Fatal Adenovirus Pneumonia in Infants

Correlation of Histologic and Electron Microscopic Observations

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Many structures morphologically identical with adenovirions were found in large numbers in typically altered respiratory epithelial cells in 10-year-old sections stained with hematoxylin and eosin of 2 cases of a histologically unique type of fatal pneumonia in infancy. These observations confirm, beyond reasonable doubt, an etiologic role of adenoviruses in the initiation and development of this type of pneumonia. The technic used, which was a modification of one previously described, is given in detail, since such methods may be useful in solving other etiologic problems. (Am J Pathol 65:543–548, 1971)

A MORPHOLOGICALLY UNIQUE TYPE of fatal nonbacterial pneumonia in infants that was associated with nuclear inclusions was described in 1939 by Goodpasture and his co-workers.[1] From the lungs of infants and young children showing the features described by Goodpasture, Chany *et al*[2] isolated adenovirus types 7a, 2 or 4. Four of their fatal cases occurred in an outbreak of respiratory illness in an overcrowded children's ward; 4 other fatal cases were found in slide files of earlier autopsies. Other sporadic cases diagnosed by virus isolation, immunologic tests, or both have been reported.[3–8] Diagnosis may be made with reasonable assurance from the histologic picture alone.[8] In 1 case,[3] a type 1 adenovirus was isolated from the lung, and its cytopathic effect in HeLa cells was specifically inhibited by antiserum to type 1. In only 1 case were inclusions reported in any organ other than the lung (the liver[7]).

Because of the ubiquity of adenoviruses, and the irregular results obtained by immunologic tests, it has been "difficult to assess the exact role of the virus in the initiation of the extensive pneumonia."[9] We report here the presence of bodies having the typical morphology of adenovirions in severely injured respiratory epithelial cells in the lungs of two typical cases of "Goodpasture's pneumonia."

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Materials and Methods

Formalin-fixed slides stained with hematoxylin and eosin (H&E) of the 2 cases (AFIP 971608 and 971609) used for a seminar [8] in 1962 were processed by a modification of the method described by Blank *et al.*[10]

1. The coverslips were removed and the slides were dipped in equal parts of propylene oxide and xylol and then in 100% propylene oxide (steps 1–6 in the paper by Blank *et al* [10]).
2. Several drops of an embedding medium consisting of 50% Epon/Araldite and 50% propylene oxide were placed on the slides, without letting them dry.
3. The propylene oxide was evaporated in 15 minutes at room temperature.
4. Slides were drained, and excess plastic wiped off, except in the areas occupied by the sections.
5. Drops of fresh embedding medium were added to the sections only. (The plastic can be built up to desired thickenss by adding more after it starts to harden in the oven.)
6. The slides, with the sections up, were put in the oven at 80 C on the smooth side of a Fullam flat embedding mold.
7. After 1 hour, when the plastic was hardened, the slides were removed from the oven and a drop of cold distilled water was added at one edge of the sections. A single-edged razor blade was forced between the slides and the plastic strips, allowing an interface of air and water to form. With continued force, the plastic sheets containing the sections were freed from the slide. (If they tended to curl, they were flattened by returning them to the oven on the Fullam mold for a few minutes and then recooling).
8. Sections were placed on slides, and under the light microscope, areas where the characteristically altered cells were particularly numerous were cut out with a razor blade and attached to a Beem capsule block of Epon/Araldite.
9. Thin sections were then cut on a Porter-Blum MT-1 microtome, and stained with alcoholic uranyl acetate and lead citrate. Electron micrographs were taken with a Phillips-300 electron microscope at 60 kV. (Original magnification was 14,000.)

Results

Histology

The characteristic picture has been amply described in previous reports.[1–8] The outstanding features are the cytolytic injury and at times complete destruction of bronchial, bronchiolar and alveolar epithelium, and the presence of typical nuclear alterations. What we wish to stress here is that typical "haloed" nuclear inclusions, with visible nuclear membranes, may be extremely difficult to find, and were absent from many slides even when the typical bronchiolar abscesses were present On the other hand, enlarged epithelial cells, with loss of nuclear membranes and migration of nuclear contents into the cytoplasm (Fig 1) were numerous in all of several hundred slides examined, 30 or more often being present in a single low-power field. These cells, which have been called "smudge" cells[8] are quite characteristic of adenovirus infection. They are most often somewhat basophilic, but may be ampho-

philic or eosinophilic. In some cells, a cytoplasmic area may be recognized, but many cells appear completely homogenized.

Electron Microscopy

Structures that were identified beyond reasonable doubt as adenovirions were readily found in both cases studied. Criteria for identification and for distinguishing them from other virions (including herpesvirions) were the overall average diameter (approximately 67 mm) the presence of a dense core representing 80–90% of the total diameter, the absence of envelopes of host cell origin, and their complete morphologic identity with type 2 adenovirions, as seen in KB cells infected *in vitro* (Average diameters were obtained by measuring individual virions, and by center-to-center measurements in linear components of viral crystals where the virions appeared to be contiguous. The two methods have nearly identical results.)

In AFIP 971609, a number of degenerating cells showed only five or six virions. Fig 2 shows a cell with relatively light nuclear infection, but with absence of the nuclear membranes. In AFIP 971608, virions in crystalline array (Fig 3) were present in several of the cells studied. In one cell (not pictured), they were admixed with mitochondria. This apparent difference between the 2 cases may have been related to the duration of illness (20 days in No. 971609 versus 10 days in No. 971608) or to irregularities in sampling.

Discussion

In No. 971609, type 3 adenovirus was isolated; in No. 971608 virus isolation was not attempted. As noted above, types 1, 2, 4 and 7 have been isolated in other reported cases. Contact with siblings suffering from epidemic keratoconjunctivitis [5] or pharyngoconjunctival fever [3] has been noted. Though Goodpasture's cases occurred mostly in children convalescing from other infections, several reported cases have occurred in previously healthy children. A morbilliform rash is often present, and is probably caused by adenovirus.[2,7]

It is probable that fatal pneumonia in infants represents only one peak of the adenoviral pneumonia iceberg. One can assume that many patients recover, with a degree of residual pulmonary damage proportional to the severity of the involvement. It therefore is important to make a diagnosis in these nonfatal cases. Since characteristic cells are desquamated into the bronchial exudate, diagnosis could probably be made by cytologic, or more precisely, by electron microscopic study. Retrospective light and electron microscopic study of microscopic slides

from autopsy files could serve an important function in the study of other disease entities—for example, sudden death in infancy.

After completing our observations, we learned by personal communication that a case briefly mentioned by Blank and Collins,[11] in which adenovirions were demonstrated in the lung, was "a fatal infantile pneumonia with inclusions and large cells which were suspicious in H&E for adenovirus pneumonia." Also, in a similar case reported in 1967, Nahmias *et al*[12] clearly showed adenovirions in formalinized lung tissue.

References

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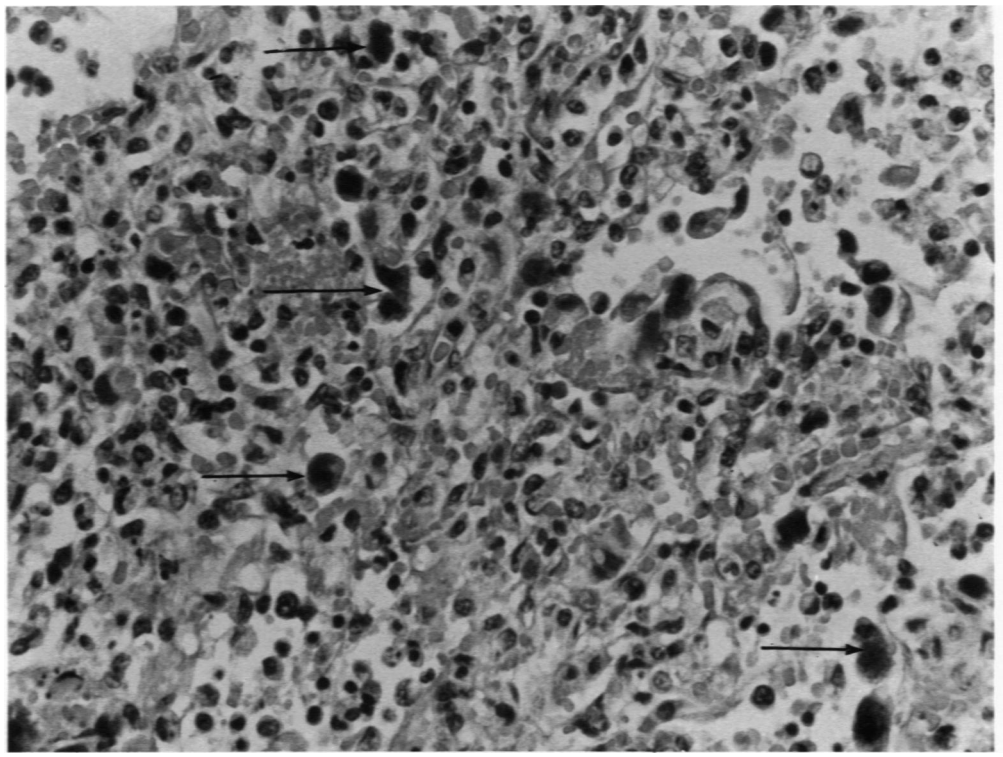

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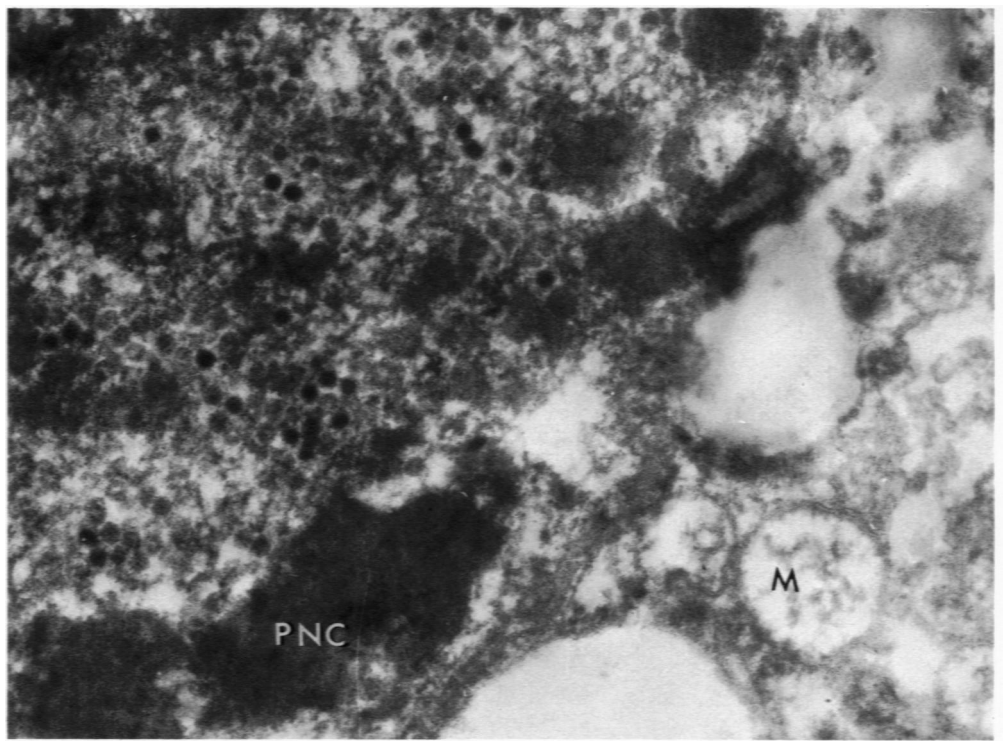

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Fig 1—AFIP 971609. Adenovirus pneumonia. Characteristic large cells with nucleocytoplasmic blurring (*arrows*) are seen in an area of interstitial pneumonia. Haloed inclusions could not be found in this section (H&E, × 650). **Fig 2**—AFIP 971609. Intranuclear virions, presumably type 3 adenovirions, in an infected respiratory epithelial cell. Peripheral nuclear chromatin (*PNC*); mitochondrion (*M*). Note absence of nuclear membranes. (A stained paraffin section was removed from the slide and processed as described in the text) (× 39,760; original magnification × 14,000).

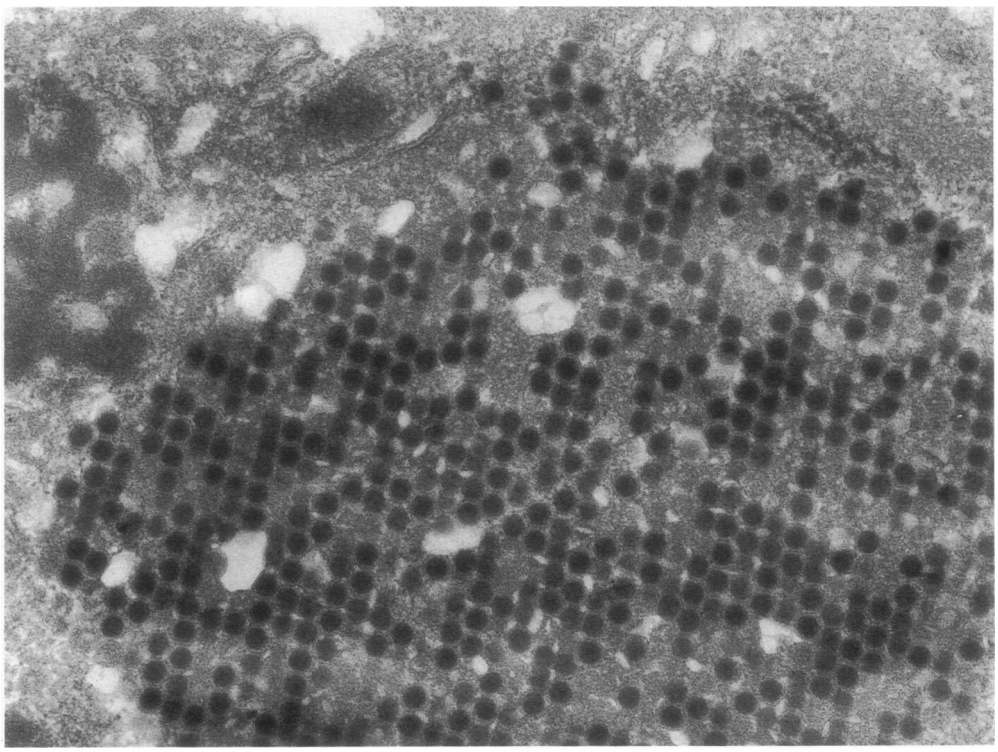

Fig 3—AFIP 971608. A similar case of fatal adenoviral pneumonia in infancy. A 10-year-old paraffin section was processed as described in the text. Virions in crystalline array are seen in a homogenized cell (× 56,000; original magnification × 14,000).