CLINICAL MICROBIOLOGY REVIEWS, Apr. 1994, p. 143–151
0893-8512/94/$04.00+0

Vol. 7, No. 2

Mixed Viral Infections: Detection and Management

JOSEPH L. WANER*

Section of Infectious Diseases, Department of Pediatrics, University of Oklahoma Health Sciences Center and The Children's Hospital of Oklahoma, Oklahoma City, Oklahoma 73190

INTRODUCTION

The occurrence of multiple virus infections in the same patient is, intuitively, not surprising and is not an unusual finding. Multiple virus infections associated with primary or secondary immunodeficiencies are relatively common. A latent infection, such as that following a primary infection with a human herpesvirus, in conjunction with other latent infections or with an acute infection with another virus also defines categories of mixed viral infections. More perplexing is the meaning of mixed, acute viral infections that occur at the same site in immunocompetent patients and involve viruses with similar expressions of disease. The incidence of such mixed infections, their clinical importance, and whether they warrant particular diagnostic or therapeutic considerations are unresolved questions. To focus on these questions, viruses of the respiratory tract were emphasized. Although multiple infections of the gastrointestinal tract are well recognized (3, 4, 72, 113), the methods of diagnosis and knowledge of pathogenesis are limited compared with those for the common respiratory viruses. The ability of viruses to be immunosuppressive and thereby affect the host relative to other viral infections is also well recognized (90) but was not considered in this review.

Respiratory virus infections are the most numerous of acute viral infections. Several viruses that cause respiratory diseases circulate concurrently in the autumn, winter, and early spring (7, 14, 29–31). There is a tendency, however, for epidemic outbreaks of respiratory viruses not to occur simultaneously in a given population (28, 30, 31). Other viruses, such as herpes simplex virus (HSV) and cytomegalovirus (CMV), are generally not considered as respiratory agents but may occur in the respiratory tract. Additionally, enteroviruses enter the host by way of the mouth and may replicate initially in associated tissues. They are often overlooked, however, as agents of respiratory disease.

* Mailing address: Pediatric Infectious Diseases, University of Oklahoma Health Sciences Center, P.O. Box 26901, Oklahoma City, OK 73190. Phone: (405) 271-5703. Fax: (405) 271-3967. Electronic mail address: JOSEPH_WANER@UOKHSC.EDU.

MIXED ACUTE VIRAL INFECTIONS IN IMMUNOCOMPETENT PATIENTS

Clinical and Epidemiologic Studies

Multiple virus infections occurring at the same site in immunocompetent patients are rarely searched for and are usually detected by serendipity, such as the simultaneous recovery of strains of influenza A and C from a patient in 1951 (66). Most of the early virus studies were oriented to associating clinical findings with particular viruses with the goal of establishing viral etiologies for the diseases (23, 39, 41, 46, 73, 75). The majority of the investigations employed serology as well as virus isolation. For example, Parrott et al. reported that 30% of patients with respiratory syncytial virus (RSV) also had serological evidence of another simultaneous viral infection (73). A similar study of 667 cases of respiratory disease noted a 5% dual infection rate as discerned serologically by rises in antibody titer; of 34 patients studied by virus isolation, 4 (11%) yielded two different viruses (39). In looking for an association of rhinovirus infection with lower respiratory disease, one report presented serological evidence showing that 38% of the study population had a concomitant viral infection (76); the authors noted the difficulty of interpreting such data when assigning an etiologic role for rhinoviruses. Portnoy et al. commented subsequently that concomitant infections in patients with respiratory disease may be relatively frequent and may thus create uncertainty regarding the assigning of an etiologic role to a particular virus (75). That study specifically addressed the question of multiple infections but failed to isolate more than one virus from any patient. As in previous reports, however, serological evidence suggested multiple infections. Serodiagnostic rises in titer to two viruses occurred in 14% of patients with lower respiratory disease and in 13% of patients with upper respiratory disease (75). In the absence of

virus isolations, however, there was no direct evidence of simultaneous infection. Technical difficulties also compromised the diagnostic efforts. Experience with cell cultures and the ability to identify virus isolates differed between laboratories. Different cell cultures were often used, which affected the sensitivity of isolating different viruses. Additionally, rapidly growing viruses such as HSV or certain enteroviruses would prevent detection of more slowly replicating viruses, such as parainfluenza or influenza viruses. Wolontis et al. noted reports of mixed viral infections in other studies (28, 91). They reported a mixed infection rate of 35% in what appeared to be simultaneous epidemics of parainfluenza type 1 and influenza (112). Importantly, they commented that the use of a battery of cell cultures for isolation and holding the cultures for 3 weeks were important considerations for identifying mixed infections; from the same nasopharyngeal swab of one patient, they isolated adenovirus type 2, mumps virus, and parainfluenza viruses type 1 and 3 in three different cell lines.

Clinical Implications

Portnoy et al. did not observe differences in the duration of hospitalization between patients with dual infections and those with a single virus (75). Nor did Maletzky et al. find clinical differences between patients infected with one and those infected with two viruses (55). Nichol and Cherry, however, noted that patients with bronchitis or pneumonia more frequently grew multiple organisms than did patients with infections of the upper respiratory tract; dual viral infections were prominent in patients with bronchitis while mixed viral-bacterial infections predominated in patients with pneumonia (68). A total of 14 mixed viral infections were identified out of 69 respiratory cases (20%) by serology and viral isolation; only 4% of a comparable control group without respiratory illness had evidence of mixed viral infections. The relative contributions of serology and virus isolation to the study were not indicated (68). Miller et al. commented that they did not observe as many dual infections as Nichol and Cherry, possibly because their patients had milder illnesses (61).

Foy et al. noted the incidence of dual infections by serology and virus isolation in 10% of pneumonia cases, 17% of bronchiolitis cases, and 5% of croup cases; the data did not discern between mixed viral infections and a viral-*Mycoplasma pneumoniae* infection; nor was there an attempt to compare degrees of illnesses (23). Similarly, Hall et al. reported dual infections with viruses in 4% of RSV-infected patients but emphasized the significance of coinfections with bacteria (35).

A single mixed infection of RSV and influenza A was reported in a concurrent outbreak of RSV and influenza in a nursing facility for the elderly; the patient developed pneumonia as did five patients with influenza only (56). A report of nine dual isolations of RSV and influenza A virus noted that there was no unusual severity of disease seen in the patients with dual infections; the same degree of illness could be seen in infections with either virus alone (33). Valenti et al. noted similar clinical findings in patients infected with rhinovirus or RSV (102).

The use of nonculture techniques for diagnosis of viral infections did not add to the knowledge base regarding the role of mixed infections in disease. Respiratory viruses were isolated and/or identified by immunofluorescence (IF) in necropsy material of 24 children who died of respiratory disease; only 1 case (4%) was a dual infection, parainfluenza and influenza (15). Meissner et al. employed viral isolation and direct immunofluorescence (DIF) of cells obtained from nasal wash specimens to study a simultaneous outbreak of RSV and parainfluenza virus type 3 (PIV 3) in a newborn nursery (59). Of 14 patients yielding virus, 2 had dual infections with RSV and PIV 3. These infants could not be distinguished clinically, however, from infants infected with RSV or PIV 3 alone.

Tristram et al. used similar methods and identified four RSV patients coinfected with adenoviruses and one RSV patient coinfected with CMV (101). Three of the four RSV-adenovirus patients died of respiratory failure. Importantly, all four of the patients had serious underlying problems that may have enhanced the likelihood of severe complications from the dual infections. In comparison, adenovirus was associated with 6 of 20 fatal cases reported in a 3-year survey of acute lower respiratory disease in 805 children. Three of the cases involved a mixed adenovirus-bacterial infection, and three were cases of adenovirus alone; both categories of infection occurred in patients with underlying conditions (6). Considering these reports together, the relative threat of an adenovirus-mixed viral infection in an immunocompetent subject can not be discerned.

A retrospective study reported that 7.6% of 666 specimens with RSV (detected by isolation and/or DIF) also contained another virus; six additional dual infections not involving RSV were also noted. No significant difference in the severity of disease was seen, however, in a case control study of patients with RSV alone versus patients with RSV and another virus (93). A prospective study involving 1,246 healthy infants also found no difference in the severity of disease in cases of mixed infection versus cases of RSV infection alone (82). The latter study concluded that when RSV is detected in previously healthy infants, there is no clinical reason to search for other concomitant viral infections.

Studies of respiratory viral infections in developing countries also reported mixed viral infections (13, 26, 43, 70, 77, 79). The incidence (<10%) was comparable to that reported in studies from industrialized countries (discussed below). Nor was there any indication of increased severity of disease. Rather, the role of mixed viral and bacterial infections was emphasized.

In summary, evidence for an association of mixed infections with increased severity of disease was not forthcoming. The primary intent of the majority of the studies was to associate agents with clinical syndromes relative to age and sex of the patients. In most instances, little more than anecdotal experiences with mixed infections were provided. Much of the data were derived from serological studies with few dual organism isolations reported. There were few instances of observations of clinical severity, and where noted, the reports did not indicate any increased severity that might be attributed to mixed infections.

MIXED VIRAL INFECTIONS IN IMMUNODEFICIENT PATIENTS

The virus-host relationship changes in the immunodeficient patient. Viruses that are normally latent in the immunocompetent host may reactivate and cause acute disease in the immunodeficient host; viral infections that are normally suppressed by the immune system may become chronic infections; and generally, the severity of disease resulting from a viral infection is likely to be greater in immunodeficient patients.

In a study of children with acute lymphoblastic leukemia, 20 of 99 (20%) patients had multiple viruses isolated from the same specimen (5); significantly, only 20 of 796 (2.5%) control patients (no malignant disease or immunosuppressive therapy) had similar mixed infections (5, 12). When HSV was excluded, only three mixed infections were identified in healthy children. However, 17 mixed infections were identified in the leukemic

group. CMV was a coisolate in six of the leukemic children but in none of the control children. The multiple isolations from leukemic patients accounted for a significantly larger proportion of mixed infections than those from normal patients ($P = <0.001$), suggesting that the leukemic children were more susceptible to mixed viral infections. Similarly, Hall et al. reported that 6 of 47 (12.8%) immunocompromised children with RSV had a mixed infection (34). Jarvis et al. noted that 3 of 12 patients with severe combined immunodeficiency had dual respiratory virus infections (47).

There was an indication of increased incidence of mixed infections in immunocompromised patients. The severity of respiratory disease in the patients with mixed infections, however, appeared comparable to that of single infections in patients with similar underlying diseases. The increased incidence may be attributable to the underlying condition of the patients. Immunocompromised patients excrete virus longer than immunocompetent patients (12, 21, 34, 47). Latent viruses, such as HSVs and CMV, are commonly reactivated and may produce chronic infections (40, 108, 109). Adenovirus infections also become prolonged and may result in serious disease (54, 116). The immunocompromised patient, therefore, represents a diagnostic dilemma for correlating laboratory findings with clinical presentation.

The above analysis indicates the need for an increased awareness of mixed infections in immunocompromised patients. Although there is no evidence that mixed infections result in enhanced disease, any single virus is likely to cause increased morbidity and/or mortality over that caused by the same virus in immunocompetent patients. Rapid diagnosis to identify all of the viruses present, therefore, becomes more critical for timely treatment with antiviral agents. Acyclovir is available for HSVs and varicella-zoster virus, ganciclovir is available for CMV; amantadine is available for influenza A; ribavirin is available for RSV; and foscarnet is available for CMV retinitis and, in some circumstances, for disseminated CMV infection. Use of immune modulating agents, such as interferon and lymphokines, also requires a specific and rapid diagnosis. Additionally, there should be an awareness of the impact of diagnosis of mixed viral infections on the pharmacology of antiviral agents. Vogt et al., for example, reported antagonism between ribavirin and azidothymidine (104).

THE INCIDENCE AND NATURE OF MIXED VIRAL INFECTIONS

Rapid Methods of Viral Diagnosis

The advent of nonculture methods for the rapid diagnosis of viral infections provided opportunities for assessing the occurrence and importance of mixed viral infections. Analogous to earlier investigations that were intended to establish etiologies for clinical syndromes, however, the studies of rapid methods for diagnosis were not intended to identify mixed viral infections. The fact that the technique under evaluation required isolation in cell culture or the use of other established techniques for confirmation of the rapid method resulted in data that contained incidences of mixed infections. The two prominent rapid methodologies studied were DIF (58) and enzyme immunoassay (EIA) (8, 16, 85, 86, 114). More recently, the shell vial technique, which was originally described for the rapid identification of CMV (27), has been used for the diagnosis of respiratory viruses (18, 49, 57, 69, 78, 89).

Data reporting only on immunocompetent patients were examined for the occurrence of mixed viral infections and for the identity of the viruses in the mixtures (1, 5, 8, 9, 13, 15, 23, 24, 28, 35, 38, 39, 41, 43, 45, 46, 50, 52, 56, 57, 59, 63, 70, 71, 74, 77–79, 81, 92–95, 97, 99, 105–107, 112). Pediatric and adult patients constituted the populations studied. No effort was made to distinguish outpatients from hospitalized patients. Mixed infections were categorized as two or more viruses identified in the same specimen by isolation in cell culture and/or antigen detection.

TABLE 1. Analysis of published reports of the occurrence of mixed virus infections

No. (%) of reports (n = 38)	% Specimens with >1 virus	
	Range	Avg
24 (63.1)	1–5	3.4
7 (18.4)	5–10	7.2
5 (13.1)	10–15	12.8
1 (2.6)	15–20	20.0
1 (2.6)	>20	35.0

Incidence. Percent mixed infection was calculated by dividing the number of specimens containing two or more viruses by the total number of specimens yielding viruses. The analysis revealed that 63.1% of the reports identified between 1 and 5% mixed viral infections (average, 3.4%), 18.4% reported mixed infections in 5 to 10% of cases, and 13.1% reported mixed infections in 10 to 15% of cases (Table 1). One study reported mixed infections in 19% of cases (94), and one study observed greater than 35% mixed infections in patients with viral infections (112); the total number of positive specimens in these studies was small, 21 and 17, respectively. The latter study was conducted during a simultaneous outbreak of parainfluenza and influenza.

Composition of mixed infections. Two hundred fifty-one mixed infections were identified in the literature reviewed (Table 2). RSV, influenza viruses, adenoviruses, and parainfluenza viruses occurred in 82.4, 26.3, 25.1, and 23.5%, respectively, of the mixed infections. The combination of RSV and influenza was most common, occurring in 25.6% of the mixed infections identified.

Differential diagnosis. The articles on rapid testing were further examined for the number of viruses identified other than the one targeted by the rapid procedure. Data were retrieved from 23 reports (1, 8, 9, 24, 36–38, 42, 45, 48–50, 60, 62, 74, 77, 78, 94, 97, 99, 105–107). In 13 studies, 15 to 41% of

TABLE 2. Analysis of 251 mixed virus infections

Virus(es) identified in mixed infections	No. (%) of occurrences in mixed infections	No. (%) of occurrences with:	
		RSV	Other viruses
RSV	207 (82.4)		207
Influenza viruses	66 (26.3)	53 (25.6)	13[a]
Adenoviruses	63 (25.1)	39 (18.9)	24[b]
Parainfluenza viruses	59 (23.5)	40 (19.3)	19[c]
Enteroviruses	34 (13.5)	27 (13.0)	7
CMV	34 (13.5)	29 (14.0)	5
Rhinoviruses	22 (8.8)	16 (7.7)	6
HSV	13 (5.2)	3 (1.4)	10[d]
Mumps	2 (0.8)	0 (0)	2

[a] Includes six adenoviruses.
[b] Includes six influenza viruses, seven parainfluenza viruses, and six HSVs.
[c] Includes seven adenoviruses and four enteroviruses.
[d] Includes six adenoviruses.

TABLE 3. Analysis of published reports in which specimens were positive for a virus other than the virus targeted by a specific rapid technique

No. of reports (n = 23)	% Positive specimens	
	Range	Avg
3	5–10	9.2
7	10–15	13.3
1	15–20	17.0
8	20–25	22.8
4	27–41	34.3

the specimens that were not reactive for the targeted virus yielded other viruses (Table 3).

The identification of other viruses was possible because multiple diagnostic methods were used in the studies, primarily isolation in cell culture or DIF. If the additional methods were not employed, the other viruses would not have been detected. This analysis demonstrated the need for a comprehensive strategy to identify both the mixed infections and, more importantly, the viruses present in specimens that would be considered negative if only one virus was targeted for diagnosis.

DIAGNOSTIC CONSIDERATIONS

This review has focused on multiple viruses that are identified in the same clinical specimen. Simultaneous viral infections, however, often occur in different body sites in the immunocompromised patient. Both categories of mixed infections require the necessary laboratory resources to effect a comprehensive diagnosis. Virus isolation in cell cultures, identification of antigen directly in specimens, and centrifugation culture (shell vial technique) are the common methods that are most applicable to the problem.

Diagnostic Strategies

Strategies for the rapid diagnosis of multiple viral infections, whether from the same specimen or from specimens originating from different anatomic sites, must take into account both feasibility and cost; a major component of cost is hands-on time. The problem of diagnosis of multiple virus infections with cell cultures was addressed by Hsuing over 25 years ago (44). A single cell culture is rarely useful for the identification of multiple viruses (Fig. 1); even if it is permissive for several viruses, one agent, such as HSV, may overgrow another. Wolontis et al. advocated the use of multiple cell cultures and the prolonged maintenance of them after inoculation (112). Recently, Fong and Landry presented data obtained prospectively to support the proposition that multiple cell cultures should be employed with other techniques to maximize the recovery of viruses from one specimen (22); the strategy is equally amenable to diagnosis of multiple viruses from different anatomical sites. Conventional isolation procedures, however, are time-consuming. Use of hemadsorption (65) or IF (92) methods before the appearance of a cytopathic effect has been advocated as an adaptation to routine cell culture to speed the rapid identification of isolates in cell cultures.

The shell vial technique combines the specificity of isolation in cell culture with the rapid diagnostic capabilities of IF. The method is particularly useful when good specimens for DIF can not be obtained. Like conventional isolation techniques, however, multiple cell lines are required to permit an adequate differential diagnosis. The use of multiple cell cultures and conventional handling of specimens for inoculation and IF staining makes the procedure labor intensive.

In the studies reporting >5% mixed infections, viruses were detected by isolation in cell culture, cell culture and DIF, or cell culture and EIA (Table 1). Mixed infections were found in only two of seven studies in which the shell vial technique was used for identification of respiratory viruses. In these studies, the mixed infection rate was 1% (57) and 3% (78). Additional experience is needed to fully evaluate the shell vial technique and its potential for detecting multiple viruses.

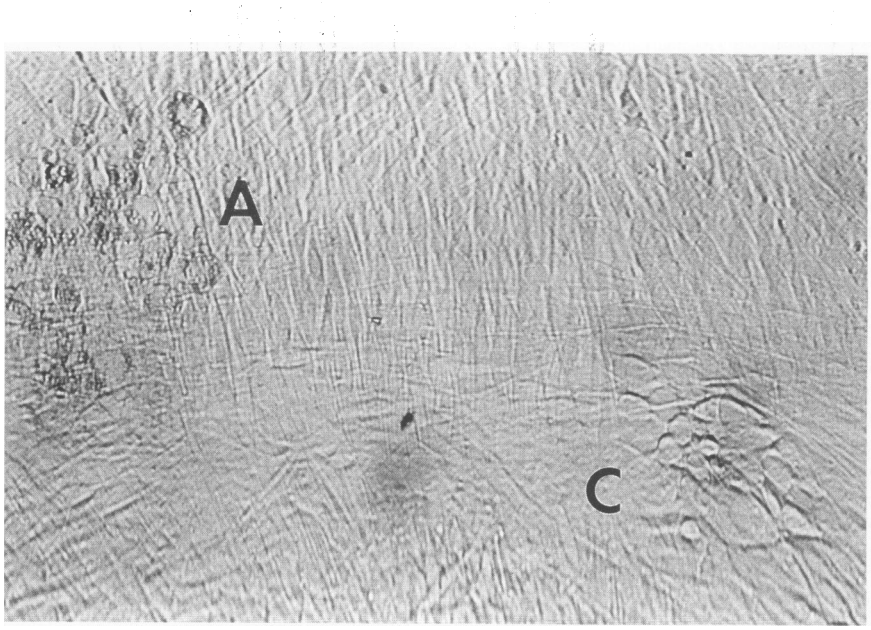

FIG. 1. Cytopathic effects of adenovirus (A) and CMV (C) in a culture of MRC-5 cells inoculated with a urine specimen.

DIF permits rapid diagnosis of multiple viruses in the same specimen. Cells obtained from a nasopharyngeal wash or aspirate, bronchial lavage, or lesion may be apportioned to numerous circumscribed wells on glass slides and stained by IF for as many viruses as the availability of cells and reagents permits (81, 106). The viruses present are thereby identified in their natural host cell(s). Preventing the interaction of two or more viruses in a cell culture eliminates the possibility of interference or interferon production, either of which could compromise a diagnosis. Currently, DIF provides the optimum requirements for rapid and cost-effective analysis of multiple infections. Good monoclonal antibodies for the common viral pathogens and accompanying anti-immunoglobulin conjugates are available commercially, and the initial investment in a fluorescence microscope is modest.

The available EIA tests are targeted for a few important agents but are not available commercially as panels for differential diagnosis. EIA tests for most viruses, however, can be constructed in the laboratory, and comprehensive panels may be assembled. The general experience suggests that to account for their variable sensitivities and specificities EIA tests should be supported by virus isolation in cell cultures and/or DIF (36, 37, 49, 74, 94, 95, 97, 107).

Future Prospects

An expanded use of cell cultures for diagnosis will require the identification of novel cell lines with enhanced abilities to support the replication of common viral pathogens and noncultivable viruses. Genetically engineered cell lines, such as 293 cells for adenoviruses 40 and 41 (96), could be developed for use as centrifugation cultures (shell vials) to provide comprehensive and rapid diagnosis of the common and fastidious agents.

Recent presentations have reported a rapid immunofluorescence adaptation to DIF that permits a diagnosis in 20 min, a time comparable to that of the rapid EIA tests (64, 100). Otherwise, DIF seems to have reached its limit of performance and remains very dependent on the quality of specimen collected and the acquired skill of the laboratory staff.

The newest adaptations to EIA have resulted in tests that produce results in 20 min (95, 105, 107). As discussed above, commercial EIA tests are focused on individual agents. Unit panels of EIA tests for the common viruses (such as a respiratory virus panel) with sensitivities and specificities comparable to those of DIF are necessary for comprehensive diagnostic screening.

Detection of viral nucleic acids provides the specificity of a genomic sequence and permits identification of a latent virus (111). The relative merits of nucleic acid detection versus antigen detection, however, are controversial (115). The procedural aspects of either hybridization on filters or hybridization in solution are not readily amenable to routine diagnosis. The sensitivity of the reaction is also dependent on the amount of viral nucleic acid present in the specimen. Additionally, radioisotope-labeled probes in hybridization formats are not practical for routine use, and nonisotope probes often do not provide the desired sensitivity. Molecular strategies for detection of multiple viruses in a single specimen have not been pursued.

Application of PCR to the detection of nucleic acids circumvents the problems of sensitivity and source of specimen (2, 84, 87). Unique segments of the target nucleic acid are amplified to obtain quantities that are easily detected by nonisotopic methods. Theoretically, multiple primer pairs directed against different viruses could be included in a single reaction mixture to amplify the various viral nucleic acids that may be present. In practice, however, different primer pairs often require specific optimum conditions that require individual reaction mixtures. A provisional diagnosis may be made in one working day, but confirmation usually requires an additional day. New variations of PCR permit rapid amplification that can be completed in minutes (110). A provocative suggestion has been to use PCR in a diagnostic panel by spotting any number of known nucleic acids onto a membrane strip to bind with amplified products (53).

MIXED VIRAL INFECTIONS IN SINGLE CELLS

Individual cells may also experience a mixed virus infection (Fig. 2) in which two infecting viruses simultaneously enact part or all of their replicative cycles (103). These infections may prove to be more clinically relevant than mixed infection of the same organ system or simultaneous infections of multiple organ systems. Findings concerning heterologous transactivation of viruses suggested that a dual infection of the same cell may have important implications for the host (98). For example, in a cell culture model of HSV latency, infection with CMV induced the synthesis of infectious HSV (11). Subsequently, it was reported that the product of the major immediate-early gene of a porcine virus, pseudorabies, activated transcription of human adenovirus genes that are normally activated by the immediate-early gene products of adenovirus (20). Thus, it was recognized that a gene product from one virus may function analogously, in *trans*, as the like product of an unrelated virus.

Coinfection with some human herpesviruses can affect human immunodeficiency virus (HIV) gene expression (25, 32, 80); certain viral antigens also stimulate cytokine production which in turn can up-regulate expression of HIV type 1 (HIV-1) (10). These findings raise the question of whether such dual infections may be associated with reactivation of latent HIV. Transactivation of HIV genes requires a dual infection. Given the common association of several viruses and HIV with lymphoid tissue, the opportunity for coinfections appears considerable (17, 51, 83).

There is evidence that dual infections of single cells occur in patients. CMV and HIV-1 were shown to coinfect brain cells (67) and retinal cells in patients with AIDS (88). In the absence of CMV, the HIV-1-infected cells within the neural retina were not associated with inflammation or necrosis (88). The rapid diagnosis of mixed infections, therefore, may be important for rapid initiation of treatment to diminish the likelihood of dual infections of individual cells.

PERSPECTIVE

Publications on respiratory virus infections were reviewed to evaluate the significance of multiple viruses found in the same clinical specimen. The review focused on immunocompetent patients and certain immunocompromised patients. A salient feature was the lack of controlled studies involving patients with dual viral infections in comparison with patients with single virus infections. The majority of reports did not identify mixed infections or reported them in the context of the total data accumulated.

The literature survey showed that 63% of the articles with data on mixed infections identified them in <5% of the total number of viral infections. RSV occurred in 82.4% of all mixed infections, with RSV and influenza being the most common virus pair identified. Of importance was the coincident finding of the effect of focusing on only one virus for diagnosis. A total

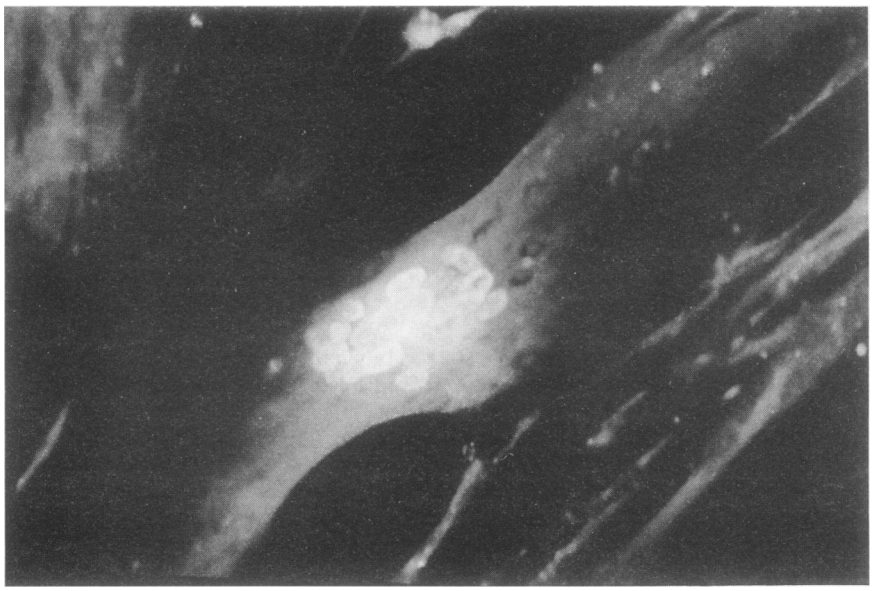

FIG. 2. Immunofluorescent stain of nuclei showing CMV immediate-early antigen and contained in RSV-induced syncytium. A nasal wash specimen was inoculated into a shell vial centrifugation culture of MRC-5 cells and stained 72 h later. Photo courtesy of Christine Robinson.

of 87% of the studies with available data identified a virus in >10% of the specimens that were negative for the virus targeted by one technique; 56.5% of the reports recognized a virus in >15% of negative specimens.

An assessment of the available reports did not indicate that mixed infections, per se, served as a clinical illness promotion factor (19) by functioning additively or synergistically in causing disease. Dual infections by certain viruses of the same cell, however, may function as a clinical illness promotion factor at the molecular level to reactivate latent infections and/or to initiate or stimulate a disease process. The clinical impact of the latter findings has yet to be determined.

A comprehensive strategy of diagnosis is necessary for (i) the diagnosis of mixed infections, (ii) the diagnosis of viral infections occurring at different anatomical sites, and (iii) the achievement of a differential diagnosis if one virus is targeted for rapid diagnosis by a single method. This approach is also relevant for choosing the appropriate antiviral agent and for monitoring its efficacy. Future directions in methodology, whether based on antibody or nucleic acid probes, should consider these requirements.

ACKNOWLEDGMENT

I gratefully acknowledge Christine Robinson, The Children's Hospital, Denver, Colo., for providing the photograph of the dual infection of RSV and CMV (Fig. 2).

REFERENCES

1. **Blanding, J. G., M. G. Hoshiko, and H. R. Stuttman.** 1989. Routine viral culture for pediatric respiratory specimens submitted for direct immunofluorescence testing. J. Clin. Microbiol. **27**:1438–1440.
2. **Bloch, W.** 1991. A biochemical perspective of the polymerase chain reaction. Biochemistry **30**:2735–2747.
3. **Brandt, C. D., H. W. Kim, W. J. Rodriguez, J. O. Arrobio, B. C. Jeffries, and R. H. Parrott.** 1986. Simultaneous infections with different enteric and respiratory tract viruses. J. Clin. Microbiol. **23**:177–179.
4. **Brandt, C. D., H. W. Kim, W. J. Rodriguez, J. O. Arrobio, B. C. Jeffries, E. P. Stallings, C. Lewis, A. J. Miles, R. M. Chanock, A. Z. Kapikian, and R. H. Parrott.** 1983. Pediatric viral gastroenteritis during eight years of study. J. Clin. Microbiol. **18**:71–78.
5. **Bruce, E., M. M. Reid, A. W. Craft, J. Kernahan, and P. S. Gardner.** 1979. Multiple virus isolations in children with acute lymphoblastic leukaemia. J. Infect. **1**:243–248.
6. **Carballal, G., M. Siminovich, P. Murtagh, M. C. Cerqueiro, M. Avila, H. Salomon, M. Catalano, and M. Weissenbacher.** 1990. Etiologic, clinical, and pathologic analysis of 31 fatal cases of acute respiratory tract infection in Argentinean children under 5 years of age. Rev. Infect. Dis. **12**(Suppl. 8):S1074–S1080.
7. **Chanock, R. M., and R. H. Parrott.** 1965. Acute respiratory disease in infancy and childhood: present understanding and prospects for prevention. Pediatrics **36**:21–39.
8. **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.
9. **Chonmaitree, T., B. J. Bessette-Henderson, R. E. Hepler, and H. L. Lucia.** 1987. Comparison of three rapid diagnostic techniques for detection of respiratory syncytial virus from nasal wash specimens. J. Clin. Microbiol. **25**:746–747.
10. **Clouse, K. A., P. B. Robbins, B. Fernie, J. M. Ostrove, and A. S. Fauci.** 1989. Viral antigen stimulation of the production of human monokines capable of regulating HIV-1 expression. J. Immunol. **143**:470–475.
11. **Colberg-Poley, A., H. Isom, and F. Rapp.** 1979. Reactivation of herpes simplex virus type 2 from a quiescent state by human cytomegalovirus. Proc. Natl. Acad. Sci. USA **76**:5948–5951.
12. **Craft, A. W., M. M. Reid, P. S. Gardner, E. Jackson, J. Kernahan, J. McQuillin, T. C. Noble, and W. Walker.** 1979. Virus infections in children with acute lymphoblastic leukaemia. Arch. Dis. Child. **54**:755–759.
13. **Cruz, J. R., E. Quinonez, A. D. Fernandez, and F. Peralta.** 1987. Isolation of viruses from nasopharyngeal secretions: comparison of aspiration and swabbing as means of sample collection. J. Infect. Dis. **156**:415–416.
14. **Denny, F. W., and W. A. Clyde, Jr.** 1986. Acute lower respiratory tract infections in nonhospitalized children. J. Pediatr. **108**:635–646.
15. **Downham, M. A. P. S., P. S. Gardner, J. McQuillin, and J. A. J. Ferris.** 1975. Role of respiratory viruses in childhood mortality. Br. Med. J. **1**:235–239.
16. **Engvall, E., and P. Perlman.** 1971. Enzyme-linked immunosor-

bent assay (ELISA). Quantitative assay of immunoglobulin G. Immunochemistry **8:**871–874.
17. **Epstein, M., and B. Achong.** 1986. The Epstein-Barr virus: recent advances. William Heineman Medical Books, London.
18. **Espy, M. J., T. F. Smith, M. W. Harmon, and A. P. Kendal.** 1986. Rapid detection of influenza virus by shell vial assay with monoclonal antibodies. J. Clin. Microbiol. **24:**677–679.
19. **Evans, A. S.** 1982. The clinical illness promotion factor: a third ingredient. Yale J. Biol. Med. **55:**193–199.
20. **Feldman, L. T., M. J. Imperiale, and J. R. Nevins.** 1982. Activation of early adenovirus transcription by the herpesvirus immediate early gene. Evidence for a common cellular control factor. Proc. Natl. Acad. Sci. USA **79:**4952–4956.
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. **Fong, C. K. Y., and M. L. Landry.** 1991. Advantages of multiple cell culture systems for detection of mixed-virus infections. J. Virol. Methods **33:**283–290.
23. **Foy, H. M., M. K. Cooney, A. J. Matelzky, and J. T. Grayston.** 1973. Incidence and etiology of pneumonia, croup and bronchiolitis in preschool children belonging to a prepaid medical care group over a four-year period. Am. J. Epidemiol. **97:**80–92.
24. **Freke, A., E. J. Stott, A. P. C. H. Roome, and E. O. Caul.** 1986. The detection of respiratory syncytial virus in nasopharyngeal aspirates: assessment, formulation, and evaluation of monoclonal antibodies as a diagnostic reagent. J. Med. Virol. **18:**181–191.
25. **Gendelman, H. E., W. Phelps, L. Feigenbaum, J. M. Ostrove, A. Adachi, P. M. Howley, G. Khoury, H. S. Ginsberg, and M. A. Martin.** 1986. Trans-activation of the human immunodeficiency virus long terminal repeat sequence by DNA viruses. Proc. Natl. Acad. Sci. USA **83:**9759–9763.
26. **Ghafoor, A., N. K. Nomani, Z. Ishaq, S. Z. Zaidi, F. Anwar, M. I. Burney, A. W. Quershi, and S. A. Ahmad.** 1990. Diagnosis of acute lower respiratory tract infections in children in Rawalpindi and Islamabad, Pakistan. Rev. Infect. Dis. **12**(Suppl. 8)**:**S907–S914.
27. **Gleaves, C. A., T. F. Smith, E. A. Shuster, and G. R. Pearson.** 1984. Rapid detection of cytomegalovirus in MRC-5 cells inoculated with urine specimens by using low-speed centrifugation and monoclonal antibody to an early antigen. J. Clin. Microbiol. **19:**917–919.
28. **Glezen, P., H. Wulff, G. A. Lamb, C. G. Ray, T. D. Chin, and H. A. Wenner.** 1967. Patterns of virus infections in families with acute respiratory illnesses. Am. J. Epidemiol. **86:**350–361.
29. **Glezen, W. P.** 1983. Viral pneumonia as a cause and result of hospitalization. J. Infect. Dis. **147:**765–770.
30. **Glezen, W. P., and F. W. Denny.** 1973. Epidemiology of acute lower respiratory disease in children. N. Engl. J. Med. **288:**498–505.
31. **Glezen, W. P., F. A. Loda, W. A. Clyde, Jr., R. J. Senior, C. I. Sheaffer, W. G. Conley, and F. W. Denny.** 1971. Epidemiologic patterns of acute lower respiratory disease of children in a pediatric group practice. J. Pediatr. **78:**397–406.
32. **Golden, M. P., S. Kim, S. M. Hammer, E. A. Ladd, P. A. Schaffer, N. DeLuca, and M. A. Albrecht.** 1992. Activation of human immunodeficiency virus by herpes simplex virus. J. Infect. Dis. **166:**494–499.
33. **Grist, N. R., K. Martin, and I. W. Pinkerton.** 1970. Double infections with R.S. virus and influenza. Lancet **ii:**1033.
34. **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 viral infection in children with compromised immune function. N. Engl. J. Med. **315:**77–81.
35. **Hall, C. B., K. R. Powell, K. C. Schnabel, C. L. Gala, and P. H. Pincus.** 1988. Risk of secondary bacterial infection in infants hospitalized with respiratory syncytial virus infection. J. Pediatr. **113:**266–271.
36. **Halstead, D. C., S. Todd, and G. Fritch.** 1990. Evaluation of five methods for respiratory syncytial virus detection. J. Clin. Microbiol. **28:**1021–1025.
37. **Harmon, M. W., and K. M. Pawlik.** 1982. Enzyme immunoassay for direct detection of influenza type A and adenovirus antigens in clinical specimens. J. Clin. Microbiol. **15:**5–11.
38. **Hierholzer, J. C., P. G. Bingham, R. A. Coombs, K. H. Johnansson, L. J. Anderson, and P. E. Halonen.** 1989. Comparison of monoclonal antibody time-resolved fluoroimmunoassay with monoclonal antibody capture-biotinylated detector enzyme immunoassay for respiratory syncytial virus and parainfluenza virus antigen detection. J. Clin. Microbiol. **27:**1243–1249.
39. **Hilleman, M. R., V. V. Hamparian, A. Ketler, C. M. Reilly, L. McClelland, D. Cornfeld, and J. R. Stokes, Jr.** 1962. Acute respiratory illnesses among children and adults. Field study of contemporary importance of several viruses and appraisal of the literature. JAMA **180:**445–453.
40. **Ho, M.** 1977. Virus infections after transplantation in man. Arch. Virol. **55:**1–24.
41. **Holzel, A., L. Parker, W. H. Patterson, C. Cartmel, L. L. R. White, R. Purdy, K. M. Thompson, and J. O. Tobin.** 1965. Virus isolations from throats of children admitted to hospital with respiratory and other diseases, Manchester 1962–1964. Br. Med. J. **1:**614–619.
42. **Hornsleth, A., B. Friis, P. Anderson, 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.
43. **Hortal, M., C. Mogdasy, J. C. Russi, C. Deleon, and A. Suarez.** 1990. Microbial agents associated with pneumonia in children from Uruguay. Rev. Infect. Dis. **12**(Suppl. 8)**:**S915–S922.
44. **Hsuing, G. D.** 1965. Problems in diagnosis of multiple virus infections, p. 138–141. *In* M. Sanders, E. H. Lennette, and W. I. Sheboygan (ed.), Applied virology. Olympic Press, Inc., Minneapolis.
45. **Hughes, J. H., D. R. Mann, and V. V. Hamparian.** 1988. Detection of respiratory syncytial virus in clinical specimens by viral culture, direct and indirect immunofluorescence, and enzyme immunoassay. J. Clin. Microbiol. **26:**588–591.
46. **Jacobs, J. W., D. B. Peacock, B. D. Corner, E. O. Caul, and S. K. R. Claske.** 1971. Respiratory syncytial and other viruses associated with respiratory disease in infants. Lancet **i:**871–876.
47. **Jarvis, W. R., P. J. Middleton, and E. W. Gelfand.** 1983. Significance of viral infections in severe combined immunodeficiency disease. Pediatr. Infect. Dis. J. **2:**187–192.
48. **Johnston, S. L. G., and H. Bloy.** 1993. Evaluation of a rapid enzyme immunoassay for detection of influenza A virus. J. Clin. Microbiol. **31:**142–143.
49. **Johnston, S. L. G., and C. S. Siegel.** 1990. Evaluation of direct immunofluorescence, enzyme immunoassay, centrifugation culture, and conventional culture for the detection of respiratory syncytial virus. J. Clin. Microbiol. **28:**2394–2397.
50. **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.
51. **Lavery, D., S. Fu, T. Lufkin, and S. Chen-Kiang.** 1987. Productive infection of cultured human lymphoid cells by adenovirus. J. Virol. **61:**1466–1472.
52. **Lefkowitz, L. B., Jr., and G. G. Jackson.** 1966. Dual respiratory infection with parainfluenza and rhinovirus: pathogenesis of transmitted infection in volunteers. Am. Rev. Respir. Dis. **93:**519–528.
53. **Lizardi, P. M., and F. R. Kramer.** 1991. Exponential amplification of nucleic acids: new diagnostics using DNA polymerases and RNA replicases. Trends Biotechnol. **9:**53–58.
54. **Ljungman, P., C. A. Gleaves, and J. D. Meyers.** 1989. Respiratory virus infection in immunocompromised patients. Bone Marrow Transplant. **4:**35–40.
55. **Maletzky, A. J., M. K. Cooney, R. Luce, G. E. Kenny, and J. T. Grayston.** 1971. Epidemiology of viral and mycoplasmal agents associated with childhood lower respiratory illness in a civilian population. J. Pediatr. **78:**407–414.
56. **Mathur, U. S. H. A., D. W. Bentley, and C. B. Hall.** 1980. Concurrent respiratory syncytial virus and influenza A infections in the institutionalized elderly and chronically ill. Ann. Intern. Med. **93:**49–52.

57. **Matthey, S., D. Nicholson, S. Ruhs, B. Olsen, M. Knock, K. Schultz, and A. Schumecker.** 1992. Rapid detection of respiratory viruses by shell vial culture and direct staining by using pooled and individual monoclonal antibodies. J. Clin. Microbiol. **30:** 1505–1508.
58. **McQuillin, J., and P. S. Gardner.** 1968. Rapid diagnosis of respiratory syncytial virus infection by immunofluorescent antibody techniques. Br. Med. J. **1:**602–605.
59. **Meissner, H. C., S. A. Murray, M. A. Kiernan, D. R. Snydman, and K. McIntosh.** 1984. A simultaneous outbreak of respiratory syncytial virus and parainfluenza virus type 3 in a newborn nursery. J. Pediatr. **104:**680–684.
60. **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.
61. **Miller, D. G., M. O. Gabrielson, and D. M. Horstman.** 1968. Clinical virology and viral surveillance in a pediatric group practice: the use of double-seeded tissue culture tubes for primary virus isolation. Am. J. Epidemiol. **88:**245–256.
62. **Mills, R. D., K. J. Cain, and G. L. Woods.** 1989. Detection of influenza virus by centrifugal inoculation of MDCK cells and staining with monoclonal antibodies. J. Clin. Microbiol. **27:**2505–2508.
63. **Minnich, L., and C. G. Ray.** 1980. Comparison of direct immunofluorescent staining of clinical specimens for respiratory virus antigens with conventional isolation techniques. J. Clin. Microbiol. **12:**391–394.
64. **Minnich, L., and J. L. Waner.** 1993. Rapid immunofluorescence (RIF) procedure for the identification of respiratory syncytial virus (RSV) in smears prepared at the patient's bedside from nasopharyngeal (NP) swabs, abstr. M9A. Program Abstr. 9th Annu. Clin. Virol. Symp.
65. **Minnich, L. L., and C. G. Ray.** 1987. Early testing of cell cultures for detection of hemadsorbing viruses. J. Clin. Microbiol. **25:**421–422.
66. **Minuse, E., and F. M. Davenport.** 1951. Simultaneous recovery of type A and type C influenza viruses from patient. J. Lab. Clin. Med. **38:**747–750.
67. **Nelson, J., C. Reynolds-Kohler, M. Oldstone, and C. Wiley.** 1988. HIV and HCMV coinfect brain cells in patients with AIDS. Virology **165:**286–290.
68. **Nichol, K. P., and J. D. Cherry.** 1967. Bacterial-viral interrelations in respiratory infections of children. N. Engl. J. Med. **277:**667–672.
69. **Olsen, M. A., K. M. Shuck, A. R. Sambol, S. M. Flor, J. O'Brien, and B. J. Cabrera.** 1993. Isolation of seven respiratory viruses in shell vials: a practical and highly sensitive method. J. Clin. Microbiol. **31:**422–425.
70. **Olson, L. C., U. Lexomboon, P. Sithisarn, and H. E. Noyes.** 1973. The etiology of respiratory tract infections in a tropical country. Am. J. Epidemiol. **97:**34–43.
71. **Paisley, J. W., B. A. Lauer, K. McIntosh, M. P. Glode, J. Schachter, and C. Rumack.** 1984. Pathogens associated with acute lower respiratory tract infection in young children. Pediatr. Infect. Dis. J. **3:**14–19.
72. **Parks, W. P., L. T. Queiroga, and J. L. Melnick.** 1967. Studies of infantile diarrhea in Karachi, Pakistan. Am. J. Epidemiol. **85:** 469–478.
73. **Parrott, R. H., A. J. Vargasko, H. W. Kim, C. Cumming, H. Turner, R. J. Huebner, and R. M. Chanock.** 1961. Respiratory syncytial virus. II. Serological studies over a 34 month period of children with bronchiolitis, pneumonia, and minor respiratory diseases. JAMA **176:**653–657.
74. **Popow-Kraupp, T., G. Kern, C. Binder, W. Tuma, M. Kundi, and C. Kunz.** 1986. Detection of respiratory syncytial virus in nasopharyngeal secretions by enzyme-linked immunosorbent assay, indirect immunofluorescence, and virus isolation: a comparative study. J. Med. Virol. **19:**123–134.
75. **Portnoy, B., H. L. Eckert, B. Hanes, and M. A. Salvatore.** 1965. Multiple respiratory virus infections in hospitalized children. Am. J. Epidemiol. **82:**262–272.
76. **Portnoy, B., H. L. Eckert, and M. A. Salvatore.** 1965. Rhinovirus infection in children with acute lower respiratory disease: evidence against etiological importance. Pediatrics **35:**899–905.
77. **Puthavathana, P., C. Wasi, V. Kositanont, S. Suwanjutha, T. Chantarojanasiri, W. Kantakamalakul, P. Kantawateera, and P. Thongcharoen.** 1990. A hospital-based study of acute viral infections of the respiratory tract in Thai children, with emphasis on laboratory diagnosis. Rev. Infect. Dis. **12**(Suppl. 8)**:**S988–S994.
78. **Rabalais, G. P., G. G. Stout, K. L. Ladd, and K. M. Cost.** 1992. Rapid diagnosis of respiratory viral infections by using a shell vial assay and monoclonal antibody pool. J. Clin. Microbiol. **30:**1505–1508.
79. **Rahman, M., F. Huq, D. A. Sack, T. Butler, A. K. Azad, A. Alam, N. Nahar, and M. Islam.** 1990. Acute lower respiratory tract infections in hospitalized patients with diarrhea in Dhaka, Bangladesh. Rev. Infect. Dis. **12**(Suppl. 8)**:**S899–S906.
80. **Rando, R. F., P. E. Pellet, P. A. Luciw, C. A. Bohan, and A. Srinivasan.** 1987. Transactivation of human immunodeficiency virus by herpesviruses. Oncogene **1:**13–18.
81. **Ray, C. G., and L. L. Minnich.** 1987. Efficiency of immunofluorescence for rapid detection of common respiratory viruses. J. Clin. Microbiol. **25:**355–357.
82. **Ray, C. G., L. L. Minnich, C. J. Holberg, Z. M. Shehab, A. L. Wright, L. L. Barton, L. M. Taussig, and the Group Health Medical Associates.** 1993. Respiratory syncytial virus-associated lower respiratory illnesses: possible influence of other agents. Pediatr. Infect. Dis. J. **12:**15–19.
83. **Rice, G. P. A., R. D. Schrier, and M. B. A. Oldstone.** 1984. Cytomegalovirus infects human lymphocytes and monocytes: virus expression is restricted to immediate-early gene products. Proc. Natl. Acad. Sci. USA **81:**6134–6138.
84. **Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich.** 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239:**487–491.
85. **Sarkkinen, H. K., P. E. Halonen, P. P. Arstila, and A. A. Salmi.** 1981. Detection of respiratory syncytial, parainfluenza type 2, and adenovirus antigens by radioimmunoassay and enzyme immunoassay on nasopharyngeal specimens from children with acute respiratory disease. J. Clin. Microbiol. **13:**258–265.
86. **Sarkkinen, H. K., P. E. Halonen, and A. A. Salmi.** 1981. Detection of influenza A virus by radioimmunoassay and enzyme-immunoassay from nasopharyngeal specimens. J. Med. Virol. **7:**212–220.
87. **Schochetman, G., C.-Y. Ou, and W. K. Jones.** 1988. Polymerase chain reaction. J. Infect. Dis. **158:**1154–1157.
88. **Skolnik, P. R., R. J. Pomerantz, S. M. de la Monte, S. F. Lee, G. D. Hsuing, R. Y. Foos, G. M. Cowan, B. R. Kosloff, M. S. Hirsch, and J. S. Pepose.** 1989. Dual infection of retina with human immunodeficiency virus type 1 and cytomegalovirus. Am. J. Ophthalmol. **107:**361–372.
89. **Smith, M. C., C. Creutz, and Y. T. Huang.** 1991. Detection of respiratory syncytial virus in nasopharyngeal secretions by shell vial technique. J. Clin. Microbiol. **29:**463–465.
90. **Specter, S., M. Bendinelli, and H. Friedman (ed.).** 1989. Virus-induced immunosuppression. Plenum Press, New York.
91. **Stott, E. J., E. J. Bell, M. B. Eadie, C. A. C. Ross, and N. R. Grist.** 1967. A comparative virological study of children in hospital with respiratory and diarrhoeal illnesses. J. Hyg. Camb. **65:**9–23.
92. **Stout, C., M. D. Murphy, S. Lawrence, and S. Julian.** 1989. Evaluation of a monoclonal antibody pool for rapid diagnosis of respiratory viral infections. J. Clin. Microbiol. **27:**448–452.
93. **Subbarao, E. K., J. Griffis, and J. L. Waner.** 1989. Detection of multiple viral agents in nasopharyngeal specimens yielding respiratory syncytial virus (RSV). An assessment of diagnostic strategy and clinical significance. Diagn. Microbiol. Infect. Dis. **12:**327–332.
94. **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.
95. **Swierkosz, E. M., R. Flanders, L. Melvin, J. D. Miller, and M. W. Kline.** 1989. Evaluation of the Abbott TESTPACK RSV enzyme immunoassay for detection of respiratory syncytial virus in nasopharyngeal swab specimens. J. Clin. Microbiol. **27:**1151–1154.

96. **Takiff, H. E., S. E. Straus, and C. F. Garon.** 1981. Propagation and in vitro studies of previously non-cultivatable enteric adenoviruses in 293 cells. Lancet **ii:**832–834.
97. **Takimoto, S., M. Grandien, M. A. Ishida, M. S. Pereira, T. M. Paiva, T. Ishimaru, E. M. Makita, and C. H. O. Martinez.** 1991. Comparison of enzyme-linked immunosorbent assay, indirect immunofluorescence assay, and virus isolation for detection of respiratory viruses in nasopharyngeal secretions. J. Clin. Microbiol. **29:**470–474.
98. **Tevethia, M. J., and D. J. Spector.** 1989. Heterologous transactivation among viruses. Prog. Med. Virol. **36:**120–190.
99. **Thomas, E. E., and L. E. Book.** 1991. Comparison of two rapid methods for detection of respiratory syncytial virus (RSV) (TestPack RSV and Ortho RSV ELISA) with direct immunofluorescence and virus isolation for the diagnosis of pediatric RSV infection. J. Clin. Microbiol. **29:**632–635.
100. **Todd, S. J., and J. L. Waner.** 1993. Rapid immunofluorescence (RIF) procedure for the diagnosis of respiratory syncytial virus (RSV), abstr. M5. Program Abstr. 9th Annu. Clin. Virol. Symp.
101. **Tristram, D. A., R. W. Miller, J. A. McMillan, and L. B. Weiner.** 1988. Simultaneous infection with respiratory syncytial virus and other respiratory pathogens. Am. J. Dis. Child. **142:**834–836.
102. **Valenti, W. M., T. A. Clarke, C. B. Hall, M. A. Menegus, and D. L. Shapiro.** 1982. Concurrent outbreaks of rhinovirus and respiratory syncytial virus in an intensive care nursery: epidemiology and associated risk factors. J. Pediatr. **100:**722–726.
103. **Verville, E., J. D. Connor, and M. M. Sigel.** 1961. Double infection of tissue culture cells with CA virus and adenovirus. Virology **13:**261–262.
104. **Vogt, M. W., K. L. Hartshorn, P. A. Furman, T. C. Chou, J. A. Fyfe, L. A. Coleman, C. Crumpacker, R. T. Schooley, and M. S. Hirsch.** 1987. Ribavirin antagonizes the effect of azidothymidine on HIV replication. Science **235:**1376–1379.
105. **Waner, J. L., S. J. Todd, H. Shalaby, P. Murphy, and L. V. Wall.** 1991. Comparison of Directigen FLU-A with viral isolation and direct immunofluorescence for the rapid detection and identification of influenza A virus. J. Clin. Microbiol. **29:**479–482.
106. **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.
107. **Waner, J. L., N. J. Whitehurst, S. J. Todd, H. Shalaby, and L. V. Wall.** 1990. Comparison of Directigen RSV with viral isolation and direct immunofluorescence for the identification of respiratory syncytial virus. J. Clin. Microbiol. **28:**480–483.
108. **Whelchel, J. D., R. F. Pass, A. G. Diethelm, R. J. Whitley, and C. A. Alford, Jr.** 1979. Effect of primary and recurrent cytomegalovirus infections upon graft and patient survival after renal transplantation. Transplantation **28:**443–446.
109. **Whitley, R. J., M. Levin, N. Barton, B. J. Hershey, G. Davis, R. E. Keeney, J. Whelchel, A. G. Diethelna, P. Kartus, and S.-J. Soong.** 1984. Infections caused by herpes simplex virus in the immunocompromised host: natural history and topical acyclovir therapy. J. Infect. Dis. **150:**323–329.
110. **Wittwe, C. T., and D. J. Garling.** 1991. Rapid cycle DNA amplification: time and temperature optimization. BioTechniques **10:**76–83.
111. **Wolcott, M. J.** 1992. Advances in nucleic acid-based detection methods. Clin. Microbiol. Rev. **5:**370–386.
112. **Wolontis, S., J. Stenbeck, and H. Fransen.** 1969. Multiple virus isolations in a group of children hospitalized with acute respiratory illness. Scand. J. Infect. Dis. **1:**119–126.
113. **Yolken, R. H., C. A. Bishop, T. R. Townsend, E. A. Bolyard, J. Bartlett, G. W. Santos, and R. Saral.** 1982. Infectious gastroenteritis in bone-marrow-transplant recipients. N. Engl. J. Med. **306:**1009–1012.
114. **Yolken, R. H., and P. J. Stopa.** 1979. Analysis and nonspecific reactions in enzyme-linked immunosorbent assay testing for human rotavirus. J. Clin. Microbiol. **10:**703–710.
115. **Yolkin, R. H.** 1988. Nucleic acids or immunoglobulins: which are the molecular probes of the future? Mol. Cell. Probes **2:**87–96.
116. **Zahradnik, J. M., M. J. Spencer, and D. D. Porter.** 1980. Adenovirus infection in the immunocompromised patient. Am. J. Med. **68:**725–732.