Respiratory Viral Antigens in Autopsy Lung Tissue Specimens from Patients with Cancer or Myocardial Infarction

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Using immunoenzyme histochemical analysis, we retrospectively examined lung tissue specimens obtained at autopsy from 118 patients with cancer who had received chemotherapy and 20 patients who had died after myocardial infarction. Respiratory viral antigens were demonstrated in lung tissue specimens from eight of 118 cancer patients and two of 20 myocardial infarction patients. Most of the patients with demonstrable viral antigens were febrile and had signs of pulmonary infection, but in no case was pulmonary viral infection considered clinically. The following viral antigens were demonstrated: influenza A virus (6 patients), respiratory syncytial virus (2), influenza B virus (1), and parainfluenza virus type 1 (1).

Many lower respiratory tract viral infections have been considered to be primarily limited to infants and children, although influenza viruses and respiratory syncytial virus (RSV) cause morbidity and mortality in the elderly [1, 2]. Immunocompromised adults have been noted to develop severe or fatal infections caused by respiratory viruses [3, 4].

Immunoenzyme histochemical analysis allows demonstration of viral antigens in formalin-fixed paraffin-embedded tissue specimens retained in pathology laboratories. In this study, we used this technique to determine if patients who had received chemotherapy for cancer had pulmonary viral infection. We chose patients who died after myocardial infarction as controls, but two of these patients also had evidence of viral infection.

Patients, Materials, and Methods

Patient selection. The records of 711 patients autopsied at the UCLA Medical Center from June 1978 through June 1980 (25 months; before the first cases of AIDS in our institution) were reviewed; 186 patients from this period who died with a malignant tumor and received chemotherapy were considered for this study. Patients were excluded for the following reasons: a single dose of a planned course of chemotherapy (1 patient), chemotherapy completed >1 year before death (3), autopsy performed >36 hours after death (3), and unavailability of formalin-fixed lung tissue specimens (4). The number of patients was then reduced to 118 by using a table of random numbers. Of these patients, 54 had leukemia or lymphoma, 46 had carcinoma, and 18 had sarcoma. In the leukemia or lymphoma group, there were 18 cases of acute myelogenous leukemia, 7 of acute lymphocytic leukemia, 7 of lymphocytic lymphoma, and <4 of any other diagnosis. In the carcinoma group, the primary organ was the breast (10 cases), lung (7), ovary (7), endometrium (3), colon (3), cervix (3), and pancreas (3). In the sarcoma group, there were 11 melanomas, 3 osteogenic sarcomas, and 1 each of sarcomas at several other sites.

All 22 patients who were autopsied during the designated period within 1 week of myocardial infarction uncomplicated by other major diseases were chosen as controls, and two of these patients were excluded from the study because of unavailability of formalin-fixed lung tissue specimens. Clinical data were obtained from patient charts, and we independently reviewed the autopsy protocols and tissue slides.

Viruses, cells, and antisera. Contemporary strains of influenza A and B viruses, parainfluenza virus types 1–3, RSV, and adenoavirus type 4 were obtained from the American Type Culture Collection (Manassas, VA). Influenza viruses were grown on MDCK cells with added trypsin, parainfluenza viruses were grown on LLC-MK2 cells, RSV was grown on HEP-2 cells, and adenoavirus was grown on HeLa cells. Antisera were prepared by infection of ferrets, which were exsanguinated 30 days later.

Immunoenzyme staining. A two-stage glucose oxidase method was used as previously described [5]. Tissue specimens with a positive staining reaction were also stained with the corresponding preimmune ferret serum and immune serum from which antibody had been removed by absorption with infected cells. Coverslips of cells infected with each of the viruses were used as positive controls in each staining batch of human tissue specimens. As an additional control, human lung tissue specimens from culture-proven cases of pneumonia due to influenza A virus, RSV, parainfluenza virus type 3, and adenovirus were used. Negative controls included normal human lung tissue specimens and uninfected cells grown on coverslips.
Review of lung lesions. The criteria for diffuse alveolar damage were those of Wright [6].

Results

Of the 118 cancer patients tested, eight had viral antigen demonstrated in the bronchi and/or alveoli as shown in table 1. Of these patients, four had undergone autologous bone marrow transplantation 1 to 19 days earlier. Of the 110 cancer patients who did not have viral antigens demonstrated, 11 had undergone bone marrow transplantation 4 days to 11 months before death (seven autologous and four allogeneic transplants), of whom only two had undergone transplantation 30 days previously. Of the 20 “control” myocardial infarction patients, two had viral antigens demonstrated.

The viral antigens demonstrated were as follows: influenza A virus (6 patients), RSV (2), influenza B virus (1), and parainfluenza virus type 1 (1). Known positive and negative tissue specimens and control tissue culture specimens yielded appropriate results. Cytomegalovirus (CMV) was isolated from patient 10 and one patient without respiratory viral antigens, but only the latter patient had morphological evidence of CMV pneumonia. One-half of the patients with demonstrable viral antigens had morphological evidence of diffuse alveolar damage, four in the exudative phase and one (patient 10) in the proliferative phase. The remaining patients had pulmonary edema, scattered pulmonary hemorrhages, or other nondiagnostic changes in the lung tissue.

Microscopic morphology of the lungs and localization of viral antigens in case 5 are illustrated in figure 1; influenza A virus was demonstrated in this case. Each of the patients with demonstrable viral antigens had adequate causes of death demonstrated at autopsy, but we believe that diffuse alveolar damage, probably induced by the viral infection, contributed to the deaths of patients 2, 7, and 10.

Nine of 10 patients with demonstrable viral antigens had clinical signs of pulmonary disease, and of these patients, eight (in whom body temperature was measured) were febrile. Six of the 10 patients were receiving corticosteroid therapy, which may have blunted the signs of infection and perhaps increased the magnitude of viral replication. All 10 patients had viral antigen in the bronchial epithelium, and six also had viral antigen in alveoli. Antigen distribution did not correlate with the presence or absence of diffuse alveolar damage.

### Table 1. Clinical and pathological findings for patients with cancer or myocardial infarction who had demonstrable viral antigens in the lung at autopsy.

| Case no. | Age (y)/sex | Diagnosis | Cause(s) of death | Cytotoxic therapy | Steroid therapy | Febrile | Clinical lung disease | Viral antigen(s) | Antigen in Bronchi | Diffuse alveolar damage | Date of death | Autologous BMT* |
|----------|-------------|-----------|-------------------|-------------------|-----------------|---------|----------------------|-----------------|-----------------|----------------------|---------------|-----------------|
| 1        | 38/M        | Colon carcinoma | Bleeding, sepsis | +                 | 0               | 0       | 0                    | Influenza A virus | +               | +                    | November 1979 | 1               |
| 2        | 18/M        | Osteogenic sarcoma | Diffuse alveolar damage | +                 | 0               | +       | +                    | Influenza A virus | +               | 0                    | September 1979 | 0               |
| 3        | 35/F        | Acute lymphocytic leukemia | Septic shock | +                 | +               | +       | +                    | Influenza A virus | +               | 0                    | January 1979 | 14              |
| 4        | 38/M        | Embryonal carcinoma of testis | Pneumonia | +                 | +               | +       | +                    | Influenza A virus | +               | 0                    | January 1979 | 14              |
| 5        | 63/M        | Lymphocytic lymphoma | Bleeding | +                 | +               | +       | +                    | Influenza A virus | +               | 0                    | May 1979      | 0               |
| 6        | 69/M        | Myocardial infarction | Cardiac shock | 0                 | 0               | +       | +                    | Influenza A virus | +               | 0                    | May 1980      | 0               |
| 7        | 2/M         | Acute myelocytic leukemia | Pneumonia | +                 | +               | +       | +                    | Influenza B virus | +               | +                    | April 1979    | 0               |
| 8        | 71/M        | Myocardial infarction | Ventricular fibrillation | 0                 | 0               | ND      | +                    | Respiratory syncytial virus | +               | 0                    | March 1980    | 0               |
| 9        | 59/F        | Hodgkin’s disease | Respiratory failure | +                 | +               | +       | +                    | Respiratory syncytial virus | +               | +                    | November 1978 | 0               |
| 10       | 22/F        | Histiocytic lymphoma | Diffuse alveolar damage, venoocclusive disease of liver | +                 | +               | +       | +                    | Parainfluenza virus type 1, CMV by culture | +               | 0                    | May 1979      | 19              |

NOTE. BMT = bone marrow transplantation; CMV = cytomegalovirus; ND = not determined; + = present.

* Number of days before death that BMT had been performed.
Discussion

In the present study, the immunoenzyme assay for viral antigen in the lungs was both sensitive and specific. Seven percent of our patients had pulmonary infections with common RNA respiratory viruses. However, it should be emphasized that no epidemiological conclusions should be drawn from the present study, because of the small sample size and the occurrence of viral antigen in two of the designated controls. Patients 6 and 8 (myocardial infarctions) acquired influenza virus and RSV infections, respectively, in the community, since they were hospitalized for a shorter time than an incubation period. Most of the infections occurred in the winter and spring, the characteristic seasonality for respiratory viral infections. It seems possible that patients 3 and 4 were infected with influenza A virus from a common source, since they were in the same unit at the same time. The only other autopsy study of pulmonary viral infections in immunosuppressed individuals looked for CMV, herpes simplex virus, Epstein-Barr virus, and adenovirus (but not RNA respiratory viruses) by in situ hybridization and found only CMV [7].

It is becoming clear that the burden of disease produced by common RNA respiratory viruses is much higher than generally appreciated in the elderly, adults with serious chronic disease, and individuals who have received immunosuppressive therapy [8]. The perceived incidence of disease is quite dependent on the sensitivity and specificity of the assays for infection, and many studies have looked for only one or a few viruses. In a broad prospective study of the elderly living in the community [9], rhinoviruses were found to be the most important cause of upper respiratory tract infections, followed by coronaviruses and more distantly by influenza viruses, RSV, and parainfluenza viruses. Surprisingly, even rhinovirus and coronavirus infections were associated with lower respiratory tract signs and symptoms.

We suggest that elderly, chronically ill, or immunosuppressed patients who are febrile and have signs of pulmonary disease be tested for the presence of respiratory viral infection. Rapid diagnostic testing based on immunofluorescence or EIAs for viruses associated with lower respiratory tract infections is sensitive and specific for pediatric patients. However, these methodologies are not sensitive for adult patients, since all such infections represent reinfections and are associated with

Figure 1. Photomicrographs of an autopsy lung tissue specimen from a patient with cancer who received chemotherapy and had demonstrable viral antigens in this specimen (case 5). A. Small bronchus (hematoxylin-eosin stain). B. Similar bronchus immunostained for the presence of influenza A virus antigen (purple color). C. Alveolar area of the same lung tissue (hematoxylin-eosin stain). D. Alveolar area stained for the presence of influenza A virus antigen. Bars = 200 μm (original magnification, ×135).
shorter periods of viral shedding and much lower virus titers [10]. Multiplex reverse transcriptase PCR assays are efficacious for adults and can test for multiple viruses in a single reaction [11]. Whether rapid diagnosis will identify influenza A virus infections early enough to be treated with amantadine or rimantadine remains to be determined, since therapy probably needs to be started within the first 2 or 3 days after infection.

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