respiratory syncytial virus serum antibodies by an ELISA system. Acta Virol. **25**:41–48.
12. **Bui, R. H. D., G. A. Molinaro, J. D. Kettering, D. C. Heiner, D. T. Imagawa, and J. W. St. Geme, Jr.** 1987. Virus-specific IgE and IgG4 antibodies in serum of children infected with respiratory syncytial virus. J. Pediatr. **110**:87–90.
13. **Busse, W. W., C. A. Swenson, E. C. Borden, M. W. Treuhaft, and E. C. Dick.** 1983. Effect of influenza A virus on leukocyte histamine release. J. Allergy Clin. Immunol. **71**:382–388.
14. **Chao, R. K., M. Fishaut, J. D. Schwartzman, and K. McIntosh.** 1979. Detection of respiratory syncytial virus in nasal secretions from infants by enzyme-linked immunosorbent assay. J. Infect. Dis. **139**:483–486.
15. **Cheeseman, S. H., L. T. Pierik, D. Leombruno, K. E. Spinos, and K. McIntosh.** 1986. Evaluation of a commercially available direct immunofluorescent staining reagent for the detection of respiratory syncytial virus in respiratory secretions. J. Clin. Microbiol. **24**:155–156.
16. **Cranage, M. P., and R. R. A. Coombs.** 1982. An indirect haemadsorption procedure (M_{RS}PAH) for detecting antibodies to respiratory syncytial virus. J. Virol. Methods **5**:199–208.
17. **Cranage, M. P., E. J. Stott, J. Nagington, and R. R. A. Coombs.** 1981. A reverse passive haemagglutination test for the detection of respiratory syncytial virus in nasal secretions from infants. J. Med. Virol. **8**:153–160.
18. **Dabbous, I. A., J. S. Tkachyk, and S. J. Stamm.** 1966. A double-blind study on the effects of corticosteroids in the treatment of bronchiolitis. Pediatrics **37**:477–484.
19. **Faden, H., T. N. Kaul, and P. L. Ogra.** 1983. Activation of oxidative and arachidonic acid metabolism in neutrophils by respiratory syncytial virus antibody complexes: possible role in disease. J. Infect. Dis. **148**:110–116.
20. **Fernald, G. W., J. R. Almond, and F. W. Henderson.** 1983. Cellular and humoral immunity in recurrent respiratory syncytial virus infections. Pediatr. Res. **17**:753–758.
21. **Fishaut, M., D. Tubergen, and K. McIntosh.** 1980. Cellular response to respiratory viruses with particular reference to children with disorders of cell-mediated immunity. J. Pediatr. **96**:179–186.
22. **Fulton, R. E., and P. J. Middleton.** 1974. Comparison of immunofluorescence and isolation techniques in the diagnosis of respiratory viral infections of children. Infect. Immun. **10**:92–101.
23. **Gardner, P. S., and J. McQuillin.** 1968. Application of immunofluorescent antibody technique in rapid diagnosis of respiratory syncytial virus infection. Br. Med. J. **3**:340–343.
24. **Gerna, G., E. Cattaneo, P. M. Cereda, M. G. Revello, and G. Achilli.** 1980. Serodiagnosis of respiratory syncytial virus infections in infants and young children by the immunoperoxidase technique. J. Clin. Microbiol. **11**:79–87.
25. **Glezen, W. P., and F. W. Denny.** 1973. Epidemiology of acute lower respiratory disease in children. N. Engl. J. Med. **288**:498–505.
26. **Glezen, W. P., A. Paredes, J. E. Allison, L. H. Taber, and A. L. Frank.** 1981. Risk of respiratory syncytial virus infection for infants from low-income families in relationship to age, sex, ethnic group, and maternal antibody level. J. Pediatr. **98**:708–715.
27. **Gray, K. G., D. E. MacFarlane, and R. G. Sommerville.** 1968. Direct immunofluorescent identification of respiratory syncytial virus in throat swabs from children with respiratory illness. Lancet **i**:446–448.
28. **Hall, C. B., R. G. Douglas, Jr., and J. B. Geiman.** 1980. Possible transmission by fomites of respiratory syncytial virus. J. Infect. Dis. **141**:98–102.
29. **Hall, C. B., J. T. McBride, C. L. Gala, S. W. Hildreth, and K. C. Schnabel.** 1985. Ribavirin treatment of respiratory syncytial viral infection in infants with underlying cardiopulmonary disease. J. Am. Med. Assoc. **254**:3047–3051.
30. **Hall, C. B., J. T. McBride, E. E. Walsh, D. M. Bell, C. L. Gala, S. Hildreth, L. G. Ten Eyck, and W. J. Hall.** 1983. Aerosolized ribavirin treatment of infants with respiratory syncytial viral infection. N. Engl. J. Med. **308**:1443–1447.
31. **Hall, C. B., K. R. Powell, N. E. MacDonald, C. L. Gala, M. E. Menegus, S. C. Suffin, and H. J. Cohen.** 1986. Respiratory syncytial virus infection in children with compromised immune function. N. Engl. J. Med. **315**:77–81.
32. **Hall, C. B., E. E. Walsh, J. F. Hruska, R. F. Betts, and W. J. Hall.** 1983. Ribavirin treatment of experimental respiratory syncytial viral infection. J. Am. Med. Assoc. **249**:2666–2670.
33. **Hemming, V. G., G. A. Prince, R. L. Horswood, W. T. London, B. R. Murphy, E. E. Walsh, G. W. Fischer, L. E. Weisman, P. A. Baron, and R. M. Chanock.** 1985. Studies of passive immunotherapy for infections of respiratory syncytial virus in the respiratory tract of a primate model. J. Infect. Dis. **152**: 1083–1087.
34. **Hendry, R. M., L. T. Pierik, and K. McIntosh.** 1986. Comparison of washed nasopharyngeal cells and whole nasal secretions for detection of respiratory syncytial virus antigens by enzyme-linked immunosorbent assay. J. Clin. Microbiol. **23**:383–384.
35. **Hendry, R. M., A. L. Talis, E. Godfrey, L. J. Anderson, B. F. Fernie, and K. McIntosh.** 1986. Concurrent circulation of antigenically distinct strains of respiratory syncytial virus during community outbreaks. J. Infect. Dis. **153**:291–297.
36. **Hierholzer, J. C., and M. S. Hirsch.** 1979. Croup and pneumonia in human infants associated with a new strain of respiratory syncytial virus. J. Infect. Dis. **140**:826–828.
37. **Hornsleth, A., N. Bech-Thomsen, and B. Friis.** 1985. Detection by ELISA of IgG-subclass-specific antibodies in primary respiratory syncytial (RS) virus infections. J. Med. Virol. **16**:321–328.
38. **Hornsleth, A., N. Bech-Thomsen, and B. Friis.** 1985. Detection of RS-virus IgG-subclass-specific antibodies: variation according to age in infants and small children and diagnostic value in RS-virus-infected small infants. J. Med. Virol. **16**:329–335.
39. **Hornsleth, A., E. Brenoe, B. Friis, F. U. Knudsen, and P. Uldall.** 1981. Detection of respiratory syncytial virus in nasopharyngeal secretions by inhibition of enzyme-linked immunosorbent assay. J. Clin. Microbiol. **14**:510–515.
40. **Hornsleth, A., B. Friis, P. Andersen, and E. Brenoe.** 1982. Detection of respiratory syncytial virus in nasopharyngeal secretions by ELISA: comparison with fluorescent antibody technique. J. Med. Virol. **10**:273–281.
41. **Hruska, J. F., P. E. Morrow, S. C. Suffin, and R. G. Douglas, Jr.** 1982. In vivo inhibition of respiratory syncytial virus by ribavirin. Antimicrob. Agents Chemother. **21**:125–130.
42. **Ida, S., J. J. Hooks, R. P. Siraganian, and A. L. Notkins.** 1977. Enhancement of IgE-mediated histamine release from human basophils by viruses: role of interferon. J. Exp. Med. **145**:892–906.
43. **Jordan, W. S., Jr.** 1962. Growth characteristics of respiratory syncytial virus. J. Immunol. **88**:581–590.
44. **Kapikian, A. Z., R. H. Mitchell, R. M. Chanock, R. A. Shvedoff, and C. E. Stewart.** 1968. An epidemiologic study of altered clinical reactivity to respiratory syncytial (RS) virus infection in children previously vaccinated with an inactivated RS vaccine. Am. J. Epidemiol. **89**:405–413.
45. **Kaul, A., R. Scott, M. Gallagher, M. Scott, J. Clement, and P. L. Ogra.** 1978. Respiratory syncytial virus infection: rapid diagnosis in children by use of indirect immunofluorescence. Am. J. Dis. Child. **132**:1088–1090.
46. **Kaul, T. N., R. C. Welliver, and P. L. Ogra.** 1981. Comparison of fluorescent-antibody, neutralizing-antibody, and complement-enhanced neutralizing-antibody assays for detection of serum antibody to respiratory syncytial virus. J. Clin. Microbiol. **13**:957–962.
47. **Kaul, T. N., R. C. Welliver, and P. L. Ogra.** 1982. Development of antibody-dependent cell-mediated cytotoxicity in the respiratory tract after natural infection with respiratory syncytial virus. Infect. Immun. **37**:492–498.
48. **Kaul, T. N., R. C. Welliver, and P. L. Ogra.** 1982. Appearance

of complement components and immunoglobulins on nasopharyngeal epithelial cells following naturally acquired infection with respiratory syncytial virus. J. Med. Virol. **9**:149–158.
49. **Kaul, T. N., R. C. Welliver, D. T. Wong, R. A. Udwadia, K. Riddlesberger, and P. L. Ogra.** 1981. Secretory antibody response to respiratory syncytial virus infection. Am. J. Dis. Child. **135**:1013–1016.
50. **Kim, H. W., J. O. Arrobio, C. D. Brandt, B. C. Jeffries, G. Pyles, J. L. Reid, R. M. Chanock, and R. H. Parrott.** 1973. Epidemiology of respiratory syncytial virus infection in Washington, D.C. I. Importance of the virus in different respiratory tract disease syndromes and temporal distribution of infection. Am. J. Epidemiol. **98**:216–225.
51. **Kim, H. W., S. L. Leikin, J. Arrobio, C. D. Brandt, R. M. Chanock, and R. H. Parrott.** 1976. Cell-mediated immunity to respiratory syncytial virus induced by inactivated vaccine or by infection. Pediatr. Res. **10**:75–78.
52. **Kim, H. W., R. G. Wyatt, B. F. Fernie, C. D. Brandt, J. O. Arrobio, B. C. Jeffries, and R. H. Parrott.** 1983. Respiratory syncytial virus detection by immunofluorescence in nasal secretions with monoclonal antibodies against selected surface and internal proteins. J. Clin. Microbiol. **18**:1399–1404.
53. **Lauer, B. A., H. A. Masters, C. G. Wren, and M. J. Levin.** 1985. Rapid detection of respiratory syncytial virus in nasopharyngeal secretions by enzyme-linked immunosorbent assay. J. Clin. Microbiol. **22**:782–785.
54. **Lenney, W., and A. D. Milner.** 1978. Alpha and beta adrenergic stimulants in bronchiolitis and wheezy bronchitis in children under 18 months of age. Arch. Dis. Child. **53**:707–709.
55. **McIntosh, K., R. M. Hendry, M. L. Fahnestock, and L. T. Pierik.** 1982. Enzyme-linked immunosorbent assay for detection of respiratory syncytial virus infection: application to clinical samples. J. Clin. Microbiol. **16**:329–333.
56. **McIntosh, K., H. B. Masters, I. Orr, R. K. Chao, and R. M. Barkin.** 1978. The immunologic response to infection with respiratory syncytial virus in infants. J. Infect. Dis. **138**:24–32.
57. **Meurman, O., O. Ruuskanen, H. Sarkkinen, P. Hanninen, and P. Halonen.** 1984. Immunoglobulin class-specific antibody response in respiratory syncytial virus infection measured by enzyme immunoassay. J. Med. Virol. **14**:67–72.
58. **Meurman, O., H. Sarkkinen, O. Ruuskanen, P. Hanninen, and P. Halonen.** 1984. Diagnosis of respiratory syncytial virus infection in children: comparison of viral antigen detection and serology. J. Med. Virol. **14**:61–65.
59. **Milner, M. E., S. M. de la Monte, and G. M. Hutchins.** 1985. Fatal respiratory syncytial virus infection in severe combined immunodeficiency syndrome. Am. J. Dis. Child. **139**:1111–1114.
60. **Mito, K., Y. Chiba, K. Suga, and T. Nakao.** 1984. Cellular immune response to infection with respiratory syncytial virus and influence of breast-feeding on the response. J. Med. Virol. **14**:323–332.
61. **Murphy, B. R., B. S. Graham, G. A. Prince, E. E. Walsh, R. M. Chanock, D. T. Karzon, and P. F. Wright.** 1986. Serum and nasal-wash immunoglobulin G and A antibody response of infants and children to respiratory syncytial virus F and G glycoproteins following primary infection. J. Clin. Microbiol. **23**:1009–1014.
62. **Peavy, D. L., C. N. Powers, and V. Knight.** 1981. Inhibition of murine plaque-forming cell responses *in vivo* by ribavirin. J. Immunol. **126**:861–864.
63. **Perrin, L. H., A. Tishon, and M. B. A. Oldstone.** 1977. Immunologic injury with measles virus infection. Part III. Presence and characterization of human cytotoxic lymphocytes. J. Immunol. **118**:282–288.
64. **Porter, D. D., K. B. Muck, and G. A. Prince.** 1980. The age dependence of respiratory syncytial virus growth in ferret lung can be shown in organ and monolayer cultures. Clin. Immunol. Immunopathol. **15**:415–423.
65. **Powers, C. N., D. L. Peavy, and V. Knight.** 1982. Selective inhibition of functional lymphocyte subpopulations by ribavirin. Antimicrob. Agents Chemother. **22**:108–114.
66. **Prince, G. A., R. L. Horswood, E. Camargo, D. Koenig, and R. M. Chanock.** 1983. Mechanisms of immunity to respiratory syncytial virus in cotton rats. Infect Immun. **42**:81–87.
67. **Prince, G. A., R. L. Horswood, and R. M. Chanock.** 1985. Quantitative aspects of passive immunity to respiratory syncytial virus infection in infant cotton rats. J. Virol. **55**:517–520.
68. **Prince, G. A., R. L. Horswood, D. W. Koenig, and R. M. Chanock.** 1985. Antigenic analysis of a putative new strain of respiratory syncytial virus. J. Infect. Dis. **151**:634–637.
69. **Prince, G. A., A. B. Jenson, V. G. Hemming, B. R. Murphy, E. E. Walsh, R. L. Horswood, and R. M. Chanock.** 1986. Enhancement of respiratory syncytial virus pulmonary pathology in cotton rats by prior intramuscular inoculation of Formalin-inactivated virus. J. Virol. **57**:721–728.
70. **Pullan, C. R., and E. N. Hey.** 1982. Wheezing, asthma, and pulmonary dysfunction 10 years after infection with respiratory syncytial virus in infancy. Br. Med. J. **284**:1665–1669.
71. **Richardson, L. S., R. H. Yolken, R. B. Belshie, E. Camargo, H. W. Kim, and R. M. Chanock.** 1978. Enzyme-linked immunosorbent assay for measurement of serological response to respiratory syncytial virus infection. Infect. Immun. **20**:660–664.
72. **Rosner, I. K., R. C. Welliver, P. J. Edelson, K. Geraci-Ciardullo, and M. Sun.** 1987. The effect of ribavirin therapy on respiratory syncytial virus-specific IgE and IgA responses following infection. J. Infect. Dis. **155**:1043–1047.
73. **Routledge, E. G., J. McQuillin, A. C. R. Samson, and G. L. Toms.** 1985. The development of monoclonal antibodies to respiratory syncytial virus and their use in diagnosis by indirect immunofluorescence. J. Med. Virol. **15**:305–320.
74. **Rutter, N., A. D. Milner, and E. J. Hiller.** 1975. Effect of bronchodilators on respiratory resistance in infants and young children with bronchiolitis and wheezy bronchitis. Arch. Dis. Child. **50**:719–722.
75. **Schauf, V., C. Purcell, M. Mizen, and S. Mizen.** 1979. Lymphocyte transformation in response to antigens of respiratory syncytial virus. Proc. Soc. Exp. Biol. Med. **161**:564–569.
76. **Scott, R., M. O. DeLandazuri, P. S. Gardner, and J. J. T. Owen.** 1976. Detection of antibody to respiratory syncytial virus by membrane fluorescence. Clin. Exp. Immunol. **26**:78–85.
77. **Scott, R., M. O. DeLandazuri, P. S. Gardner, and J. J. Owen.** 1977. Human antibody-dependent cell-mediated cytotoxicity against target cells with respiratory syncytial virus. Clin. Exp. Immunol. **28**:19–26.
78. **Scott, R., A. Kaul, M. Scott, Y. Chiba, and P. L. Ogra.** 1978. Development of *in vitro* correlates of cell-mediated immunity to respiratory syncytial virus infection in humans. J. Infect. Dis. **137**:810–817.
79. **Sims, D. G., M. A. P. S. Downham, P. S. Gardner, J. K. G. Webb, and D. Weightman.** 1978. Study of 8-year-old children with a history of respiratory syncytial virus bronchiolitis in infancy. Br. Med. J. **1**:11–14.
80. **Sullivan, J. L., R. E. Mayner, D. W. Barry, and F. A. Ennis.** 1976. Influenza virus infection in nude mice. J. Infect. Dis. **133**: 91–99.
81. **Sun, C. S., P. R. Wyde, S. Z. Wilson, and V. Knight.** 1983. Cell-mediated cytotoxic responses in lungs of cotton rats infected with respiratory syncytial virus. Am. Rev. Respir. Dis. **127**:460–464.
82. **Swenson, P. D., and M. H. Kaplan.** 1986. Rapid detection of respiratory syncytial virus in nasopharyngeal aspirates by a commercial enzyme immunoassay. J. Clin. Microbiol. **23**:485–488.
83. **Taber, L. H., V. Knight, B. E. Gilbert, H. W. McClung, S. Z. Wilson, H. J. Norton, J. M. Thurson, W. H. Gordon, R. L. Atmar, and W. R. Schlaudt.** 1983. Ribavirin aerosol treatment of bronchiolitis associated with respiratory syncytial virus infection in infants. Pediatrics **72**:613–618.
84. **Treuhaft, M. W., J. M. Soukup, and B. J. Sullivan.** 1985. Practical recommendations for the detection of pediatric respiratory syncytial virus infections. J. Clin. Microbiol. **22**:270–273.
85. **Walsh, E. E., C. B. Hall, M. Briselli, M. W. Brandriss, and J. J. Schlesinger.** 1987. Immunization with glycoprotein subunits of respiratory syncytial virus to protect cotton rats against viral infection. J. Infect. Dis. **155**:1198–1204.
86. **Waner, J. L., N. J. Whitehurst, S. Jonas, L. Wall, and H.**

Shalaby. 1986. Isolation of viruses from specimens submitted for direct immunofluorescence test for respiratory syncytial virus. J. Pediatr. **108**:249–250.

87. **Welliver, R. C., A. Kaul, and P. L. Ogra.** 1979. Cell-mediated immune response to respiratory syncytial virus infection: relationship to the development of reactive airway disease. J. Pediatr. **94**:370–375.
88. **Welliver, R. C., T. N. Kaul, and P. L. Ogra.** 1980. The appearance of cell-bound IgE in respiratory-tract epithelium after respiratory-syncytial-virus infection. N. Engl. J. Med. **303**: 1198–1202.
89. **Welliver, R. C., T. N. Kaul, T. I. Putnam, M. Sun, K. Riddlesberger, and P. L. Ogra.** 1980. The antibody response to primary and secondary infection with respiratory syncytial virus: kinetics of class-specific responses. J. Pediatr. **96**:808–813.
90. **Welliver, R. C., T. N. Kaul, M. Sun, and P. L. Ogra.** 1984. Defective regulation of immune responses in respiratory syncytial virus infection. J. Immunol. **133**:1925–1930.
91. **Welliver, R. C., M. Sun, D. Rinaldo, and P. L. Ogra.** 1985. Respiratory syncytial virus-specific IgE responses following infection: evidence for a predominantly mucosal response. Pediatr. Res. **19**:420–424.
92. **Welliver, R. C., D. T. Wong, M. Sun, E. Middleton, Jr., R. S. Vaughan, and P. L. Ogra.** 1981. The development of respiratory syncytial virus-specific IgE and the release of histamine in nasopharyngeal secretions after infection. N. Engl. J. Med. **305**:841–846.
93. **Wohl, M. E. B., and V. Chernick.** Bronchiolitis. Am. Rev. Respir. Dis. **118**:759–781.
94. **Wyde, P. R., R. B. Couch, B. F. Mackler, T. R. Cate, and B. M. Levy.** 1977. Effects of low- and high-passage influenza virus infection in normal and nude mice. Infect. Immun. **15**:221–229.