Detection of SV40-induced T Antigen in Peripheral Leukocytes of Tumor-bearing Hamsters

Richard M. Jamison

Department of Pathology, University of Colorado Medical Center, Denver, Colorado 80220

SUMMARY

SV40-induced T antigen has been detected by immunofluorescent techniques in leukocytes of hamsters bearing the homologous tumor. The identity of the cells obtained from peripheral blood that contained this antigen is obscure.

INTRODUCTION

The T antigen induced by papovavirus SV40 has been detected in almost every SV40-induced tumor or in vitro transformed cell line (1, 2, 5, 6). To date, cells derived from all primary tumors induced by inoculation of suckling hamsters with SV40 virus have been shown to contain large amounts of this characteristic intranuclear antigen. As a result of bearing the SV40 tumor and its consequent exposure to the T antigen, the hamsters develop high titers of antibody(ies) to the T antigen (3, 9). These antibodies can be demonstrated by immunofluorescence and complement fixation. Although it is reasonable to assume that these antibodies arise as a consequence of liberation of the T antigens into the peripheral circulation of the hamster, to date, such free circulating T antigen has not been demonstrated. The present brief communication reports the first incidence of location of T antigen in the host animal outside of the primary lesion.

MATERIALS AND METHODS

Materials

**Tumor Lines.** The animals used in this study were bearing tumors of 5 different derivations: (a) an SV40-induced tumor obtained commercially (Flow Laboratories, Rockville, Md.), (b) tumors started from the in vitro transformed tumor cell line H-50 (1), (c) an SV40-induced tumor line obtained from Dr. A. Sabin, and (d) a polyoma virus-induced tumor obtained commercially (Flow Laboratories). All SV40 tumor lines had been passaged in vivo at least 7 times in this laboratory.

**Sera.** Blood was collected by cardiac puncture from either normal hamsters or hamsters bearing tumors 5 to 15 cm in diameter. The blood was allowed to coagulate at 4°C and the clot was removed by centrifugation. The serum was collected, pooled, and stored at -20°C until needed. All anti-T sera used had a titer of ≥1:64 when tested against commercially available (Flow Laboratories) homologous virus-induced T antigen. Antihamster rabbit globulin and SV40 antiserum were obtained from commercial sources (Flow Laboratories and Colorado Serum Company, Denver, Colo.). Aliquots of these sera were conjugated with FITC by the method of Riggs et al. (7). Unconjugated fluorescein was removed after conjugation by adsorption to a DEAE-cellulose column.

**Leukocytes.** Blood was obtained by cardiac puncture from either normal hamsters or hamsters bearing tumors 5 to 15 cm in diameter. Blood was drained from the animals into a heparinized syringe. This heparinized blood was centrifuged in a clinical centrifuge at high speed in Winthrobe tubes for 30 min. At this time, the buffy coat was collected and either smeared onto glass slides for direct study or cultured for 18 to 24 hr at 37°C on glass coverslips either in Leighton tubes or in 60-mm Petri dishes. The Petri dishes were incubated in the gas mixtures described by Saunders (8). The medium used for in vitro leukocyte culture was that of Saunders (8).

Methods

**Direct Fluorescent Antibody Test.** The specimens (either a leukocyte smear obtained from auffy coat or a coverslip of in vitro cultured leukocytes) were air dried, fixed in absolute acetone for 3 min, and then again air dried. They were then coated with FITC-conjugated serum and incubated in a moist chamber at 37°C for 30 min. Following staining the specimens were washed for 10 min in 3 changes of PBS (pH 7.2, 0.01 M) and mounted in buffered glycerol. Control slides were preincubated with unconjugated anti-T sera for 20 min prior to staining with FITC-conjugated anti-T sera.

**Indirect Fluorescent Antibody Test.** Specimens were air dried, fixed in absolute acetone for 3 min, and again air dried. They were then covered with the unconjugated serum (either normal hamster serum or anti-T serum)

---

1 This research was supported in part by grants from the Damon Runyon Memorial Fund for Cancer Research, Inc. (DRG-965), and the Milheim Foundation for Cancer Research, as well as by USPHS Grant CA 5164 from the National Cancer Institute.

Received July 22, 1969; accepted November 5, 1969.

2 The abbreviations used are: FITC, fluorescein isothiocyanate; PBS, phosphate-buffered 0.9% NaCl solution.
Richard M. Jamison

Table 1

<table>
<thead>
<tr>
<th>Reagents used to stain</th>
<th>Specimen</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>FITC-conjugated SV40 anti-T serum</td>
<td>Buffy coat smear*</td>
<td>All preparations yield positive cells. Usually &lt;1% positive</td>
</tr>
<tr>
<td></td>
<td>Cultured leukocytes*</td>
<td>1-10% of cells examined were positive</td>
</tr>
<tr>
<td>FITC-conjugated normal hamster serum</td>
<td>Buffy coat smear*</td>
<td>No positive cells seen</td>
</tr>
<tr>
<td></td>
<td>Cultured leukocytes*</td>
<td>No positive cells seen</td>
</tr>
<tr>
<td>Nonconjugated T serum followed by FITC-conjugated serum</td>
<td>Buffy coat smear*</td>
<td>No positive cells seen</td>
</tr>
<tr>
<td></td>
<td>Cultured leukocytes*</td>
<td>No positive cells seen</td>
</tr>
<tr>
<td>FITC-conjugated SV40 antiserum</td>
<td>Buffy coat smear*</td>
<td>No positive cells seen</td>
</tr>
<tr>
<td></td>
<td>Cultured leukocytes*</td>
<td>No positive cells seen</td>
</tr>
</tbody>
</table>

*Leukocytes obtained from a hamster bearing an SV40-induced tumor.

†Leukocytes from hamsters bearing an SV40 tumor after being cultured in vitro for 18 to 24 hr.

Table 2

<table>
<thead>
<tr>
<th>Reagents used to stain</th>
<th>Specimen</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-T serum + FITC-conjugated antihamster globulin</td>
<td>Buffy coat smear*</td>
<td>1-10% positive cells</td>
</tr>
<tr>
<td></td>
<td>Cultured leukocytes*</td>
<td>10-20% of cells are positive</td>
</tr>
<tr>
<td></td>
<td>Cultured leukocytes*</td>
<td>No positive cells seen</td>
</tr>
<tr>
<td>Normal hamster serum + FITC-conjugated antihamster globulin</td>
<td>Buffy coat smear*</td>
<td>No positive cells seen</td>
</tr>
<tr>
<td></td>
<td>Cultured leukocytes*</td>
<td>No positive cells seen</td>
</tr>
<tr>
<td></td>
<td>Cultured leukocytes*</td>
<td>No positive cells seen</td>
</tr>
</tbody>
</table>

*Leukocytes obtained from a hamster bearing an SV40-induced tumor.

†Leukocytes from hamsters bearing an SV40 tumor after being cultured in vitro for 18 to 24 hr.

‡Leukocytes from normal hamsters after being cultured in vitro for 18 to 24 hr.

and incubated at 37° for 30 min in a moist chamber. After this incubation, all specimens were washed for 10 min in PBS and drained. Each coverslip was then flooded with FITC-conjugated antihamster rabbit globulin and incubated in a moist chamber for 20 min. At the end of this period, the leukocytes were washed in 3 changes of PBS for a total of 30 min.

Microscopy and Photomicrography. All specimens were examined in a Leitz Ortholux fluorescence microscope at a magnification of 400×. For observation of the stained slides, an ultraviolet exciter filter UG1 and barrier filter GG9 were used. Photomicrographs were obtained on Anscochrome 500 film with the use of a microphotometer resulting in an exposure of approximately 1 min. Black and white copies of the color transparencies were prepared by the usual photographic techniques.

RESULTS AND DISCUSSION

Leukocytes from hamsters bearing transplanted fibrosarcomas originally induced by papovavirus SV40 can be readily shown to contain the T antigen characteristic of tumors induced by this virus. Tables 1 and 2 demonstrate the results of staining such leukocytes by immunofluorescent techniques. Although specific fluorescence can be demonstrated by directly staining these cells with FITC-conjugated anti-T serum (Table 1), the number of positive cells seen can be increased by using the more sensitive (although less specific) indirect technique (Table 2). In no instance could SV40 viral antigen be demonstrated in the leukocytes by the direct technique. Leukocytes (either from buffy coat smears or in vitro cultures) from hamsters bearing tumors induced by polyoma virus did not react with any antiserum used.

Figs. 1 and 2 illustrate leukocytes cultured in vitro and stained by the direct technique. The specific antigen appears to occur in large granules scattered throughout the cytoplasm (Fig. 1) or as an area of more diffuse fluorescence (Fig. 2). Fig. 3 illustrates 2 leukocytes which do not contain T antigen. (The negative from which Fig. 3 was derived was deliberately overexposed so as to obtain the contrast necessary to illustrate a nonfluorescent cell.)

Although granulocytes are notorious as containing non-specific fluorescent materials (4), the specific nature of the fluorescence demonstrated in Figs. 1 and 2 is confirmed by the controls illustrated in Table 1. Application of nonfluorescent anti-T serum to the leukocytes prior to staining with FITC-conjugated anti-T serum completely blocked the reaction. Further, the anti-SV40 T serum did not react specifically with leukocytes from hamsters bearing tumors induced by polyoma virus.

The identity of those cells collected from the peripheral circulation which contain T antigen is obscure. The number of positive cells seen in the buffy coat smears was much too low to permit correlation with cells identified by Wright's stain of other aliquots. Those leukocytes adhering to the glass coverslips and cultured for 18 to 24 hr were large mononucleate cells, presumed to be macrophages.

REFERENCES


2. Black, P. H., Rowe, W. P., Turner, H. C., and Huebner, R. J. A


Fig. 1. Leukocyte stained for the presence of T antigen by the direct fluorescent antibody technique. The antigen occurs as relatively large granules distributed throughout the cell. Figs. 1 to 3 are black and white reproductions of the original color transparencies.

Fig. 2. Leukocyte stained for the presence of T antigen by the direct fluorescent antibody technique. The antigen is diffuse and is distributed through the cell.

Fig. 3. Two nonfluorescing leukocytes from the same preparation as Fig. 2. The original negative was greatly overexposed so as to demonstrate the nonfluorescing cells.
Detection of SV40-induced T Antigen in Peripheral Leukocytes of Tumor-bearing Hamsters

Richard M. Jamison


Updated version  Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/30/5/1541

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.