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Cell Biology

# Somatic cell hybrids producing antibodies specific for the tumor antigen of simian virus 40

(mouse myeloma/primed spleen cells/cell fusion/BK virus)

JOANNE MARTINIS AND CARLO M. CROCE

The Wistar Institute of Anatomy and Biology, 36th Street at Spruce, Philadelphia, Pennsylvania 19104

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**ABSTRACT** **We have produced somatic cell hybrids between mouse myeloma cells deficient in hypoxanthine phosphoribosyltransferase (IMP:pyrophosphate phosphoribosyltransferase; EC 2.4.2.8) and spleen cells derived from mice primed with either syngeneic or allogeneic cells transformed by simian virus 40. Such hybrids produced antibodies specific for simian virus 40 tumor (T) antigen. Only four of twelve independent hybrid cell cultures produced antibodies against simian virus 40 T antigen that crossreacted with the T antigen induced by BK virus, a human papovavirus isolated from patients who had undergone immunosuppressive therapy.**

The availability of somatic cell hybrids (hybridomas) that produce large amounts of monoclonal antibodies of the desired specificity and homogeneous binding characteristics would greatly facilitate the analysis of complex antigens (1–4). We have shown that somatic cell hybridization between mouse myeloma cells and virus-primed mouse spleen cells results in the formation of hybrid cells that produce monoclonal antibodies specific for viral antigens (3, 4). In this study we intended to determine whether it is possible to generate hybridomas that produce antibodies against the tumor (T) antigen(s) of simian virus 40 (SV40) (5). The availability of hybrids producing large amounts of monoclonal antibodies against different determinants of this antigen could allow its antigenic and biochemical characterization. In addition, since it has been shown that the T antigen induced by a human papovavirus, BK virus, crossreacts with SV40 T antigen (6), we wanted to determine whether the antibodies produced by different independent hybridomas would all crossreact with BK virus T antigen or whether some of these antibodies would be specific for antigenic determinants unique to SV40 T antigen and not shared with BK virus T antigen.

## MATERIALS AND METHODS

**Immunization of Mice.** BALB/c or C57BL/6J mice were immunized intraperitoneally with either $30 \times 10^6$ syngeneic or allogeneic SV40-transformed cells. Animals were either hyperimmunized 4–11 times at approximately 1-week intervals or immunized once and boosted a single time 2–4 weeks after immunization. Spleens were taken 3–6 days after the final injection. The anti-T antigen titer of the serum from the spleen donors was generally 1:100 or 1:200.

**Production of Hybrid Cells.** Spleen cell suspensions were prepared in phosphate-buffered saline and depleted of erythrocytes by hypotonic shock. The origin and growth properties of the myeloma parental cells (P3 × 63 Ag8), deficient in hypoxanthine phosphoribosyltransferase (IMP:pyrophosphate phosphoribosyltransferase; EC 2.4.2.8), and the method of fusion of spleen cells and myeloma cells in the presence of polyethyleneglycol 1000 have been described (3, 4). After fusion, cells were suspended in hypoxanthine/aminopterin/thymidine selective medium (7) and seeded in 75-cm$^2$ Falcon flasks or individual wells of Linbro FB16-24TC plates.

Table 1. Cell lines used in this study

| Abbreviation | Description |
|---|---|
| LN-SV | SV-transformed human fibroblasts |
| HT1080-6TG | Human fibrosarcoma derived cells |
| HEK | Human embryo kidney cells |
| F5-1 | SV40-transformed Syrian hamster fibroblasts |
| B1 | Syrian hamster fibroblasts |
| C57SV | SV40-transformed C57BL/6J mouse fibroblasts |
| C57MEF | C57BL/6J embryonic mouse fibroblasts |
| MKSBu100 | SV40-transformed BALB/c mouse kidney cells |
| BALB MEF | BALB/c embryonic mouse fibroblasts |
| BALB 3T3 | BALB/c embryonic mouse fibroblasts |
| HKBK-DNA-4 | Syrian hamster kidney cells transformed by BK virus DNA |

**T Antigen Assay.** Expression of SV40 T antigen was detected by indirect immunofluorescence (8). Acetone-fixed cells were reacted with control mouse anti-T antigen antiserum for 30 min, washed, then reacted with fluorescein-tagged rabbit anti-mouse immunoglobulin. A distinctive pattern of nuclear fluorescence indicates the presence of T antigen. The hybrid cells were tested for the production of anti-T antibody by substituting hybridoma culture fluids for the control anti-T antiserum on test cells known to be positive for T antigen. The anti-T titer of a serum or culture fluid is the last dilution that gives 100% staining of nuclei of SV40-transformed cells. Culture fluids from P3 × 63 Ag8 mouse myeloma cells do not contain any anti-SV40 T antigen activity. In addition, sera and ascites from BALB/c mice carrying P3 × 63 Ag8 myeloma tumors were also negative for anti-SV40 T antigen activity.

**Test Cells.** The test cells used in this study and their origins are given in Table 1. The HKBK-DNA-4 cells are hamster kidney cells transformed by BK virus DNA and were the kind gift of Giuseppe Barbanti Brodano, University of Ferrara, Italy (9).

## RESULTS

### Production of hybrid cell cultures

From 2–3 weeks after the polyethyleneglycol-induced fusion of hypoxanthine phosphoribosyltransferase-deficient P3 × 63

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Abbreviations: SV40, simian virus 40; T antigen, tumor antigen.

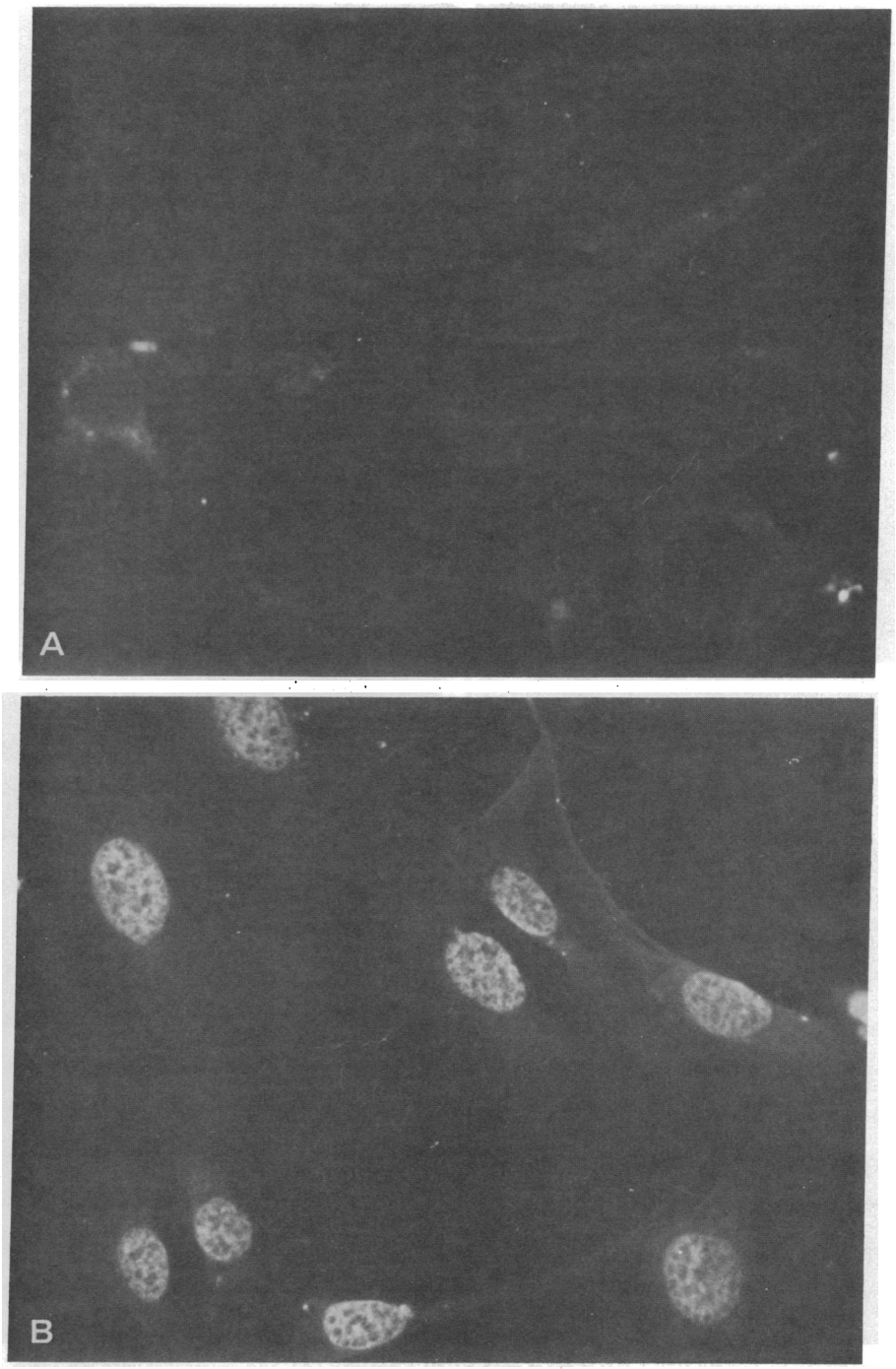


FIG. 1. Staining of T antigen expressed in SV40-transformed human cells (LN-SV) with hybridoma culture fluids. (*A*) Hybrid A17.2#2 does not produce detectable amounts of anti-SV40 T antigen antibodies. (*B*) Hybrid A25.1#1B3 produces anti-T antigen antibodies.

Ag8 mouse myeloma cells with spleen cells derived from either BALB/c mice hyperimmunized with C57SV cells (series A17.2) or C57BL/6J mice immunized with C57SV cells (series A25.1) or BALB/c mice immunized with MKSBu100 cells (series B16.1) (Table 1), hybrid cells growing in hypoxanthine/aminopterin/thymidine selective medium appeared and were subcultured weekly in the selective medium. One hundred forty-six independent hybrid cell cultures from 20 different fusions were obtained and were then tested for the production of antibodies specific for SV40 T antigen.

## Specificity of antibodies produced by hybrids

Only 13 of the 146 hybrid cell cultures, 10 of which were independently derived from the same fusion experiment (B16.1), produced antibodies against SV40 T antigen (Fig. 1). As shown in Table 2, the antibodies produced by the hybridomas and a control mouse antiserum raised against SV40 T antigen reacted with SV40-transformed human, hamster, and mouse cells, as determined by indirect immunofluorescence, but did not react with normal or malignant cells derived from these same species. In order to establish whether the antibodies produced by the hybridomas crossreact with the T antigen of BK virus, culture fluids derived from 11 different hybrid cell lines and the serum from a mouse injected with an additional hybrid cell line were tested for the presence of anti-SV40 and anti-BK virus T antigen antibodies using SV40-transformed human cells (LN-SV) and

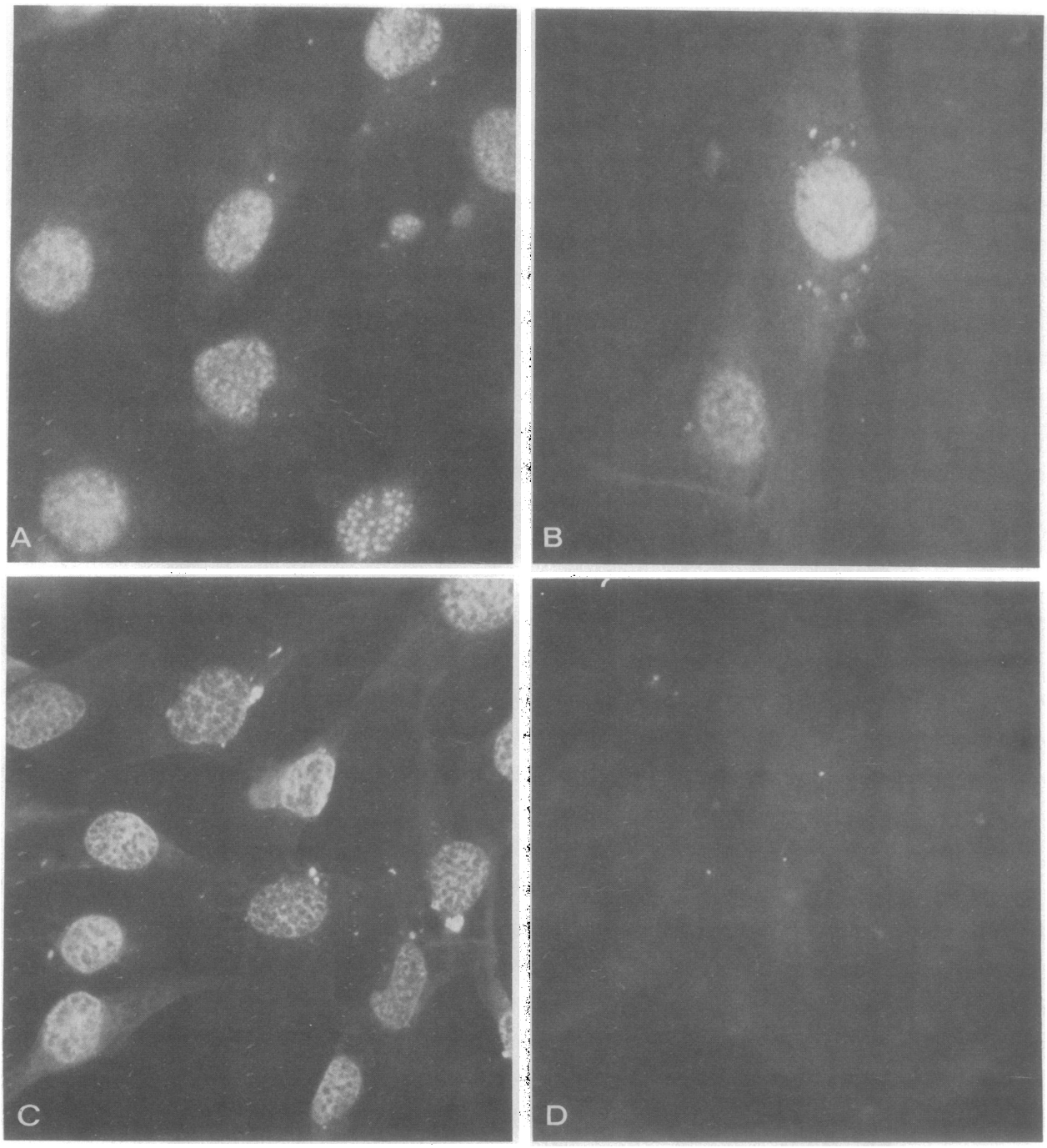


FIG. 2. Antibodies produced by hybrid B16.1#1C4 react with SV40 T antigen (*A*), and crossreact with BK virus T antigen (*B*). However, the antibodies produced by hybrid B16.1#1B6 react only with SV40 T antigen (*C*) and not with BK virus T antigen (*D*).

BK virus DNA-transformed hamster kidney cells (HKBK-DNA-4) as test cells. As shown in Table 3, only four of the hybridoma antibodies crossreacted with BK virus T antigen, although, as in the control serum, the intensity of the fluorescence was generally weaker (Fig. 2).

## Stability of antibody production by hybrid cells

At the beginning of this study, we had isolated only three hybrid cell cultures that produced anti-SV40 T antigen antibody: A17.2#1, A17.2#6, and A25.1#1B3. Of these, after 3 months in tissue culture, only hybrid A25.1#1B3 was still producing detectable amounts of anti-T antigen antibodies in the culture fluid, as determined by indirect immunofluorescence (Table 4). This hybrid has also been grown as a solid tumor and as ascites in *nude* mice, with a marked increase in titer (Table 4). The mass cultures A17.2#1 and A17.2#6 rapidly lost detectable anti-T activity in the culture fluids. In addition, culture fluids from 56 clones derived from the mass hybrid culture A17.2#1 were negative for anti-T antigen activity. However, when these hybrids were injected in BALB/c mice, one of two animals injected with A17.2#6 cells developed a weak anti-T antigen response (undiluted) and two of three animals injected with A17.2#1 cells developed an anti-T antigen response (titers 1:16 and 1:50). One of the tumors, obtained from the animal with higher antibody titer (1:50), was transferred *in vivo* and was also adapted to tissue culture. Tissue culture cells derived

Table 2. Antibody produced by hybrid cultures tested on various cell lines

| Test cells | Source of antibody: Anti-T serum* | A25.1 #1B3† | A17.2 #1‡ | B16.1 #1B2§ |
|---|---|---|---|---|
| LN-SV | + | + | + | + |
| HT1080-6TG | − | − | − | − |
| HEK | − | − | − | − |
| F5-1 | + | + | + | + |
| B1 | − | − | − | − |
| C57SV | + | + | + | + |
| C57MEF | − | − | − | − |
| MKSBu100 | + | + | + | + |
| BALB MEF | − | − | − | − |
| BALB 3T3 | − | − | − | − |

* 1:100 dilution of control serum from SV40 T antigen immune mouse.
† Undiluted culture fluid.
‡ 1:20 dilution of serum from animal bearing a tumor induced by A17.2#1 hybrid cells.
§ Undiluted culture fluid. All the anti-SV40 T antigen antibody-producing hybrids derived from the B16.1#1 fusion behaved in identical fashion on these test cells.

from this tumor produced antibodies against SV40 T antigen at a titer of 1:5.

## DISCUSSION

The results presented in this paper indicate that it is possible to obtain somatic cell hybrids between mouse myeloma cells and primed spleen cells that produce antibodies against the T antigen of SV40. Some of the hybrids produce antibodies that recognize only SV40 and not BK virus T antigen; others produce antibodies that crossreact with BK virus T antigen. These results indicate that the different hybrids recognize different antigenic determinants, only some of which are shared by the SV40 and BK virus T antigens.

Very recently it has been shown that cells productively infected with SV40 or transformed by this virus produce two different proteins that are recognized by antisera for SV40 T antigen, one with a molecular weight of approximately 95,000 and the other with a molecular weight of approximately 17,000 (10, 11). These two different proteins seem to be coded for by two spliced early mRNAs, one 2200 nucleotides long and the other 2500 nucleotides long, that are coded by overlapping DNA sequences (12).

Table 3. Crossreactivity between SV40 and BK virus T antigens

| Source of antibodies* | Test cells: LN-SV | HKBK-DNA-4 |
|---|---|---|
| Anti-T serum† | + | + |
| A17.2#1‡ | + | − |
| A25.1#1B3 | + | + |
| B16.1#1B2 | + | − |
| B16.1#1B6 | + | − |
| B16.1#1C1 | + | − |
| B16.1#1C5 | + | − |
| B16.1#2A2 | + | + |
| B16.1#2A3 | + | − |
| B16.1#2A5 | + | − |
| B16.1#2C4 | + | + |
| B16.1#2D2 | + | − |
| B16.1#2D5 | + | + |

* Except where noted, undiluted culture fluids from hybrid cultures were used for testing.
† 1:100 dilution of control serum from SV40 immune mouse.
‡ 1:20 dilution of serum from animal bearing a tumor induced by A17.2#1 hybrid cells. Culture cells from the A17.2#1 tumor produced anti-SV40 T antigen antibodies that did not crossreact with BK virus T antigen.

Table 4. Titer of anti-SV40 T antigen antibodies produced by hybridomas in culture fluids, serum, and ascites

| Hybridomas | Titer: Culture fluid | Serum | Ascites |
|---|---|---|---|
| A25.1#1B3 | 1:5 | 1:3200 | 1:3200 |
| B16.1#2A2 | 1:2 | ND* | ND |
| B16.1#2C4 | 1:10 | 1:800 | ND |
| B16.1#2D5 | 1:2 | ND | ND |
| B16.1#1B6 | 1:20 | 1:6400 | 1:3200 |
| B16.1#1C5 | 1:10 | ND | 1:3200 |
| B16.1#2A3 | 1:5 | 1:6400 | 1:1600 |
| B16.1#1B2 | 1:2 | ND | ND |
| A17.2#1† | Negative | 1:50 | ND |

* ND, mice were not injected with hybridoma cells.
† This hybrid was originally a producer of anti-SV40 T antigen antibodies, but became negative after subculture.

It would be of interest to determine whether monoclonal antibodies directed against SV40 T antigen would recognize antigenic determinants specific for either the large T antigen or the small T antigen and whether some of the hybridomas produce antibodies against antigenic determinants which are shared by these two proteins.

The use of monoclonal antibodies produced in large amounts by the different hybridomas and directed against different antigenic determinants of the SV40 T antigens should result in their immunological and biochemical characterization.

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