On the Occurrence of Virus-Like Bodies in Human Leukemia

By H. Braunsteiner, K. Fellinger and F. Pakesch

DURING the last years, electron microscopic studies of leukemic tissues in chicken and mice have revealed the presence of intra- and extra-cellularly located virus-like bodies. The bodies are very similar to those found in solid tumors of animals, such as Rous' sarcomas or breast carcinomas. Some particles were also found in animals without evidence of leukemia or tumor.

It might be expected that these results would intensify the search for virus-like particles in human leukemias. Positive results in single cases have been reported recently.

Our research group has looked for evidence of viruses in human neoplastic conditions for about 10 years. One of us published a report with the Oberling group in Paris in 1950 in which the presence of virus-like bodies in spread leukemic cells was discussed. It was felt, however, that the method did not permit discrimination between virus-like bodies and microgranulations in leukemic cells.

Since 1952, after the introduction of ultra-thin sectioning, these researches have been intensified and have become almost a routine procedure in our department. A rather extensive number of patients with acute or chronic leukemia, Hodgkins' disease, myeloma and lymphosarcoma were examined. In a previous study published in this journal the cytologic features were described. The present study gives a short quantitative survey of our work in relation to "positive findings of virus-like bodies." It is believed that the quantitative aspect must be discussed before such findings can be interpreted.

METHODS

The pathologic material was obtained by bone marrow puncture or lymph node biopsy and immediately fixed, embedded and sectioned as previously described. A Philips electron microscope model 100 A with modified lenses and a resolution power of about 25 A was used.

We have examined the bone marrow of 16 patients (13 adults and 3 children) with acute leukemia, of 5 patients with chronic lymphatic leukemia and of 8 patients with plasmacytic myeloma. We have further examined lymph nodes of 2 patients (1 child) with acute leukemia, of 1 patient with chronic lymphocytic leukemia, of 3 patients with lymphosarcoma and of 6 patients with Hodgkins' disease.

We have tried to evaluate the number of cells which we have studied of the patients with acute leukemia in the course of this program. These naturally have varied from case to case, but on the average we have cut 6 different blocks of specimen at 3 different levels with 20 to 50 (average 35) serial sections, that is, approximately 100 serial sections in a block. Each section was about 200 to 400 A thick and included fields of about 10 different cells.

Aided by a grant of the Alfred P. Sloan Foundation.
Submitted Apr. 25, 1959; accepted for publication Sept. 27, 1959.

476
We, therefore, have seen more than 100,000 sections of single leukemic cells. We must consider, however, that a suitable section represents only 1/300 of a whole cell. To rule out the presence of viral bodies in a cell, we must divide the number of examined sections at least by 100. In total we have gained, therefore, a picture which would correspond to 1000 leukemic cells. Naturally, we have seen far more individual cells, but with our technic we have not seen serial sections through more than approximately one-fifth of a single cell. Each preparation contains 10 serial sections or 100 cell sections. In case of any doubt photomicrographs were taken, a very rapid procedure with our Philips microscope. The average observation time for 1 section was 15 minutes. The total amount of time for microscopic examination sums up to 250 hours or 100 days' work.

RESULTS

Our results, so far as virus-like bodies are concerned, were completely negative in all cases of chronic leukemia, of lymphosarcoma and of Hodgkin's disease. They were also negative in 15 patients with acute leukemia.

Only in the bone marrow of one adult patient with acute leukemia could one group of virus-like particles be found. This is shown in figure 1. These particles were located extracellularly in an interstitial space, and there was no relation to neighboring cells. They measured 750 to 950 Å in diameter and had a distinct limiting membrane and a dense central body. They strongly resembled virus-like bodies described in animal tumors and leukemias.

DISCUSSION

In an intense effort carried out over several years it was possible to detect only a single group of virus-like particles in 1 case of acute leukemia of 16
patients with acute leukemia and 27 patients with other malignant blood diseases. It is obvious that this result can in no way be accepted as an evidence for the viral origin of the disease.

In this connection we have to report a recent observation which introduces another complicating element. We have tried to cultivate the virus of serum hepatitis in tissue cultures of the so-called Chang strain,7 which is derived from an explant of human liver. In three of eight trials we have found particles in the infected and noninfected cultures which are very similar to those observed in our positive preparation of leukemia (figs. 2 and 3). In direct observations of liver cells from hepatitis patients, particles of this size had never been found. We are continuing to observe the Chang strain in the hope of finding out whether this is only a saprophytic virus or if this might be related to a tumor virus. As is often observed with cultured cells, our strain shows evidence of malignancy.

We have published these data to give a quantitative evaluation of our work. We are forced to conclude that a single group will not likely obtain significant results. If other research groups working on the same problem will publish similar surveys, a better understanding might be gained.

SUMMARY

Electron-microscope observations on bone marrows and lymph nodes of 16 patients with acute leukemia and 27 patients with various malignant blood diseases are reported. The approximate number of cells from the cases of acute leukemia which could be examined is reported. In a single instance of acute leukemia a group of virus-like bodies was found. Similar particles, however, have also been observed in several tissue cultures of a Chang strain from human liver.

SUMMARIO IN INTERLINGUA

Es reportate observationes a microscopio electronic in le medulla ossee e le nodos lymphatic de 16 patientes con leucemia acute e 27 patientes con varie maligne morbos del sanguine. Es estimate que le studio ha includite le examine de plus que 100.000 sectiones de cellulas leucemic individual. Corpores virusoide esseva notate in un sol caso de leucemia acute. Tamen, simile particulares ha etiam essite observate in plure histoculturas de cellulas epithelioidne del typo Chang.

REFERENCES


On the Occurrence of Virus-Like Bodies in Human Leukemia

H. BRAUNSTEINER, K. FELLINGER and F. PAKESCH