Search for antigens and antibodies crossreactive with type C viruses of the woolly monkey and gibbon ape in animal models and in humans

(type-C viral antigen expression/radioimmunoassay/virus isolation from human cells)

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ABSTRACT: Several reports have indicated the presence of type-C viral antigens in human tumors and of viruses closely related to those of the woolly monkey and gibbon ape in cultured human cells. In the present study, attempts to detect woolly monkey viral antigens in human tissues, or antibodies directed against structural polypeptides of woolly monkey viruses in human sera, were unsuccessful. In contrast, it was possible to demonstrate viral antigens in tissues and antibodies reactive to viral components in several animal and even primate model systems. Further evidence against the presence of woolly monkey viruses in humans is our inability to identify spontaneous or chemically induced viruses of this group in more than 300 individual cultures of human origin examined. These findings argue against the likelihood that viruses closely related to the woolly monkey virus are associated with human tumors or are common infectious agents of man.

Type-C RNA viruses are genetically transmitted within the DNAs of many vertebrate species. The detection and subsequent isolation of these viruses from primates, including the woolly monkey (1), gibbon ape (2, 3), and baboon (4-6) has provided impetus to the search for analogous viruses of humans. Wooly monkey and gibbon ape virus isolates have been shown to be closely related immunologically (7-10) and biochemically (11, 12), but can be readily distinguished from type-C RNA viruses of baboon origin. Whereas the baboon virus has been demonstrated to be an endogenous virus of the baboon (13), viruses of the woolly monkey/gibbon ape group do not appear to be genetically transmitted in those primates (14). Instead they are infectious viruses that are etiologically associated with a high frequency of lymphoid neoplasia in certain gibbon ape colonies (15).

Recently, there have been reports of the detection in human tissues of antigens related to the reverse transcriptase (16, 17) and the major structural polypeptide (p30) (18, 19) of the woolly monkey/gibbon ape virus group. Antibodies to the gp70 and p30 polypeptides of this virus group have also been reported to be widespread in the human population (20). Moreover, several laboratories have isolated type-C viruses closely related to these viruses from cultured human cells (21-26). Such findings could have important implications toward defining a viral etiology to human cancer.

For several years, our laboratory has been involved in the search for evidence of type-C RNA viruses of humans. In the present study, we report the lack of detectable major structural antigens of the woolly monkey/gibbon ape virus group in human tissues as well as the absence of antibodies in human serum directed against structural polypeptides of these viruses. Furthermore, attempts to isolate viruses related to woolly monkey/gibbon ape viruses from a large number of cultured human cells have been unsuccessful. Thus, the present findings do not substantiate previous studies indicating the common occurrence of viral antigens or of viruses closely related to woolly monkey/gibbon ape virus group in humans.

MATERIALS AND METHODS

Cells. Cells were grown in Dulbecco’s modification of Eagle’s medium supplemented with 10% fetal calf serum (Colorado Serum Co., Denver, Colo.). Human fibroblasts derived from explants of embryonic tissues and from skin punch biopsies, and human tumor cell lines derived in this laboratory were used and have already been reported (27). An established line of Mus caroli embryo cells (28) was obtained from H. Coon, National Cancer Institute.

Tissues and Sera. Human normal liver and tumor tissues and human sera were obtained from J. Gruber and L. Sekely, Office of Resources and Logistics, National Cancer Institute. Additional tissues from patients with acute myelogenous leukemia (AML) were provided by R. Gallo, National Cancer Institute. Sera from cancer patients immunized with formalin- killed Rauscher murine leukemia virus (R-MuLV) (29) were provided by E. Hersh, M.D. Anderson Hospital, Houston, Texas. Mouse tissues of the NIH Swiss strain were obtained from the National Institutes of Health, Bethesda, Md. Domestic cat (Felis catus) tissues were provided by R. Amity, Fairfax County Animal Shelter, Fairfax, Va. Baboon tissues (Papio cynocephalus) were from S. Kalter, Southwest Foundation for Research and Education, San Antonio, Texas; rhesus monkey (Macaca mulatta) tissues were provided by D. Dalgaard, Hazleton Laboratories, Vienna, Va. Sera from gibbon apes (Hylobates lar) from high leukemia-incidence colonies were kindly provided by T. Kawakami, University of California, Davis.

Radioimmunoassays. Double antibody precipitation and competition radioimmunoassays for the p30 polypeptides of woolly monkey virus, R-MuLV, and the endogenous P. cynocephalus baboon virus were performed as previously described (9).

Purification of Type-C Viral p30s from Cell Extracts. Viral antigens were purified from cell extracts by sequential DEAE ion-exchange chromatography and agarose-gel filtration in the presence of 6 M guanidine-HCl as described in detail previously (31, 32).

RESULTS

Analysis of Viral p30 Antigen Expression in Cell Extracts of Mammalian Species with Known Endogenous Type-C Viruses. Previous studies demonstrated that structural com-

Abbreviations: R-MuLV, Rauscher murine leukemia virus; AML, acute myelogenous leukemia.
ponents of an endogenous mouse type-C virus could be isolated from mouse cells in the absence of detectable virus release and shown to be biochemically and immunologically indistinguishable from known mouse type-C viral polypeptides (31, 32). To determine the feasibility of attempts to isolate viral p30 related to that of woolly monkey/gibbon ape viruses from human cells, we initially investigated M. caroli cells. A type-C virus, immunologically related to the woolly monkey/gibbon ape virus group, is inductive from virus-negative cells of this species (28). As shown in Fig. 1, DEAE-cellulose chromatography of M. caroli cell extracts led to elution of antigen cross-reactive with woolly monkey virus p30 at 0.07-0.09 M KCl. The fractions containing the greatest amounts of woolly monkey viral p30 crossreactive antigen were subjected to agarose gel filtration. As shown in Fig. 2, the woolly monkey viral p30 crossreactive antigen cochromatographed with 125I-labeled woolly monkey viral p30 at a molecular weight of 28,000-30,000 relative to standards. As summarized in Table 1, the final extent of viral p30 purification from M. caroli cells achieved by sequential ion-exchange chromatography and agarose-gel filtration was 28-fold. Analogous methods led to similar degrees of purification (32-75-fold) of type-C viral antigens from liver tissues of several other species including cat, rhesus monkey, and baboon (Table 1).

The antigenic activity partially purified from M. caroli cells was immunologically characterized. As shown in Fig. 3A, this antigen competed efficiently in an immunoassay for woolly monkey virus p30 and was poorly, if at all, reactive in immunoassays for either baboon virus or mouse type-C viral p30 antigens. In contrast, the viral p30 antigenic activity purified from rhesus monkey liver tissue (Fig. 3B) competed with greatest efficiency in the baboon virus p30 assay and showed no detectable reactivity in either mouse or woolly monkey viral p30 assays. A very similar pattern of antigenic reactivity was also observed with p30 isolated from P. cynocephalus baboon liver (data not shown). These findings indicate that p30 antigens detectable at very low levels in cells of several mammalian species could be purified and shown to possess physical and immunologic characteristics indistinguishable from those of the p30 polypeptides of endogenous viruses present in those cells.

Absence of Detectable Woolly Monkey Type-C Viral p30 in Human Tissues. Utilizing purification procedures described above, we investigated the question of woolly monkey viral antigen expression in human tissues. Of 130 normal and neoplastic tissues tested individually, the majority were not reactive even at very high protein concentration (1 mg/ml) in immunoassays for woolly monkey viral p30. Crude extracts of 24 tissues exhibited significant reactivity in this assay. However, most of these extracts were also reactive in immunoassays for mouse or baboon type-C viral p30s. To determine whether the immunologic reactivity detected was due to expression of a virus related to the woolly monkey isolate, we subjected the extracts to DEAEx-exchange chromatography. When indi-

**Fig. 1.** Partial purification of endogenous viral p30 antigen from M. caroli cells. About 1 g of cell extract containing 2.1 x 10^4 cpm of 125I-labeled woolly monkey viral p30 (<1 ng of viral antigen) was subjected to DEAE ion-exchange chromatography as described in Materials and Methods. Protein concentrations of individual fractions were determined by the method of Lowry et al. (30). Fractions were pooled in groups of five, concentrated by lyophilization, and tested for p30 antigenic reactivity in an immunoassay for p30 of the woolly monkey virus. Immunoassay results (p30 antigenic reactivity) are expressed as the reciprocal of the highest antigen dilution at which 50% displacement of 125I-labeled p30 was achieved and represent mean values from three separate determinations. A, KCl concentration.

**Fig. 2.** Agarose-gel filtration analysis of p30 antigenic reactivity partially purified from M. caroli cells. Fractions obtained by DEAE-chromatography (Fig. 1, fractions 100-115) that contained the highest levels of p30 antigenic reactivity were pooled, concentrated by lyophilization, and subjected to agarose-gel filtration in the presence of 6 M guanidine-HCl. Fractions were pooled in groups of five, concentrated by lyophilization, and assayed for p30 antigenic reactivity as described in the legend to Fig. 1.

**Fig. 3.** Immunologic analysis of p30 antigenic reactivities partially purified by sequential ion-exchange chromatography and agarose-gel filtration from (A) M. caroli cells and (B) rhesus monkey liver cell extract. Immunoassays included those for the p30 antigens of woolly monkey virus ( ), P. cynocephalus baboon virus (O--O), and R-MuLV ( ).
Table 1. Partial purification of viral p30 polypeptides of known endogenous type-C RNA viruses from virus-negative cells

<table>
<thead>
<tr>
<th>Cell extract prepared from:</th>
<th>Endogenous viral p30 antigen assayed</th>
<th>Increase in specific activity following purification by*:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DEAE-chromatography</td>
</tr>
<tr>
<td></td>
<td></td>
<td>125I-Labeled p30 antigen</td>
</tr>
<tr>
<td>Mouse</td>
<td>Wooly monkey virus</td>
<td>Mouse</td>
</tr>
<tr>
<td>M. caroli</td>
<td>R-MuLV</td>
<td>3.6</td>
</tr>
<tr>
<td>M. musculus</td>
<td>RD114</td>
<td>5.6</td>
</tr>
<tr>
<td>Cat (F. catus)</td>
<td>Baboon virus</td>
<td>5.0</td>
</tr>
<tr>
<td>Rhesus (M. mulatta)</td>
<td>Baboon virus</td>
<td>3.5</td>
</tr>
<tr>
<td>Baboon (P. cynocephalus)</td>
<td>Baboon virus</td>
<td></td>
</tr>
</tbody>
</table>

* Cell extracts were partially purified by sequential DEAE 52 ion-exchange chromatography and agarose-gel filtration in the presence of 6 M guanidine-HCl. The degree of purification at each step was determined by measuring the increase in specific activity relative to cellular protein of both 125I-labeled p30 marker added to the original extract and p30 immunologic relativity of the known endogenous virus, as indicated. Results are expressed as the number of fold-increase in specific activity achieved.

Individual column fractions were tested in the immunoassay for woolly monkey viral p30, reactivity was dispersed at very low levels throughout the column without evidence of a discrete peak.

To increase the probability of detection of woolly monkey viral p30 crossreactive antigen, we pooled the column fractions containing marker 125I-labeled viral p30 and subjected them to agarose-gel filtration. As shown in Table 2, no woolly monkey viral p30 reactivity was detected following this additional purification step in any of the human tissues extracted. Thus, under conditions where endogenous type-C viral p30 could be partially purified from virus-negative cells of several species, none of the 130 human tissues tested exhibited evidence of woolly monkey viral p30.

Analysis of Human Sera for Antibodies to Woolly Monkey Virus p30. As another approach to the question of whether a type-C virus similar to the woolly monkey virus is an infectious agent of humans, human sera were tested for antibody to 125I-labeled woolly monkey virus p30 (9) or gp70 (33). As shown in Table 3, sera obtained from several gibbon apes infected with viruses of the woolly monkey/gibbon ape group demonstrated readily detectable antibody to woolly monkey viral p30 and gp70. In contrast, sera from patients with a variety of solid and hematopoietic neoplasms as well as from noncancer patients were uniformly nonreactive. In other studies, over 100 sera collected from a random sampling of the population in Southeast Asia also lacked detectable antibodies to either woolly monkey virus p30 or gp70 (data not shown). That the methods were capable of detecting antibodies to type-C viral antigens in humans was shown by tests of sera from tumor patients immunized with R-MuLV (29). These sera demonstrated heightened precipitation antibodies against both R-MuLV p30 and gp70 (Table 3).

Attempts to Isolate Type-C Viruses Related to Woolly Monkey/Gibbon Ape from Cultured Human Cells. Tissue specimens of human origin were explanted and grown in tissue culture. These included 96 fibroblast strains from tumor patients, 53 fibroblast strains from human embryos, 37 from newborns, and 62 from normal controls of other age groups. Fibroblast cultures were transferred at weekly intervals for at least 6 months, and many were passaged until they reached the end stage of fibroblastic growth. In addition, 32 human tumors of different morphologic types, including carcinomas, sarcomas, melanomas, and glioblastomas (27), were established as permanent lines and propagated for 2–5 years in continuous culture. Both normal and tumor cells were tested at monthly intervals for spontaneous type-C virus release by reverse transcriptase assay. In addition, 55 fibroblast cultures and each of the human tumor lines were assayed for type-C virus release following treatment with idodeoxyuridine, a known chemical inducer of endogenous type-C viruses in several other species. None of the human cultures tested was found to release type-C virus specific activity following purification by*:

Table 2. Analysis of normal and neoplastic human tissues for expression of woolly monkey viral p30 antigen

<table>
<thead>
<tr>
<th>Cell extract prepared from:</th>
<th>No. tested</th>
<th>Increase in specific activity following prepurification by: *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DEAE-chromatography</td>
</tr>
<tr>
<td></td>
<td></td>
<td>125I-Labeled p30 antigen</td>
</tr>
<tr>
<td>Normal liver</td>
<td>3</td>
<td>4–5</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>6</td>
<td>3–6</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>3</td>
<td>3–5</td>
</tr>
<tr>
<td>Chronic myelogenous leukemia</td>
<td>2</td>
<td>3–5</td>
</tr>
<tr>
<td>Acute myelogenous leukemia</td>
<td>3</td>
<td>4–6</td>
</tr>
<tr>
<td>Spleen</td>
<td>2</td>
<td>3–7</td>
</tr>
<tr>
<td>Peripheral white blood cells</td>
<td>5</td>
<td>2–8</td>
</tr>
</tbody>
</table>

* Extracts prepared from preselected human tissues with apparent reactivity in the woolly monkey virus p30 immunoassay were partially purified and assayed as described in Table 1.
virus either spontaneously or following chemical induction by
these techniques.

**DISCUSSION**

Several laboratories have recently reported the isolation of
type-C viruses, closely related to infectious viruses of the woolly
monkey and gibbon ape, from cultured human cells. Evidence
for the presence of type-C viral antigens related to this virus
group has also emerged from radioimmunologic tests of type-C
viral p30 antigens in relatively crude extracts of human tissues
(18, 19). Often, in these studies reactivities in immunoassays
for the p30s of other viruses including baboon/RD114 viruses
or even mouse viruses have been reported. One recent study
has indicated the lack of detectable reactivity of several human
tissues tested in an immunoassay for interspecies determinants
of p30 (34). The approach used in the present studies to detect
p30 related to woolly monkey/gibbon ape viruses in human
tissues was based upon methods shown to be successful in the
partial purification of viral p30s from cells of several mammalian
species. These included the cat (F. catus), baboon (P.
cynocephalus), rhesus (M. mulatta), and two mouse species,
M. musculus and M. caroll. In each case, the antigenic cross-
reactivity with viral p30 initially detected in crude tissue ex-
tracts was shown to cochromatograph with tracer quantities of
125I-labeled viral p30 during sequential purification steps and
to demonstrate antigenic determinants indistinguishable from
those of a single known type-C viral p30 in the partially purified
material.

In contrast, the majority of human tissues tested in the present
studies lacked any detectable antigenic crossreactivity with
woolly monkey virus p30. In each case where reactivity was
detected, it was shown to be nonspecific. One of the tissue ex-
tracts exhibiting such nonspecific reactivity was from a patient
with AML, patient HL-8, whose cells were previously reported
to contain woolly monkey virus p30 antigen (19). These findings
indicate the lack of detectable woolly monkey virus p30 in
human tissues and demonstrate that the detection of reactivity
in competition immunoassays in which relatively crude tissue
extracts (18, 19, 35) are used, can lead to erroneous conclusions
concerning the presence of type-C viral antigens.

Direct evidence for the presence of woolly monkey/gibbon
ape-like viruses in humans has come from several recent reports
of the isolation of these viruses from cultured human cells
(21–26). In one case, virus isolated from cultured myeloid cells
of a patient with AML (21, 22, 26) has been shown by highly
specific immunologic and biochemical tests to contain a virus
indistinguishable from the woolly monkey virus (36). This
preparation has also been found to contain a second virus very
closely related or identical to the endogenous virus of the P.
cynocephalus baboon (36). Two other laboratories (23, 25) have
recently isolated type-C viruses from a high proportion of
cultured normal fibroblasts (23) or malignant human tumor
cells (25). Several structural polypeptides (p12, p30, and gp70)
of these viruses have been characterized and shown to be in-
distinguishable from those of the prototype woolly monkey
virus (J. R. Stephenson et al., unpublished observations). The
absence of chemically inducible and/or spontaneous type-C
virus release from more than 200 fibroblast strains and 32
human tumor cell lines examined in the present study appears
to be incompatible with the reported high frequency isolation
of these viruses from cultured human cells (23, 25).

There are conflicting reports concerning the extent of nu-
clotide sequence homology of woolly monkey and gibbon ape
type-C viruses with primate cellular DNA (14, 19, 28). How-
ever, there is agreement among several laboratories regarding
the absence of their nucleic acid sequences in human cellular
DNA (14, 37, 38). Thus, if woolly monkey-like viruses are
present in the human population, it must be reasoned that they
are horizontally transmitted rather than endogenous viruses.
It is known that gibbon apes naturally exposed to the gibbon
ape virus can acquire high-titered neutralizing antibodies to
this virus (15). The present studies demonstrate that sera from
such gibbon apes also precipitate woolly monkey viral p30 and
gp70 at high titer. Independently, Charman et al. (39) have
shown antibodies to woolly monkey/gibbon ape viral p30 in
sera from gibbon naturally exposed to this virus. In contrast,
tests of more than 400 normal human sera and sera from pa-

**Table 3. Analysis of human sera for antibodies to woolly monkey virus and R-MuLV structural polypeptides**

<table>
<thead>
<tr>
<th>Serum obtained from:</th>
<th>No. tested</th>
<th>Antiserum titers for binding 125I-labeled antigens of:*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solid tumors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcoma</td>
<td>45</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>36</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Melanoma</td>
<td>18</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Hemopoietic tumors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute myelogenous leukemia</td>
<td>83</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Chronic myelogenous leukemia</td>
<td>52</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>30</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Noncancer controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinically normal</td>
<td>160</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Lupus erythematosus</td>
<td>35</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Positive controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-MuLV immunized humans</td>
<td>4</td>
<td>NT</td>
</tr>
<tr>
<td>Gibbon apes exposed to gibbon ape virus</td>
<td>3</td>
<td>3200–12800</td>
</tr>
</tbody>
</table>

* Antiserum titers are expressed as the reciprocal of the dilution required to precipitate 10% (500–1000 cpm above a background of 40 cpm) of the indicated 125I-labeled viral polypeptide. The second antibody utilized was directed against partially-purified human IgG and the data thus reflect titers of this antibody class. NT, not tested.
tients with tumors failed to demonstrate any immunologic reactivity against woolly monkey viral p30 or gp70. Because of these results, it can be argued that horizontal infection of humans with type-C viruses of the woolly monkey/gibbon ape group is not a common occurrence.

In species such as the chicken (40) and cat (41), infectious type-C viruses, that replicate at high titer in the animal, have been shown to cause naturally-occurring tumors. The absence of detectable spontaneous release of type-C virus from any of a large number of human cell cultures examined here, suggests that animal models in which infectious type-C viruses can be readily isolated may not be pertinent to the study of type-C virus-induced tumors in man. It should be emphasized, however, that the absence of replicating type-C virus does not exclude their covert presence in humans or the potential role of type-C viruses in disease.

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