Virologic Studies in Human Leukemia and Lymphoma: The Herpes-type Virus

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SUMMARY

Selected background information is presented and discussed in the light of newer facts and leads from studies constituting a broad effort aimed at determining whether viruses similar (or different) to those known to be associated with the induction of leukemia and lymphoma in laboratory and domestic animals are also involved in the etiology of human leukemia and lymphoma. Emphasis was placed on studies concerning the establishment and experimental use of hematopoietic cell lines and of a herpes-type virus frequently associated with the cultures. Based on information published or contributed to the Special Virus-Leukemia Program of the National Cancer Institute, as of July 1967, over 125 cell strains have been established from patients with cancer or other diseases and from nondiseased controls. At least 61 of the “cancer” and 5 of the “noncancer” derived lines show herpes-type virus by electron microscopy. The virus(es) has been detected in cultures from patients and controls in laboratories in England, United States, Africa, Australia, New Guinea, Japan, Sweden, and in the United States from several chimpanzees and 1 rhesus monkey—the latter with myeloid leukemia.

That the significance of these and other findings to cancer etiology remains unknown is discussed in relation to key studies that rapidly advancing technology and production of industrial quantities of virus will make possible.

INTRODUCTION

The primary purpose of this paper is to present a brief review of recent background and current facts and leads emerging from studies on the detection and characterization of viruses and virus-like particles in human leukemia and lymphoma materials. Of the biologic agents, antigens, or antibodies most frequently detected or isolated from human leukemia and lymphoma patients to 1967, the unidentified herpes-type virus (HTV) and type “C” particles are those which have received the attention of most investigators who seek to determine whether viruses similar (or different) to those known to be associated with the induction of leukemia and lymphoma in laboratory and domestic animals are also involved in the etiology of human leukemia and lymphoma. In consideration of other papers presented at this symposium, and in view of the intense current interest in recently established human hematopoietic cell lines and an associated virus, particular emphasis is given to the “state of understanding” of the herpes-type virus. Information and technical procedures developed in studies of animal leukemia and candidate human “C” type particles which appear to have particular relevance to the detection of HTV are included.

DETECTION AND DISTRIBUTION OF HTV

Epstein et al. (13) in 1964 reported successful tissue culture propagation of cells from several Burkitt lymphoma biopsies. This was followed nearly concurrently by Iwakata and Grace (42) who were able to culture cells derived from the peripheral blood of a patient with chronic myeloid leukemia. Both patient sources yielded cell lines in which were detected particles having morphologic and other characteristics of herpes-type viruses. Over 125 cell lines from cancer and noncancer patients have been established by investigators in laboratories in England, Australia, New Guinea, Japan, Sweden, Africa, and the United States (e.g., 11, 13–19, 25, 39, 42, 43, 45, 50, 53, 54, 59, 62, 68, 72, 74; B. Clarkson, personal communication). Table 1 presents data which approximate the number of lines which had been established as of July 1967 according to disease and nondisease categories and according to the presence or absence of a herpes-type virus. Viruses other than HTV have not been reported in these lines. It is important to note that the numbers and percentages of this table are approximate. The data were kindly provided by many investigators throughout the world known to be working in this area and in particular working within or related to the Special Virus-Leukemia Program of the National Cancer Institute. The cells of nearly all of these cultures are large, mononucleated, and resemble lymphoblasts. They usually do not attach to the surface of the tissue culture vessel and appear to grow best in static suspension cultures. However, Dr. George Moore at Roswell Park Memorial Institute has had considerable success in growing large quantities of some cell lines in spinner cultures (53).

Comparative immunofluorescent and electron microscopy studies have shown that only 1–5% of the cells of most of these lines are positive at any one time for the antigen and the virus particle, respectively (22, 24, 35, 36, 47). This apparently low incidence of infected cells has made it extremely difficult to extract and purify adequate quantities of virus for further characterization work. Faced with these and other problems imposed by relatively small numbers of apparently infected cells, investigators at Roswell Park attempted to increase virus production by selecting clones of more heavily
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method.

associated with human leukemia and lymphoma. A number of investigators have shown that both the direct
and indirect immunofluorescence techniques detect cells which
produce herpes-type virus particles. Earlier work by Fink
et al. (22, 24) and Henle and Henle (35) revealed a good
correlation between electron microscopic determination of virus
producing cells and immunofluorescence. This firm but inde
pendent correlation was surprising since the Fink-Malnagren
direct reagent was made in rabbits to centrifugal concentrates
of human leukemic plasma containing type "C" particles while
Henle and Henle used an indirect test which employed selected
pools of human gamma globulin. In addition, it was thought
that since approximately $10^6$ virus particles were necessary for
an electron microscopic search to be positive, this particle
visualization technique would be far less sensitive in the detection
of small quantities of virus than either immunofluorescence

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mentary detection technique to electron microscopy, but also
because the reagent and the procedure held promise of bridging
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widely different groups of "viruses" (C and HTV) might be
produced by the same patient and up to 6 lines have been started from the same patient of which only
1 or 2 are positive.

infected cells (39). Using a cloning technique in semisolid agar,
49 sublines were derived from the P3H Burkitt lymphoma line.
Following 4 months of propagation, one of these sublines, des
ignated P3HR-1, showed 15-40% of the cells infected. When
the cells were incubated at 35°C or 32°C for 9-15 days with
out refedding, up to 75% of the cells became immunofluorescent
positive and showed particles by electron microscopy.

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2 widely different groups of "viruses" (C and HTV) might be
associated with human leukemia and lymphoma.

Fink et al. (23) employed antigen derived from leukemic
plasma for the induction of antibodies in heterologous hosts
(rabbits and monkeys). The antibody was then conjugated
with fluorescein isothiocyanate and used as a specific stain for
the detection of antigen in cells of the buffy coat, bone mar
row and biopsies, or tissue culture explants thereof, derived
from human patients with leukemia and lymphoma.

The plasma antigen used for inducing the antibody reagent
was a pooled concentrate separated by differential ultracen-
trifugation from 5 cases of human leukemia in which "virus-
like" particles had been observed by A. J. Dalton, but in which
mycoplasma-like particles were not seen on electron micro
scopic examination. Absorption of the serum reagent was car
ried out with various constituents of normal blood—erythrocytes representing the known ABO blood groups and major
Rh factors, platelets, and plasma derived from 10-14 humans
—until no reaction with normal blood factors could be demon
strated by hemagglutination or micro double-diffusion studies
and the presence of a slight excess of human plasma proteins
could be demonstrated. In addition to controls on the technical
procedures, serum controls included: (a) rabbit antiserum
prepared against the concentrated plasmas of 5 normal human
subjects in which "virus-like" particles had not been observed;
(b) antibody prepared against the plasma of one leukemic
patient, in which "virus-like" particles were not demonstrated,
but in which many "mycoplasma-like" forms were easily iden
tified by electron microscopy; and (c) antibody prepared
against a mouse leukemia virus (Rauscher strain). In addi
tion, reagent antibody was prepared in adult rhesus monkeys
against a second pool of human plasmas containing "virus-
like" particles.

Control studies on antigens included testing of the various
antiserum against: (a) 11 different human strains of myco
plasma; (b) cells infected in tissue culture with pure cultures
of the Yaluo, herpes simplex, herpes zoster, and influenza
viruses, and the Eaton PPLO agent; and (c) 25 bone marrow
specimens from either normal human volunteers or patients
ill with other diseases.

Of 72 leukemic patients tested (23), 49 (68%) showed re
action with the antihuman leukemic fluorescent antibody.
Eight of them also showed positive reactions to the anti
Rauscher virus fluorescent antibody, as did 8 other patients
who failed to react positively with the antihuman leukemic
reagent. None of the specimens tested showed positive reac
tions against other control sera, nor did any of the antigen
control specimens react positively with the antihuman leu
kemia fluorescent antibody. These studies were confirmed

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**Table 1**

<table>
<thead>
<tr>
<th>Category</th>
<th>Patients cultured/ Patients attempted*</th>
<th>Patients HTV + / Patients cultured</th>
<th>Lines HTV + / Lines cultured</th>
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<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Percent</td>
<td>No.</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>31/61</td>
<td>51</td>
<td>19/31</td>
</tr>
<tr>
<td>Lymphoid leukemia</td>
<td>15/111</td>
<td>14</td>
<td>4/15</td>
</tr>
<tr>
<td>Myeloid leukemia</td>
<td>34/138</td>
<td>25</td>
<td>14/34</td>
</tr>
<tr>
<td>Sarcoma-carcinoma</td>
<td>25/54</td>
<td>46</td>
<td>7/25</td>
</tr>
<tr>
<td>Total (cancer)</td>
<td>105/364</td>
<td>29</td>
<td>44/105</td>
</tr>
<tr>
<td>Other diseases</td>
<td>5/54</td>
<td>9</td>
<td>1/5</td>
</tr>
<tr>
<td>Normal</td>
<td>14/81</td>
<td>17</td>
<td>3/14</td>
</tr>
<tr>
<td>Total (noncancer)</td>
<td>19/135</td>
<td>14</td>
<td>4/19</td>
</tr>
</tbody>
</table>

*Approximate. Successful attempts understandably are nearly always reported; nonsuc
cesses, usually not.

These numerators are not identical because several positive lines have been started from the same patient of which only
1 or 2 are positive.

Summary: Herpes-type virus (HTV), prototype Epstein's EBI, and human cell "lines."
substantially by Dabieh et al. (8), Levine et al. (46), and in part by Yohn and Grace (73).

One of the most recent and most comprehensive studies to assess the significance of electron microscopy and immunofluorescent findings in relationship to the stage of disease was reported by investigators of Roswell Park Memorial Institute, Buffalo, New York. Levine et al. (46) examined 128 blood samples from 73 patients with leukemia and from 11 nonleukemia patients. The samples were studied by electron microscopy using thin sections of plasma pellets and by immunofluorescence in peripheral blood cells. Their results showed that 29% of acute lymphocytic leukemia, 45% of acute myelocytic leukemia, and 17% of chronic myelocytic leukemia patients had at least 1 buffy coat specimen interpreted as immunofluorescent positive. The difference between acute myelocytic leukemia and chronic myelocytic leukemia is statistically significant. This could mean that there was less chronic myelocytic leukemia specific antigen in the plasma pellet used to prepare the antiserum for the fluorescent antibody test, or that chronic myelocytic leukemia cells contain less detectable antigen. Another interesting comparison is that 27% of the total specimens collected from all 3 types of leukemia were fluorescent antibody positive, 52% of the type “C” particle positive (by electron microscopy) specimens were fluorescent antibody positive, and only 21% of the electron microscopy negative specimens were fluorescent positive. This indicates a significant correlation between the presence of type “C” particles in plasma and fluorescent antigen in buffy coat cells of leukemia patients. Within this same group of patients, 7% of those in remission and 19% of those in relapse were positive for virus-like particles. Comparatively, 20% of the bone marrow cells of patients in remission and 35% of patients in relapse showed positive immunofluorescent reactions.

Of considerable additional interest is the recent report by Yohn and Grace (73) that pooled normal marrow is capable of completely absorbing the Fink type immunofluorescent antibody prepared against human “C” type virus-like particles. This finding suggested the following alternatives for consideration: (a) the immunofluorescent antibody prior to absorption with bone marrow is capable of detecting an antigen of immature leukocytes which under ordinary conditions rarely appears in the peripheral blood, or (b) a virus or virus antigen related to virus-like particles recovered from human leukemic plasma was present in the pool of adult human bone marrow. The latter consideration would be consistent with the reasonable assumption that while less than 1% of the population can be expected to develop leukemia, the vast majority of people are infected with a virus(es) which may be capable of inducing leukemia. This assumption would also be in line with the known relationship between infection and disease in polio and other “necrotizing” viruses. If this relationship between infection and disease with necrotizing viruses were true of leukemia viruses, then it would be reasonable to expect that the bone marrow may be the site for latent retention of virus and/or of virus antigen. This latter hypothesis, while attractive, does not appear to be supported by Fink et al. (23), who failed to detect immunofluorescent antigens in the bone marrows of normal individuals or of patients with diseases other than leukemia or lymphoma. One would expect that if sufficient antigen were present in bone marrow to allow complete adsorption of immunofluorescent antibody, there should also be enough antigen to be detectable with the unadsorbed immunofluorescent antibody. Fink (20), however, suggests that “the bone marrow of normal individuals might be expected to contain latent leukemia virus, present in an amount so small as to be undetectable when only a few cells were examined, but in an amount sufficient to reduce the titer of specific antibody when measured in dry weight in grams.”

Further studies by Fink et al. (21) with the antihuman “C” type fluorescent antibody showed that cells in tissue culture derived from biopsies of 9 separate cases of Burkitt lymphoma and leukocyte cell lines derived from 6 cases of human leukemia all contained positive fluorescent reactions and “herpes-like” viral particles when examined by electron microscopy.

Fink et al. (22) interpret their findings to support the thesis that the antigen detected by immunofluorescence with the antihuman leukemia antibody “is concerned with the leukemic process.” Although these data are concerned with the hypothesis of a viral etiology of human leukemia, it remains to be determined whether the antigen being detected is of viral origin.

Further similar studies by Henle et al. (38) and Mayyasi et al. (49) using an indirect immunofluorescent method showed that human sera which were immunofluorescent positive contained antibodies directed against the virion as evident from electron microscopic determination of antibody coating and agglutination of virus particles extracted and concentrated from Burkitt cell lines. Additional studies by Henle and Henle (34) revealed that on pulse exposure of cells containing HTV to thymidine-3H both viral and cellular DNA became labeled. Later, fluorescent cells showed the label to be present in the cytoplasm, whereas in nonfluorescent cells it remained restricted to the nucleus. The labeling of cellular DNA was substantially lower in cells lethally X-irradiated 4—7 days prior to exposure to the isotope. This treatment, however, did not prevent the labeling of immunofluorescent cells. Unequivocal proof that immunofluorescent cells are those that contain HTV was reported by zur Hausen et al. (75), who were able to pick immunofluorescent cells for electron microscopic examination. The results showed clearly that immunofluorescent negative cells revealed the presence of virus particles.

Several groups of investigators have employed virus-containing Burkitt tumor cells to detect antibodies in human sera by immunofluorescence (35), complement-fixation (1, 26), immunodiffusion (55), and antibody coating techniques (38, 40). Using appropriate immunofluorescence tests with Burkitt lymphoma cell lines, Henle and Henle (34) have shown that the age distribution of antibodies in noncancer American and African children revealed a pattern similar to that for other common viruses. The incidence of positive sera was high (70%) at age 0—3 months, declined between age 4—24 months, and then at age 4 years rose to 50% where it remained until adolescence. Greater than 80% of sera from people at age 40 showed antibodies to HTV. Various investigators using different serologic detection techniques have shown that nearly all sera from Burkitt patients contain HTV antibodies and in most cases at levels
higher than sera from nondiseased controls. There is some indication that Burkitt patients in remission contain generally higher levels of antibody, whereas on reoccurrence of the tumor, the antibody level declined. Very intriguing information was reported by Old et al. (55), who showed an unusually high incidence and level of HTV antibodies in the sera of patients with postnasal carcinoma. Gerber and Birch (26) using a sensitive complement-fixture test with partially purified HTV prepared from the Burkitt P3 line by investigators of the Pfizer Company, Maywood, New Jersey, showed that 90% of sera from healthy adults and patients with malignant diseases had antibodies to HTV. They also tested 5 species of nonhuman primates and found that all except baboons had a high incidence of complement-fixing antibodies to the P3 antigen and that none of the sera from 9 species of laboratory or domestic animals reacted with this antigen. Their finding that 10 of 10 chimpanzees had relatively high levels of HTV antibody is particularly interesting in view of the report by Landon et al. (45), who have established hematopoietic cell lines from these un inoculated chimpanzees, all of which contain a herpes-type virus. The virus in the chimpanzee cell lines is antigenically similar, if not identical, to HTV contained in the human lines (J. L. Landon, personal communication). Studies by various investigators have shown clearly that HTV is not related antigenically to any of the known human or animal herpes viruses, nor is it related to other nonherpetic human and animal viruses that have been detected. Although additional work needs to be done, there is good evidence to suggest that the herpes-type viruses in at least some of the Burkitt lymphoma lines from patients in Africa and the United States, as well as cell lines started from American leukemic patients, are related (49). It is obviously important to determine whether more than one major antigenic type of the virus exists in the more than 60 virus-containing human cell lines already established.

HTV has been detected in the peripheral leukocytes or tissues of several leukemia patients and in peripheral leukocytes from 1 normal person prior to propagation of cells in tissue culture and in biopsy materials from cases of Burkitt lymphoma (30). In one study, investigators of the Pfizer Company, Maywood, New Jersey, E. Jensen et al., personal communication) detected by electron microscopy HTV in peripheral cells from a child with congenital leukemia. The virus persisted in the cells following propagation in tissue culture. HTV was also detected in the peripheral blood of the mother but not of the father of this child. In this regard, Moore et al. (personal communication) and Gerber and Birch (26) have established cell “lines” from buffy coat cells of normal, nondiseased donors. Several of these “lines” were shown to contain HTV. In view of this, it may be necessary to reevaluate the previous assumption that the cells of lines started from human leukemia and lymphoma patients were neoplastic rather than normal or non-neoplastic. Definitive karyotypic analyses have, however, been done with several of these lines, the results of which suggest that some of the cells of at least some of these lines are neoplastic. As an example, the chronic myeloid leukemia patient from which the M-2 (11) line was established was shown to be positive for the Philadelphia chromosome (48). Periodic chromosome analyses on the cultured cells showed retention of the Philadelphia marker.

Perhaps related to these observations is the report by Griffin et al. (28, 29), who detected by electron microscopy the presence of virus particles morphologically of the herpes type in the pulp of a tooth removed from a Burkitt lymphoma patient with an active maxillary tumor. Similarly, they detected herpes particles in the tooth of an apparently normal child, who 5 years previously had been successfully treated for a Burkitt lymphoma of the maxilla. Unfortunately, virus isolation and identification tests were not performed, and therefore it is not known whether these particles represented herpes simplex virus, other known herpes viruses, or HTV. Herpes simplex virus has, in fact, been isolated from biopsy specimens of Burkitt jaw tumors (3, 65, 71) as well as from the throats of normal children resident in the same area of Africa (65).

**BIOLOGIC ACTIVITY OF HTV**

Until very recently, infectivity or other kinds of biologic activity had not been shown with any of the HTV isolates. The virus appeared to replicate (persist) only in the tissue culture cells in which it was originally detected and numerous attempts to extract HTV infective for other cell cultures and for animals were unsuccessful. More recently, however, several investigators have succeeded in showing infectivity of HTV and in several cases apparent disease induction in animals following their inoculation with cell and cell-free materials containing the virus. Stewart et al. (67) showed that hamsters developed a progressive encephalitic syndrome leading to death following intracerebral inoculation with human cells or cell extracts known to contain HTV. Investigators of the Pfizer Company, Maywood, New Jersey (F. Durr, personal communication) confirmed this observation and showed in addition that various strains of mice developed a similar disease following intracerebral inoculation. Grace et al. (personal communication) at Roswell Park Memorial Institute showed that newborn kittens also developed this disease following intracerebral inoculation with HTV. In none of these studies, however, were virus particles detected by electron microscopy in thin sections of neural tissue.

Henle et al. (37) have detected a reasonably frequent chromosome aberration in tissue culture lines begun from human leukemia and lymphoma patients and known to contain HTV. This modification of chromosome No. 10 and replicating virus particles also appeared in some normal human cell lines following exposure to HTV.

At this point it appears important to reiterate that while particles of the HTV generally are indistinguishable in morphology and size to other known members of the herpes virus group, there are several quantitative and qualitative differences. First, Dalton et al. (personal communication) reported apparent morphologic differences between the unidentified herpes-type virus and known members of the herpes group. Particles associated with tissue cultures of human leukemia and lymphoma cells acquired a finely granular coat as distinct from an envelope formed from the nuclear or plasma membrane. No such coat was found to develop in relation to the known herpes viruses.
Increased electron density of peripherally condensed chromatin and beading of mitochondria were also characteristic of cells of the human leukemia and lymphoma tissue cultures which contain virus particles. Such features are not seen in cells infected with known herpes viruses. These investigators cautioned, however, that the latter phenomena could be nonspecific host-cell reactions to viral infection. Secondly, the usual findings in attempts to replicate more common herpes viruses, especially herpes simplex virus, is that the particles nearly consistently contain nucleoids (J. T. Grace et al., personal communication). Conversely, a persistent finding in the human leukemia and lymphoma cell lines is that many to most of the particles of HTV are devoid of nucleoids. Thirdly, most attempts to show HTV infectivity have been initiated with partially purified virus following various procedures, including density gradient centrifugation, sonication, freeze-thaw cycles, and chromatographic column separation. Based upon the reasonable assumption that these factors and procedures may have been deleterious to infectivity, if not antigenicity, it appears, in retrospect, not unusual that attempts to show infectivity have been almost invariably unsuccessful. A fourth and perhaps equally important factor is that even when apparently intact particles were separated from cells, the amount of virus (complete and "incomplete") available per unit inoculum was low and, in fact, may have been too low to initiate infection. In this regard, it is important to recall the original observation of Bryan et al. (5) who showed with the Rous sarcoma virus that ability to infect, latent period, severity of disease, and recoverable virus were dependent on the amount of virus contained in the infecting dose. These observations were confirmed by others (31, 63) and most recently were shown to apply to at least one murine leukemia virus (58).

Zeve et al. (*In vitro* Cell Free Transfer of Burkitt Associated Virus to Human Leukocytes. Submitted for publication to Science, September 1967) successfully transferred HTV from a Burkitt cell line (MOB-8) to another human buffy coat culture (RPMI-2367) the latter of which has been negative for virus and virus antigen when examined electron microscopically and immunofluorescently, respectively. Using a Belco dual spinner flask the MOB-8 cells were placed on one side of a millipore filter (220 mμ, GS) and the RPMI-2367 cells on the opposite side. Following cultivation for 164 hours, the RPMI-2367 cells were shown by electron microscopy to contain particles morphologically identical to HTV. Appropriate tests to determine the integrity of the millipore filter strongly suggested cell-free infection by HTV of the recipient cell line.

Grace et al. (personal communication) at Roswell Park Memorial Institute very recently showed that a virus-free line of cells started from buffy coat of a myeloid leukemia patient (6410) rapidly declined in viability with death 5 days later after exposure to high multiplicities of HTV (derived from the HRI-K clone of the P3J Burkitt lymphoma cell line) for the order of 500-1,000 virus particles per cell. Electron microscopy and direct immunofluorescent staining indicated that almost all cells were infected and that large amounts of new virus particles were produced. A virus carrier state of 6410 cells was observed when these cells were infected with doses of virus ranging from 1-250 virus particles per cell. In these experiments, therefore, a clear dose response was observed. With lower multiplicities of virus input as long as 3–4 weeks were required for the appearance of immunofluorescence in 30–50% of the cells. For further studies these investigators reasoned that since virus adsorption is the first step of infection, an assay for adsorption may help to determine the target cell capable of supporting viral replication. Following this approach, they further confirmed biologic activity of HTV by successful infection of 3 additional human cell lines, all of which were negative by immunofluorescence and electron microscopy prior to infection. Their studies indicate that among the cells tested thus far by immunofluorescence and electron microscopy, only human and monkey lymphocytes or lymphoblast-like cells have the ability to adsorb virus. All such immunofluorescent positive cells when observed with phase contrast microscopy were mononucleated, uniform in size, approximately 12 microns in diameter with the nucleus occupying 75% of the cell. These and other findings seem to indicate an affinity of the virus for lymphocytic or lymphoblastic type cells of primate origin. These conclusions appear to be supported by the work of Gerber and Birch (26), who by complement-fixation detected antibodies to HTV in human, chimpanzees, and 4 species of monkeys but not in rats, guinea pigs, cats, dogs, goats, sheep, pigs, horses, and cows.

**DISCUSSION**

Since no DNA virus has yet been found to induce leukemia or lymphoma, and since all of the known leukemia and lymphoma viruses are of the same subgroup of RNA agents, it would appear logical, from the animal prototype diseases, to concentrate the search for a counterpart human agent on viruses of the medium-sized, membrane-bound, RNA variety. However, at the present stage of knowledge and experience, it would be unwise to exclude viruses of any type as potential candidates for leukemogenesis simply because no member of the group has thus far been discovered which induces the usual forms of leukemia or lymphoma. The facts that the medium-sized, membrane-bound, RNA viruses of avian leukemia can cause kidney carcinomas as well as leukemia and that fibrosarcomas of nearly identical gross and microscopic morphology can be induced in hamsters with both polyoma (DNA) and Rous sarcoma (RNA) viruses further caution against stereotyping all oncogenic virus-host interactions on the basis of the usually predictable tumor responses of laboratory animals to laboratory strains of tumor viruses.

After successful propagation of a virus in sufficient quantities to support its further investigation, the next step in the search for viruses associated with human leukemia and lymphoma is the demonstration of the capacity to induce neoplasia in some laboratory test-animal or tissue culture system. Testing in animals of the homologous species is impractical. Although the production of neoplasia in other animals would not constitute proof for oncogenic potential in man, it would demonstrate that this basic property is possessed by the virus and would represent important evidence to support the possibility of a similar action in man (6). In like manner, the induction of *in vitro* cytomegalias (6) in human or other cell lines would
provide valuable supporting evidence, but would not alone constitute proof for oncogenicity of the agent in man.

The oncogenic dose of Rous sarcoma virus is several orders of magnitude higher for hosts of foreign species than for the natural host (60, 61); this type of quantitative relationship may also hold for human candidate viruses tested in other animal species.

The problems of testing for oncogenicity in animal and tissue culture systems have been described by many others. The induction of leukemia in mice, inoculated when newborn with specimens from human leukemia, has been reported by investigators from 3 different laboratories (4, 12, 64), but others (27, 52) have failed to confirm this finding. Approximately 700 primates mostly rhesus monkeys, but including animals of 9 species (cynomolgous, African green, baboon, pig-tail macaque, stump-tail macaque, Galago, Bonnet, marmosets, and chimpanzees), have been inoculated when newborn with specimens from human leukemia and lymphoma by investigators both at the National Cancer Institute and at other institutions collaborating in the Special Virus-Leukemia Program of the National Cancer Institute (F. J. Rauscher and A. J. Pallotta, unpublished data). No neoplastic responses have been observed thus far. However, only about 220 of these animals are more than 1 year of age, and the oldest are only about 4 years old. If a leukemogenic agent is responsible for the age-peak which occurs at 3–4 years in human childhood leukemia, and if susceptible primates approach the human host in time-to-induction of disease, the primate animals would have to be observed for several additional years before their responses could be interpreted as definitely negative.

Since most of the studies presented in this paper were based on materials derived from patients with Burkitt's lymphoma, it is important to note that herpes simplex (3, 65, 71), vaccinia (10), Bunyamwera (70), an unidentified agent (9), and Reo viruses (3, 70), in addition to HTV, have been detected in materials derived from Burkitt lymphoma patients. It is of interest and possibly significant that only HTV has been detected or isolated from American leukemia patients and from Burkitt lymphoma patients studied in the United States. Of the above agents and in addition to HTV, Reo viruses, particularly type 3, appear to be most uniformly present in Burkitt lymphoma patients. In one study, Reo viruses were isolated from biopsy material from 25 of 90 cases of Burkitt's tumor in Uganda and Tanzania, whereas this virus was isolated from only 1 of 21 other patients with non-Burkitt tumors (3). Neutralizing antibody studies using type 3 Reo virus have shown that there is a relationship between this virus and Burkitt's tumor (2). Antibody was found in 53 of 72 cases of Burkitt's tumor, but in only 12 of 65 controls consisting of a variety of other tumor cases in normal children. Comparable results were obtained using hemagglutination-inhibition tests on another series of serum specimens (47). Stanley et al. (66) reported the induction of lymphomas in mice following their inoculation with spleen cells from a mouse presenting with chronic lesions induced after neonatal Reo virus 3 infection. The disease appears to be a manifestation of an autoimmune disease, although some features are reported as resembling Burkitt's tumor (56). These preliminary results and the report by Parker et al. (57) on the isolation of Reo 3 virus from mosquitoes, while suggestive of a more than casual relationship with Burkitt's tumor, must obviously await confirmation. It seems clear from the studies of Kajima and Pollard (44) and Huebner (40) that mice of all strains carry viruses capable of inducing leukemia and lymphoma. Since these leukemia viruses are known to be helpers (33, 41) for at least one of the known murine sarcoma viruses (32, 51), it would appear unwarranted to ascribe an etiologic role to a human isolate of a Reo virus and the induction of a lymphoma in a laboratory mouse.

The case for assigning an oncogenic role to HTV at the present time is certainly as tenuous as that concerned with other viruses. Incineration of HTV may be strengthened by the apparent relationship of a herpes virus to the induction of renal carcinomas in the frog, and by the recent report of Churchill and Biggs (7) and Burmester (personal communication) that the agent of Marek's disease may be a herpes virus. Similarly, cocarcinogenic properties have been reported for herpes simplex virus when inoculated intradermally into mice in conjunction with multiple applications of 3-methylcholanthrene (69). While these and other studies presented in this paper and at this symposium certainly do not detract from the high priority need for expanded studies on HTV, they obviously have not yet definitively determined whether HTV is (a) an international ubiquitous passenger virus or (b) etiologically related, even indirectly, to human cancer.

REFERENCES


HERPES-TYPE VIRUS

JULY 1968

1317
59. Pulvertaft, R. J. V. A Study of Malignant Tumours in Nigeria.


Virologic Studies in Human Leukemia and Lymphoma: The Herpes-type Virus

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