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Report of a Workshop on Respiratory Viral Infections: Epidemiology, Diagnosis, Treatment, and Prevention

Kenneth McIntosh, Pekka Halonen, and Olli Ruuskanen

From the Division of Infectious Diseases, Department of Pediatrics, The Children's Hospital, Harvard Medical School, Boston, Massachusetts; and the Departments of Virology and Pediatrics, University of Turku, Turku, Finland

An international workshop to review the epidemiology, diagnosis, treatment, and prevention of respiratory viral infections was held in Turku, Finland, in May 1991. This workshop emphasized the following points. (1) In epidemiological studies of influenza virus, serological, clinical, and gene-sequencing methods have been used to produce a full picture of genetic evolution. Less complete information exists about other viruses, although new data on respiratory syncytial virus are emerging. (2) Tools for the diagnosis of respiratory viral infections have been developed in conjunction with the use of solid-phase immunoassays. A role remains for tissue culture in surveillance and epidemiological studies. Detection of bacterial involvement in respiratory infections has been more difficult. (3) Treatment of infections due to respiratory viruses has advanced with the use of amantadine and aerosolized ribavirin. On the other hand, many viruses remain refractory to treatment. Means for preventing influenza are established, but barriers to the development of other viral vaccines—including the existence of multiple serotypes, imperfect natural immunity, and paradoxical hypersensitivity—have proven difficult to surmount.

Of all the common acute infectious diseases of humans, acute respiratory infections (ARIs) remain the most refractory to prevention and treatment. In every region of the world and in persons of all ages, upper respiratory tract infections, along with their complications of sinusitis and otitis, are the leading cause of acute infectious morbidity. Lower respiratory tract infections, particularly at the extremes of age, are among the most common reasons for hospitalization in Europe and North America [1]. In the developing world, acute respiratory infections—in particular, viral and bacterial pneumonia—rival diarrhea as the leading cause of death in children <5 years of age [2, 3]. Much progress has been made over the past several decades in elucidating the microbial causes of ARI and the epidemiology of many of its etiologic agents. The problem nevertheless remains enormous, and modes of diagnosis, treatment, and prevention have developed slowly.

In recognition of the global importance of ARI; of the necessity for interaction of investigators in the fields of molecular biology, epidemiology, diagnostic microbiology, clinical infectious diseases, the treatment of these diseases, and vaccine development; and of the recent rapid advances in some areas of research on ARI, a 2-day international workshop was convened in Turku, Finland, in May 1991 as a forum for the consideration and discussion of selected topics related to respiratory infections. The workshop was organized and supported by the Sigrid Juselius Foundation, the largest foundation in Finland supporting medical research. While the emphasis of the workshop was on the viral causes of ARI, the subject of ARI was considered as a whole, with particular emphasis on the interactions of viruses and bacteria in the causation and pathogenesis of pneumonia and other respiratory diseases.

The following review of selected topics covered during the workshop highlights those presentations describing new discoveries and a new synthesis of ideas. The participants at the workshop are listed at the end of the text.

Epidemiology of Respiratory Viral Infections

Although the classical epidemiology of respiratory viral infections has been carefully and thoroughly described in studies from many regions of the world, only influenza virus has been the subject of highly detailed antigenic and molecular analyses. These surveys, which have illuminated many features of the genetic evolution and spread of influenza virus, may provide important insights into the behavior of other respiratory viruses, including respiratory syncytial virus (RSV) and the parainfluenza viruses.

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Grant support: The Sigrid Juselius Foundation.
Reprints or correspondence: Dr. Kenneth McIntosh, Division of Infectious Diseases, The Children's Hospital, 300 Longwood Avenue, Boston, Massachusetts 02115.

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Influenza Virus

The description of the evolutionary behavior of both influenza A and influenza B viruses has been made possible through two types of analysis of influenza strains: rapid immunologic characterization of the HA1 protein by hemagglutination inhibition and detailed RNA analysis by oligonucleotide fingerprinting and sequencing. The combination of these two techniques has allowed epidemiologists to draw detailed maps of changes in both antigenicity and genetic composition over time and space.

Several useful generalizations can be drawn from data obtained over the past few decades. Type A influenza viruses show two patterns of global spread. The first pattern describes the spread of strains representing major antigenic shifts; for example, in the cases of H1N1 virus in 1977 and H3N2 virus in 1968, the virus first appeared in the Far East and within 6–9 months had spread to all corners of the globe. The second pattern describes the spread of strains representing significant antigenic drift; for instance, the new strain A/Beijing/353/89 appeared first in China, remained in that country for about 6 months, was found about a year later in the Southern Hemisphere, and in the following winter reappeared in the Northern Hemisphere. As part of this pattern, widespread activity is often introduced by a "herald wave," that is, the appearance of a new strain for a few weeks at the end of one respiratory season followed by extensive epidemic spread in the next [4]. Because such epidemic behavior is common and consistent, it appears likely that these strains somehow remain hidden in the population over the summer months rather than being carried to colder climates and then returning with travelers during the next respiratory season.

The evolution of influenza viruses is by no means as simple and predictable as was previously thought. To begin with, the epidemiological behavior of both A/Swine influenza strains since 1976 and A/H1N1 strains since 1977 has disproven beyond doubt the previously held dogma that an antigenically "new" strain inevitably causes a pandemic or, at the very least, displaces existing strains. Detailed analysis of A/H1N1 and B strains has further indicated that the evolution of influenza viruses is complex and nonlinear. For example, the A/H1N1 virus that appeared in 1977 was genetically almost identical to virus circulating in 1950. (This similarity is the basis for the suspicion, never proven, that the virus emerged from some research worker's freezer in China in 1977.) The evolution of the virus during the years after each of those two dates has been studied by RNA sequence analysis [5], and the evolutionary changes from 1950 to 1957 have turned out to be completely different from those occurring from 1977 to 1983 (figure 1). These changes also occurred more slowly during the latter period; this difference was attributed to the fact that the virus was subject to less antigenic "pressure" during this period, although this theory has not been proven.

Further evidence of complex evolution is that, in 1978–1979, viruses appeared that were proven by genetic analysis to be reassortants of H3N2 and H1N1 viruses, containing four RNA segments from each parent and bearing the H1N1 phenotype on the surface. These viruses circulated for only a few years and then disappeared, being survived by pure H1N1 viruses. Similar reassortants between H1N1 and H3N2 influenza A strains have emerged on other occasions. Additional studies have shown that neither A nor B strains of influenza virus necessarily evolve along a single path. There is clear evidence of branching and therefore of cocirculation of evolutionarily different strains of both influenza A/H1N1 virus [6] and influenza B virus [7]. A recent example related to the latter virus was the coexistence in Europe during 1990 of influenza viruses B/Yamashita/88 and B/Finland/90; these two viruses had evolved separately from a common parent dating back to 1983 [7].

It appears paradoxical that, on the one hand, influenza viruses are genetically unstable and constantly mutating and evolving, while, on the other, "evolutionarily successful" strains can travel throughout the world undergoing no—or, at most, a very few—changes in the base sequence of the HA gene. To what extent these two apparently opposing tendencies are influenced by chance, the error rate of the RNA polymerase, or antigenic pressure is not clear.

As has been mentioned, many of the principles delineated for influenza virus may also apply to other respiratory viruses. Information is emerging that will probably reveal to what extent they apply to RSV and the parainfluenza viruses. RSV has been the subject of the most extensive studies in this regard. Advances have been slow since, in contrast to that of influenza virus, antigenic analysis of RSV with polyclonal sera is difficult, reassortment is (of course) impossible, and sequencing (at least up to the present) has been difficult.

Respiratory Syncytial Virus

RSV clearly differs clinically and pathogenetically from influenza virus in ways that may be important to its epidemiology and genetic evolution. First, reinfection is very common, even with strains that are antigenically closely related or identical. Second, infection takes place even in the presence of maternal antibody, although high titers clearly can modify infection or even prevent it entirely. Third, it seems likely from existing data that the mode of spread is through fomites or large-particle aerosols [8], probably resembling that of rhinoviruses [9] rather than that of influenza virus, which can be transmitted by droplet nuclei [10]. These characteristics may translate into slower spread of RSV than of influenza virus (which can cause explosive epidemics under the right circumstances) and into a pattern of genetic evolution that is less governed by the need for antigenic change in order to escape from immunity in the population.

The antigenic variability of RSV has now been well

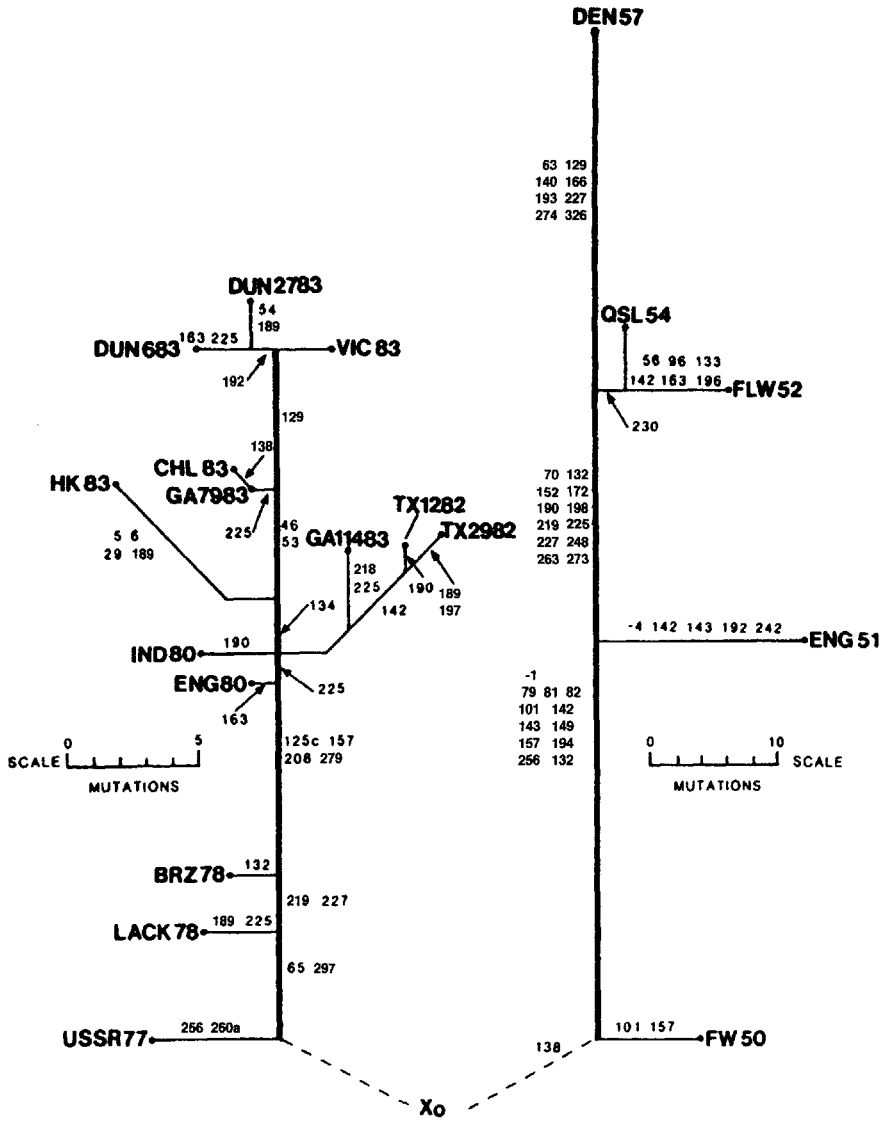

Figure 1. The divergent evolutionary pathway of the 1950–1957 and 1977–1983 strains of influenza A virus, as deduced from the nucleotide changes encoding the HA1 domain of the hemagglutinin. The two main branches are drawn to different scales and derive from the common precursor X_0. Distances between strains are the minimal mutational distances, including both coding and silent changes. Numbers refer to the amino acid residues that become "fixed" in all subsequent strains when these residues are on the mainstream (*bold line*) or to strain-specific amino acid residues (*branches*). (Reprinted with permission from [5].)

shown, largely through the analysis of strains with monoclonal antibodies. The two antigenic subgroups, A and B, differ primarily in the surface glycoprotein responsible for attachment (the G protein). Although the amino acid sequences of the G proteins of members of these two subgroups are very different (only 53% identical) and although these proteins are recognized as very different antigenically by the immune systems of both humans and rodents [11, 12], neutralizing antibody after primary infection in infants "sees" the two strains as 31% related [12]. The antigenic diversity of the two RSV subgroups probably has some influence on the susceptibility of infants to sequential RSV infection [13]; reinfection by members of the opposite subgroup is more likely than homologous reinfection. On the other hand, this effect of antigenic diversity probably does not last beyond the first reinfection, and repeated infections with either subgroup can and do occur throughout life [14].

Thus, in contrast to what has been observed for influenza virus, the influence of immunity on the larger epidemiology of RSV and on the circulation of strains of subgroups A and B may not be strong; temporary immunity in large populations may be responsible for the sometimes-acute nature of seasonal epidemics of RSV infection, with spread through a community and apparent "exhaustion of susceptibles." In addition, in some countries (Finland being the best example), there have recently been discernible patterns of epidemics that alternate between the A and B subgroups, with 2-year cycles [15]. As first described by Carlsen et al. in Norway [16], RSV has appeared in double-humped outbreaks, the first hump of the cycle representing a smaller outbreak in the late spring (of 1981, 1983, 1985, etc.) and the second hump representing a larger outbreak beginning in the autumn of that same year and extending through February or March of the next year (1982, 1984, 1986, etc.). In Finland [15] both humps of each cycle have been predominantly (but not exclusively) either subgroup A or subgroup B, with alternation at 2-year intervals.

On the whole, however, the epidemic pattern of the A and

Table 1. Predominant RSV subgroups in different geographic areas.

Epidemiological year*	Predominant subgroup of RSV in indicated location [reference]†							
	Boston, MA [17]	St. Louis, MO [19]	Hunting, WV [18]	Sapporo, Japan [20]	Montevideo, Uruguay [21]	Newcastle, UK [22]	Rochester, NY [23]	Turku, Finland [15]
1974–1975						A + B		
1975–1976						A	A	
1976–1977						A	B	
1977–1978						A + B	A	
1978–1979			A			A + B	A	
1979–1980			A			A + B	A + B	
1980–1981			A + B	A		A	A	
1981–1982	A	A	A			A + B	A + B	A + B
1982–1983	A	A	A	B		A	A	B
1983–1984	A + B	A + B	A			A	A	A + B
1984–1985	A + B	A + B	B	B	B	A + B	A + B	A
1985–1986	A	A	A	B	A	A + B	A	A
1986–1987	B		A	A	A	A + B	B	B
1987–1988			A			A	A	B
1988–1989							A + B	A + B
1989–1990							A	A

* Usually October through May.
† A = >75% subgroup A; B = >75% subgroup B; A + B = each subgroup, 25%–75%.

B subgroups elsewhere (for example, in the United States and northern England) is less regular than has been observed in Finland. In any one outbreak in any one location, if a sufficiently large number of strains are analyzed (i.e., more than 75), both A and B strains are found; this observation suggests the type of cocirculation of antigenically different strains that has been observed for influenza virus in recent years [15, 17–23]. Moreover, although there is a tendency for the number of predominantly subgroup A outbreaks to exceed the number of predominantly subgroup B outbreaks, the sequence of subgroup predominance in any one area (except Finland) is apparently random (table 1). No Scandinavian countries other than Finland have been studied in this regard. The epidemiological behavior of RSV subgroups suggests that, at least outside Scandinavia, immunologic forces exert somewhat less control over seasonal patterns than is the case with influenza virus.

The most detailed genetic analysis of RSV to date is that by Storch and colleagues [24] using RNA fingerprinting by base-pair mismatch and RNase protection. These interesting studies have shown widespread variation of the G protein gene among strains obtained from a single outbreak in a single geographic area. On the other hand, strains that are clearly epidemiologically related (for example, isolated from members of a family or from patients in a small nosocomial outbreak) have identical G protein genes. Moreover, identity has been described in a few instances among strains that apparently are not epidemiologically related, either in time or in space. Thus, as with influenza virus, there appears to be a paradox of genetic stability on the one hand and genetic instability on the other.

Laboratory Diagnosis of Respiratory Viral Infection

Microbial diagnosis of ARIs has traditionally depended on the detection either of the microbe during the course of the illness or of a rise in antibody titer in acute- and convalescent-phase sera; recently, the former methodology has been strongly emphasized. Diagnostic technology has moved more rapidly for viral than for bacterial respiratory infections; both methodologies are clearly important—most of all in pneumonia. While this review emphasizes viral diagnosis, recent studies have introduced new methods for the detection of bacterial infections, and these methods will be addressed briefly in the section on clinical aspects of respiratory infections.

Diagnostic Confirmation by Culture

For the detection of viruses in clinical samples obtained from the respiratory tract, there seems little doubt that the use of an aspirate of nasopharyngeal secretions is most efficient. This statement holds for all methods presently available and essentially for all viruses, with the possible exception of adenovirus.

Despite advances in antigen detection, culture for virus remains an important standard procedure that is essential in large, full-service diagnostic laboratories. The reasons are many: culture is the only dependable system for detection of many viruses, including rhinoviruses; culture must be used as the reference method for evaluation of the specificity and sensitivity of all newer methods; and isolates are frequently needed for further genetic or biochemical characterization.

The most important recent advance in culture methodology has been the brief centrifugation of cells and specimens to enhance sensitivity, short incubation for viral multiplication and antigen development, followed by staining with monoclonal antibodies. The technique was first developed for rapid diagnosis of infection due to herpes simplex virus [25], but, with the recent availability of monoclonal antibodies to multiple respiratory viruses, it has been shown to be more widely applicable. Its advantages are a clear increase in sensitivity, the early availability of test results (24–48 hours), and the possibility for further subtyping with type-specific (herpes simplex types 1 and 2) or subgroup-specific (RSV subgroups A and B) monoclonal antibodies [15, 26].

Immunofluorescence for Rapid Antigen Detection

Of all methods for rapid antigen detection, immunofluorescence was the first to be developed and remains one of the most useful. Monoclonal reagents are now available for many respiratory viruses, and immunofluorescence is frequently the most convenient rapid diagnostic technique for laboratories that process a limited number of respiratory aspirate specimens on a daily basis. A further advantage of the immunofluorescence technique over other, newer methods for antigen detection is that it permits continuous evaluation of the quality of specimens and of the handling of these specimens. In addition, if properly stored, slides can be reviewed at a later date to verify the accuracy of diagnosis.

Recent extensive experience in laboratories in the developing world has demonstrated that, with suitable training and quality control, immunofluorescence techniques can be applied directly to nasopharyngeal secretions for the detection of several respiratory viruses, particularly RSV and parainfluenza virus type 3, with adequate sensitivity and specificity [27].

Improved microscopes, filters, and light sources have increased both the sensitivity and the specificity of immunofluorescence and have greatly reduced the time required to train microscopists in the technique. Nevertheless, even with the best monoclonal conjugates, careful testing of each new batch of reagents is necessary, and proper controls must be employed in each test. Even experienced technologists may sometimes require retraining when new monoclonal antibodies are introduced since the pattern of fluorescence may change with alterations in epitope specificity.

Solid-Phase Immunoassays for Rapid Antigen Detection

As in the field of immunofluorescence, monoclonal antibodies have had a profound effect on the development of solid-phase immunoassays for the detection of antigens in ARIs. Recent advances have been particularly applicable to the techniques of enzyme immunoassay (EIA) and time-resolved fluoroimmunoassay (TR-FIA). The benefits of these technologies are obvious: bulk testing is possible in laboratories where large numbers of specimens are handled daily; specimens can be transported over long distances since no intact cells or intact viral particles are required and the soluble viral proteins reactive in the tests are relatively stable; advantage is taken of the large amounts of excess viral antigen produced in infected epithelial cells in most respiratory viral infections; and reading of the print-out results on paper is simple and rapid and requires only limited training. On the other hand, quality control remains of paramount importance and is rendered more difficult by the lack of morphological criteria (such as those that are available to the immunofluorescence microscopist) and the apparent simplicity of the method. Very high standards must be maintained in dealing with all reagents, including the water used to make solutions, and washing devices and spectrophotometers must be checked and serviced regularly.

Monoclonal one-step TR-FIAs have now been developed for the detection of respiratory viruses [28–32] and are the most sensitive and convenient solid-phase assays presently available. Their simplicity and speed derive from the fact that the specimen is incubated simultaneously—and for just 1 hour—with both capture antibody and europium chelate–labeled indicator antibody. Monoclonal one-step biotin EIAs have similar clinical sensitivity and can detect almost the same number of positive specimens as TR-FIAs, but their capacity to measure very small amounts of purified viral protein is often 10 times lower than the capacity of similarly designed TR-FIAs. In addition, biotin EIAs require overnight incubation of the specimen with solid-phase capture antibody.

One-step TR-FIAs have been used in Finland for many years for the detection of seven respiratory viruses: RSV; influenza A and B viruses; parainfluenza virus types 1, 2, and 3; and adenoviruses. The rate of positive specimens overall has been 20%–25%; during RSV and influenza epidemics, it has risen to nearly 50%. At the Centers for Disease Control in Atlanta, TR-FIAs have been developed for echovirus 70 and coxsackievirus A 24var; together with TR-FIAs for adenoviruses, these assays can be used to detect the etiologic agents of hemorrhagic conjunctivitis [31]. In addition, similar assays are now available for the detection of parainfluenza virus types 4A and 4B and coronaviruses 229E and OC43 in respiratory infections [31]. For the present, the use of these TR-FIAs is limited to large reference laboratories because of the expense of the required equipment.

Nucleic Acid Detection

With such extraordinarily sensitive methods for detection of the antigens of many respiratory viruses, it is unlikely that —at least for these viruses—nucleic acid detection methods will be widely used. Nevertheless, for some viruses, antigen detection is not possible because of the multiplicity of sero-

types. When, in addition, culture is difficult or time-consuming, genome detection becomes a much more attractive alternative. Rhinoviruses are a case in point.

Nucleic acid hybridization has a sensitivity of 10^5–10^6 target molecules; however, when the polymerase chain reaction is used to expand the available material, this sensitivity can be lowered to fewer than 10 original target molecules. Primers can be constructed within the 5′ noncoding region of rhinoviruses for group-reacting assays that will detect all rhinoviruses as well as all enteroviruses or picornaviruses [33, 34]. Sandwich hybridization in solution and affinity capture onto solid phase in microtiter wells have been developed to increase the sensitivity of hybridization assays [35], and TR-FIA technology can be used for the detection of nonradioactive hybrids with high sensitivity [36].

One other potential application of nucleic acid techniques for the detection of viruses involves the identification of one or several nucleotide changes determining important phenotypic features, such as antiviral resistance. Either minisequencing [37] or the polymerase chain reaction with labeled, sequence-specific probes may be used in such studies; both techniques have been employed directly with clinical samples in the study of the resistance of human immunodeficiency virus to drugs [38].

Serological Methods

There will always be a need for serological techniques in the study of respiratory infections, both because antigen detection and culture are of value only during the acute phase of the illness and because no single technique is 100% sensitive. In addition, serological diagnosis remains useful for the evaluation of new diagnostic methods and for trials of vaccines and antiviral drugs in which the total number of infections, including subclinical infections, must be determined.

In respiratory infections the EIA for IgG-specific antibodies in paired serum specimens is the most sensitive serological method and is superior to the complement fixation test. All serological results, however, depend on the quality of the antigens used, and in this regard recombinant protein expression holds great promise for the future. Current examples of such proteins are those developed for the serological diagnosis of human immunodeficiency virus and rubella virus infections [39, 40]. Recombinant nucleoprotein of influenza A virus has been produced in *Escherichia coli* and successfully used for the EIA detection of antibody to influenza virus [41]. Expression of influenza A and B virus nucleoprotein in baculovirus has also shown potential in the serodiagnosis of influenza [42]. In light of the high specificity of these reagents, it seems likely that similar antigens for other respiratory viruses will prove equally valuable in the future.

Clinical Aspects of Respiratory Infections

The association of the various respiratory viruses with acute respiratory syndromes in children has been the subject of numerous studies, and most of the patterns are well established. The role of rhinoviruses in more serious disease of the lower respiratory tract, which was described several decades ago [43], has become increasingly clear in recent studies [44–48]. While this role is not universally accepted and cannot be definitively proven without more extensive sampling of the lower respiratory tract, the sheer number of relevant studies demands that further attention be given to these very common viruses as causes of pneumonia in children.

Viral-Bacterial Interactions

The role of bacteria in many acute respiratory syndromes, particularly in children, and the interrelationships of bacteria and viruses have been confusing and controversial. The syndromes of otitis media, sinusitis, and pneumonia are all believed to be commonly initiated by viral infection but to be frequently or occasionally complicated by bacterial coinfection or superinfection. The most controversy has surrounded pneumonia since direct sampling of the lung and bronchioles for bacteria is difficult. In contrast, the middle ear is more readily cultured, and otitis media represents a possible model for viral-bacterial interactions in respiratory infection.

Studies of acute otitis media have indicated that middle-ear fluid contains culturable bacteria in 50%–70% of cases. In addition, at the time of diagnosis, more than 90% of patients with otitis have symptoms of upper respiratory tract infection, probably virally induced. Between 40% and 50% of patients have detectable virus in the nasopharynx at this time [49], although viruses are found in the middle-ear fluid in only 17% of cases and are cultured in the absence of accompanying bacteria in only 6% [50]. Two recent studies have suggested that virus in the middle ear may complicate the response to antibiotics and account for apparent failures of treatment [51, 52].

A similarly prominent role for bacteria in pneumonia has been described by investigators studying hospitalized children in the developing world; this bacterial prominence is supported by the results of cultures of lung aspirates from children with severe, often life-threatening illness. The subject has been reviewed by Pio et al. [53], by Berman and McIntosh [54], and by Shann [55]. When viruses have been studied, they have been detected in an average of 23% of children, whereas bacteria have been isolated from lung aspirates from an average of 63% of children with previously untreated cases [56]. The organisms most commonly isolated from lung aspirates have been the same as those isolated from the middle ear—namely, *Streptococcus pneumoniae* and *Haemophilus influenzae* (both typable and nontypable strains); the occasional isolates of other bacteria have in-

cluded *Staphylococcus aureus*, gram-negative enteric bacilli, and *Moraxella catarrhalis*.

Clearly, lung aspiration must be reserved for special circumstances. These descriptions of pneumonia in children from the developing world are considered to represent such a special situation in terms of selection for severity and (possibly) of important but poorly understood risk factors, such as malnutrition, crowding, vitamin A deficiency, and exposure to environmental pollutants [57]. However, recent studies using more indirect measures of bacterial infection have raised important questions concerning the possible prominence of bacterial infection in pneumonia among children with less severe disease and among children of Europe and North America. These investigations have used a combination of antigen detection in respiratory secretions, serum, and/or urine and measurement of antibody conversion in serum. For the latter purpose, various bacterial antigens have been used, including crude lysates of nontypable *H. influenzae* or *M. catarrhalis* [58], purified pneumolysin from *S. pneumoniae* [59], and mixtures of purified pneumococcal polysaccharides [60].

The precise validity of these indirect measures of bacterial infection has not been easy to assess. In one recently published study of pneumonia in the Gambia [61, 62], culture-proven bacterial infections were frequent, and—most important—a high proportion of infants underwent lung aspiration as well as measurement of pneumococcal and *Haemophilus* antigens in serum and urine and titration of antibody responses to pneumococcal pneumolysin, *H. influenzae*, and *M. catarrhalis*. Because *S. pneumoniae* was frequently cultured from the lungs or the blood in this unusual population, this study probably represents one of the best opportunities thus far for the validation of some of the recently developed indirect tests for pneumococcal infection in childhood pneumonia.

Some evidence for pneumococcal involvement was found in 45 (61%) of 74 children between 1 year and 9 years of age who had severe or very severe pneumonia, with positive cultures of blood or lung aspirates in 17 cases. In fact, however, the agreement among different tests for *S. pneumoniae* was disappointingly weak, as is shown in the Venn diagram in figure 2. Nevertheless, it appears from other studies that when these techniques are applied by experienced laboratories, their association with illness of some sort is strong; that is, the results are very rarely positive when the tests are applied to samples from healthy children. For this reason, although further validation still is clearly needed, it is important to consider carefully the results of these studies, applying them to evaluations of community-acquired pneumonia and other acute lower respiratory syndromes in children and adults.

Data from the published studies of pneumonia in children are shown in table 2 [44, 48, 61–71]. Many of these studies were performed in Scandinavian countries, and further application of the methods in other locations, with further correlations between laboratory and clinical data, are needed. The findings raise the possibilities that bacterial invasion occurs during the course of childhood pneumonia more commonly than has been previously thought and that in pneumonia, as in otitis media, secondary bacterial infection may develop commonly in the wake of viral infection.

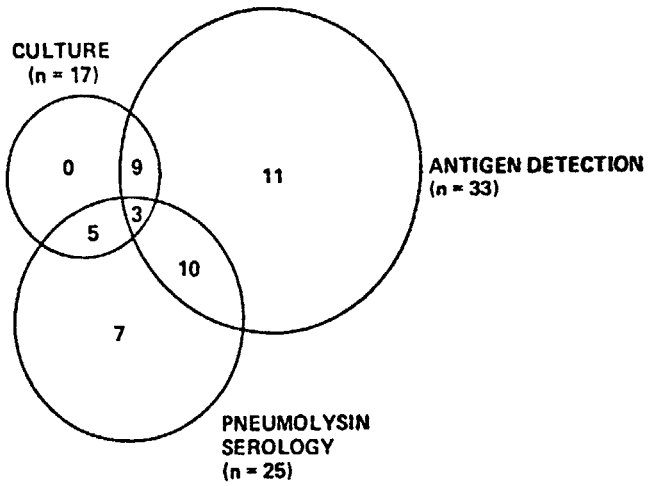

Figure 2. Venn diagram showing interrelationships of different methods used to diagnose infection with *S. pneumoniae* in Gambian children hospitalized with severe, acute infection of the lower respiratory tract. Paired sera for the measurement of antibodies to pneumococcal pneumolysin were not available from 15 patients. (Reprinted with permission from [62].)

Treatment and Prevention

Both the treatment and the prevention of respiratory viral infections are fields still in their infancy despite the availability of moderately protective influenza vaccines since the early 1940s and the licensure of amantadine, a highly active and minimally toxic anti-influenza drug, in the United States for almost 25 years. Research on both drugs and vaccines to be used against respiratory viruses has been active and intense during this time.

Treatment of respiratory viral infections. The greatest successes in chemotherapy for respiratory viral infections have been with amantadine and related drugs against influenza [72] and with ribavirin against RSV infection [73]. Yet even in these instances there have been major difficulties, in the first case because of the development of viral resistance and in the second because of environmental concerns.

The most striking aspects of the use of amantadine and the closely related drug rimantadine in treatment of influenza A are, on the one hand, the potent activity of these agents against influenza A viral strains [74] and, on the other, the rapid and predictable emergence of drug-resistant viruses after only 2–3 days of treatment [75]. The mechanism of

Table 2. Etiology of lower respiratory tract infections in children.

Year [reference]	No. of patients	Viral techniques	Bacterial techniques*	Percentage of patients with indicated isolate			
				Viruses	Bacteria	Mixed (viruses and bacteria)	Potential pathogen
1984 [44]	102	Isolation, RSV antigen, CF serology	HIB, *Streptococcus pneumoniae* antigen (CIE); pertussis tests; *Chlamydia trachomatis* culture, serology	74	38	27	85
1986 [63]	62	Isolation, CF serology	HIB, *S. pneumoniae* antigen (LA, CA); *Mycoplasma pneumoniae* culture, serology	33	29	8	48
1987 [64]	98	Isolation, CF serology, RSV antigen	HIB, *S. pneumoniae* antigen (CIE)	39	19	10	48
1989 [65]	167	Antigen detection,† CF serology, RSV IgG	*S. pneumoniae*, HIB, *M. pneumoniae*, *C. trachomatis*, ntHI, *Legionella* serology	29	22	2	49
1989 [66]	51	Isolation, antigen detection, CF serology	ntHI, *Moraxella catarrhalis* serology; *S. pneumoniae* antigen (LA)	100	. . .	37	. . .
1990 [67]‡	204	Isolation, antigen detection	HIB, *S. pneumoniae* antigen (CIE)	36	13	5	41
1990 [68]‡	1,492	Isolation, antigen detection	. . .	37	35	15	51
1990 [69]‡	1,003	Isolation, antigen detection, CF serology	*M. pneumoniae*, pertussis culture	30	9	2	37
1991 [70]	195	Antigen detection, CF serology	*S. pneumoniae*, ntHI, *M. catarrhalis* serology; *S. pneumoniae*, HIB antigen (LA)	35	32	17	50
1991 [71]	121	Antigen detection, EIA serology	*S. pneumoniae*, ntHI, *M. catarrhalis*, *Chlamydia pneumoniae* serology; *S. pneumoniae*, HIB antigen (LA); *M. pneumoniae* EIA, RNA	46	45	20	69
1991 [61]	90	Isolation, CF serology, RSV EIA serology	*S. pneumoniae*, ntHI, *M. catarrhalis*, *M. pneumoniae*, *C. trachomatis*, *C. pneumoniae* serology; *S. pneumoniae*, HIB antigen (CIE, LA); *C. trachomatis* isolation	49	30	15	69
1991 [62]	74	Isolation, CF serology, RSV EIA serology	*S. pneumoniae*, ntHI, *M. catarrhalis*, *M. pneumoniae*, *C. trachomatis*, *C. pneumoniae* serology; *S. pneumoniae*, HIB antigen (CIE, LA); *C. trachomatis* isolation	34	77	24	81
1992 [48]	50	Isolation, antigen detection, EIA serology	*S. pneumoniae*, ntHI, *M. catarrhalis*, *C. pneumoniae* serology; *S. pneumoniae*, HIB antigen (LA); *M. pneumoniae* EIA, RNA	60	62	34	88

NOTE. Abbreviations: CF = complement fixation; HIB = *Haemophilus influenzae* type b; CIE = counterimmunoelectrophoresis; LA = latex agglutination; CA = coagglutination; ntHI = nontypable *H. influenzae*.

* All studies included bacterial cultures of blood and pleural fluid.

† Antigens of RSV; adenovirus; parainfluenza virus types 1, 2, and 3; and influenza A and B viruses were sought.

‡ Studies were conducted in a developing country.

resistance is related to the function of the M2 protein, one of several transmembrane proteins, and to changes in single amino acid residues of this protein at position 27, 30, or 31 [76, 77]. Resistant virus is, unfortunately, fully infectious and pathogenic [78]. For some reason this phenomenon does not appear to inhibit the drug's beneficial effect on the course of the disease in the treated patient. It does, however, abolish prophylactic efficacy in situations where the both the index case and susceptible contacts are treated, since resistant virus spreads from index to contacts and causes breakthrough disease.

In spite of the fully virulent properties of amantadine-resistant influenza viruses, they have never been found in nature or in any situation except those clearly related to the recent use of amantadine or rimantadine. Amantadine-resistant strains of influenza A virus are also resistant to rimantadine and to other new antiviral agents with similar multiple-ring structures. Such strains, however, are still fully susceptible to ribavirin, which has been shown to be beneficial when administered as an aerosol to influenza virus–infected patients [79].

In contrast to the strong activity of amantadine and rimantadine against influenza viruses, ribavirin possesses only moderate activity against the important respiratory viruses [80].

Nevertheless, when delivered by aerosol, ribavirin reaches high levels in the respiratory secretions—levels that are inhibitory in vitro to the growth of a wide range of respiratory viruses, including influenza A and B viruses, RSV, and parainfluenza viruses. Aerosolized ribavirin shows good activity against influenza virus and RSV in murine models [81, 82] as well as a measurable and useful beneficial effect against either influenza or RSV infection in humans [73, 83].

Resistance to ribavirin has not been described thus far, possibly because, at levels required to inhibit the growth of virus in vitro, this drug is thought to have more than one mechanism of action [84]. There has recently been concern, however, about the environmental hazards of the aerosolized preparation since, under the conditions of use in mist tents or oxygen hoods, easily measurable quantities of drug are found in the surroundings of the treated patient [85]. Nevertheless, with few exceptions, investigations of health-care workers have failed to detect the drug in either blood or urine. Proper barrier methods and scavenger devices can successfully control environmental contamination, and most experts still recommend the use of ribavirin for infants and children with demonstrated RSV infection who have underlying risk factors for severe or fatal disease or whose disease has progressed to the point of impending respiratory failure [86]. Moreover, recent evidence favors the use of ribavirin (when environmental concerns are diminished because of containment by the mechanical ventilatory system) for infants with RSV infections who have been intubated and ventilated for apnea or respiratory failure [87].

Investigations of the treatment of rhinoviruses have included numerous trials of antiviral compounds as well as attempts to modify the response of the host. Certain antiviral agents have exhibited promising activity both in vitro and in vivo—notably, WIN 51711 (Sterling Winthrop) and R61837 (Janssen Pharmaceuticals), both of which probably function by binding to the receptor-interacting portion of the viral surface [88]. Studies in volunteers have shown that, if R61837 is to have significant effects on clinical symptoms and shedding of virus, it must be administered by intranasal aerosol beginning very soon after inoculation of virus and continuing for prolonged periods [89]. R61837 also stimulates the emergence of resistant viruses [90]. Interferons α and β were shown early on to have antiviral activity, but both the requirement for very large doses and local irritant effects have limited their usefulness [91]. In contrast, interferon γ actually exacerbates symptoms, perhaps through its capacity to up-regulate the production of ICAM 1, the receptor for most serotypes of rhinovirus [92].

In controlled trials a number of possible modifiers of the host response have been disappointing in their effects; these agents include NPC 567, a bradykinin antagonist [93], and certain drugs designed to enhance local immunity, such as muramyltripeptide-dipalmytoylphosphatidyl ethanolamine (MTP-PE) and 7-thia-8-oxoguanosine [94, 95], neither of which has a discernible effect on the course of experimental rhinovirus infection. Corticosteroids [96], nedocromil (which inhibits mast cell degranulation) [97], and oral zinc [98] have all had marginal effects on the course of induced infection of the upper respiratory tract. Controlled trials of vitamin C have been disappointing [99].

Respiratory viral vaccines. Prevention of respiratory viral infections through the use of vaccines has also been difficult. While both inactivated and live attenuated influenza vaccines have been successful (albeit underused), it has been problematical to apply the information gathered from their success to the other respiratory viruses, including RSV and the parainfluenza viruses. The inactivated influenza vaccines are solidly protective in healthy adults, with prevention rates of >90% when the epidemic strain closely matches the strain included in the vaccine. In practice, however, protective efficacies have been considerably lower, in part because the target populations are frequently either elderly adults [100] or very young children [101] and in part because circulating strains of influenza virus frequently differ significantly from the vaccine strains used.

After much experimentation, live attenuated influenza vaccines have finally been honed to the point that they should soon be manufactured and licensed for general use. Empirical attenuation through cold adaptation produced "master strains" with multigenic (and therefore presumably stable) attenuation [102]. The segmented RNA genome of influenza virus then permitted the relatively simple transfer of the genes promoting attenuation (in this instance, the RNA segments coding for the PA, PB1, PB2, and M proteins) to viruses bearing the hemagglutinin and neuraminidase of prevalent strains; the result is a vaccine with the desired characteristics of antigenicity, infectiousness, and attenuation [103].

The transfer of this technology to either RSV or parainfluenza viruses has been difficult. Natural infections of infants with these viruses, particularly RSV and parainfluenza virus type 3, immunize poorly, probably considerably less well than those with influenza virus [104, 105]. This characteristic does not augur well for the efficacy of live attenuated vaccines—at least those that, like measles, mumps, and rubella vaccines, are administered in a single dose. Moreover, to protect against most severe illness, vaccines would have to be used in early infancy, i.e., at a time when both maternal antibody and the immaturity of the immune system would likely blunt the efficacy of attenuated viruses, inactivated viruses, or purified antigens [106]. Nevertheless, a significant goal may be merely to prevent or lessen the severity of the first infection in early childhood, and attenuated vaccines may have a role in achieving this goal.

In a broader sense, however, to confer solid immunity, it appears that a successful vaccine will have to improve on nature itself. Fortunately, there is ample precedent for this type of effect in several bacterial vaccines, including tetanus

toxoid and the newer conjugate haemophilus vaccines, which, when administered during early infancy, clearly induce immunity superior to that which follows natural infection.

The technological obstacles encountered in work with paramyxoviruses are also great. Reassortment is not possible in the absence of a segmented genome, and this powerful tool is therefore unavailable for the analysis and manipulation of viral genes. Moreover, the resources of genetic engineering through cDNA intermediates have remained out of reach because of the difficulty of initiating infection with nucleic acid information alone.

The experience with RSV vaccines has also been discouraging. Early efforts to develop an inactivated vaccine resulted in the now widely recognized production of paradoxically severe RSV infections in vaccinees exposed to wild virus [107]. It appears likely that the surface antigens of the virus had been altered by formalin treatment or by other manipulations in a manner that stimulated a combination of poorly functional antibody and excess T cell (possibly T-helper cell) reactivity [108, 109]. Attenuated RSV vaccines have also failed, in part because of genetic instability [110] and in part because of their (predictable) failure to provide protection after a single dose [111].

Present research on inactivated or component RSV and parainfluenza virus vaccines is focused on the development of products containing antigens preserved in a form as close as possible to their native state. In practice, this goal has meant the purification of surface antigens with minimal manipulation or the insertion of surface protein genes in various vectors (such as enterically replicating adenoviruses) that might make acceptable live vaccine products. Because research on influenza and other viruses is continuing to shed light on the mechanism of attenuation and because it is likely that manipulation of the genes of parainfluenza viruses and RSV will eventually become a reality, live attenuated vaccines will almost surely emerge over time for use either alone or in combination with component or inactivated vaccines.

Another promising approach, as has already been mentioned, is to use inactivated or component vaccines with the limited aim of protecting infants for only the first few critical months of life, when they are most vulnerable to severe disease of the lower respiratory tract. This aim could theoretically be achieved by immunization of mothers during late pregnancy. Immunization of household members might be undertaken as well. Such an effort might not only produce partial immunity in early infancy but also lessen the likelihood of transmission of infection to infants from family members.

Because of the recognized difficulty of developing vaccines and because natural immunity to several respiratory viruses (including RSV) can be attained through the acquisition by the infant of high titers of maternally transferred IgG antibody, there is interest in protecting selected high-risk infants through administration of high-titered immune serum globulin. This alternative to active immunization circumvents the difficulties of working with very young infants as well as the problems of inducing a balanced and protective immune response through injections of extracted (and possibly therefore altered) viral antigens. While final results from the relevant trials are not yet available, this approach is promising. In the future, it appears likely that highly active and pure antibodies will be produced by recombinant techniques, allowing prophylaxis—and possibly treatment—with very small quantities of total antibody protein.

Conclusion

Although much progress has been made over the last decade in the epidemiological investigation, diagnosis, treatment, and prevention of respiratory viral infections, there still is clearly much to do. These infections remain among the most common in both children and adults and in both the developed and the developing world, exacting an enormous toll in terms of human disease and misery, mortality, and economic burden. Many of the fruits of the application of modern biology to this field have yet to be enjoyed. Despite advances in the diagnosis of viral involvement during lower respiratory tract illness, the role of bacteria in common respiratory infections still requires further and more accurate description. In addition, neither practical and economical treatments nor modes of prevention through vaccination have emerged from the many epidemiological studies, clinical trials, and investigations of laboratory and animal models of infections with the major viral pathogens, the possible exception being influenza. We look forward to many further advances in this important field.

Appendix: Participants in the Workshop on Respiratory Viral Infections

Pertti Arstila, Department of Virology, University of Turku, Turku, Finland; Heikki Arvilommi, National Public Health Institute, Turku; James D. Cherry, Department of Pediatrics, UCLA School of Medicine, Los Angeles, California; Robert B. Couch, Department of Microbiology and Immunology, Baylor College of Medicine, Houston, Texas; Floyd W. Denny, Department of Pediatrics, School of Medicine, University of North Carolina, Chapel Hill, North Carolina; Elliot C. Dick, Department of Preventive Medicine, University of Wisconsin/Madison Medical School, Madison, Wisconsin; Monica Grandien, Department of Virology, National Bacteriological Laboratory, Stockholm, Sweden; Karl-Otto Habermehl, Institute for Clinical and Experimental Virology, Free University of Berlin, Berlin, Germany; Pekka Halonen, Department of Virology, University of Turku; Maurice W. Harmon, Connaught Laboratories, Inc., Swiftwater, Pennsylvania; Frederick G. Hayden, Department of Internal Medicine, University of Virginia, Charlottesville, Virginia; John C. Hierholzer, Centers for Disease Control, Atlanta,

Georgia; Tapani Hovi, National Public Health Institute, Turku; Veijo Hukkanen, Department of Virology, University of Turku; Timo Hyypiä, Department of Virology, University of Turku; Alan P. Kendal, Centers for Disease Control; Hilkka Kettinen, Department of Virology, University of Turku; Igor Kharitonenkov, Ivanovsky Institute of Virology, Academy of Medical Sciences USSR, Moscow, USSR; Christian Kunz, Institute of Virology, University of Vienna, Vienna, Austria; Pauli Leinikki, National Public Health Institute, Helsinki, Finland; Erik Lycke, University of Göteborg, Göteborg, Sweden; C. R. Madeley, Department of Virology, University of Newcastle, Newcastle upon Tyne, United Kingdom; Kaija Maher, Centers for Disease Control; Helena P. Mäkelä, National Public Health Institute, Helsinki; Kenneth McIntosh, Division of Infectious Diseases, The Children's Hospital, Boston, Massachusetts; Jussi Mersola, Department of Pediatrics, University of Turku; Olli Meurman, Department of Virology, University of Turku; Jukka Nikoskelainen, Department of Medicine, University of Turku; Hanna Nohynek, National Public Health Institute, Helsinki; Erling Norrby, Department of Virology, Karolinska Institute, Stockholm; Pearay L. Ogra, Department of Pediatrics, The University of Texas, Galveston, Texas; Nils Oker-Blom, Sigrid Juselius Foundation, Helsinki; Ivor Ørstavik, Department of Virology, National Institute of Public Health, Oslo, Norway; Heikki Peltola, Department of Pediatrics, University of Helsinki; Anne Putto-Laurila, Department of Pediatrics, University of Turku; Marjut Ranki, Orion Pharmaceutical Biotechnology, Helsinki; Olli Ruuskanen, Department of Pediatrics, University of Turku; Petri Ruutu, Department of Medicine, Helsinki University Central Hospital; Aimo Salmi, Department of Virology, University of Turku; Theodor Scheinin, Sigrid Juselius Foundation; David A. J. Tyrrell, Public Health Laboratory Service, Centre for Applied Microbiology and Research, Porton Down, Salisbury, United Kingdom; Matti Uhari, Department of Pediatrics, University of Oulu, Oulu, Finland; Raija Vainionpää, Department of Virology, University of Turku; Timo Vesikari, Department of Virology, University of Tampere, Tampere, Finland; Matti Waris, Department of Virology, University of Turku; Thedi Ziegler, Department of Virology, University of Turku; Barry Ziola, Department of Microbiology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

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