JOURNAL OF VIROLOGY, Mar. 1986, p. 1168–1172
0022-538X/86/031168-05$02.00/0

Vol. 57, No. 3

Antigenic Binding Sites of Monoclonal Antibodies Specific for Simian Virus 40 Large T Antigen

ELIZABETH G. GURNEY,[1]* SUSAN TAMOWSKI,[1] AND WOLFGANG DEPPERT[2]

Department of Biology, University of Utah, Salt Lake City, Utah 84112,[1] and Department of Biochemistry, University of Ulm, D7900 Ulm, Federal Republic of Germany[2]

Received 13 June 1985/Accepted 15 November 1985

We isolated 16 new monoclonal antibodies that recognize large T antigen of simian virus 40 and mapped the epitopes to three distinct regions of the large T antigen. Also, 3 of the 16 recognized the large T antigen of the human papovavirus BKV.

The simian virus 40 (SV40) large T antigen plays important roles in SV40 infection and transformation of cells; however, the quantities of large T antigen per cell are tiny, about 10^{-5} that of cellular protein. Hence, specific antibodies have been required for research on large T antigen. The most common source of antibodies has been "tumor serum," which is antiserum from rodents injected with SV40-transformed cells. Tumor sera provide variable polyclonal sets of antibodies specific for determinants on native large T antigen, presumably sites exposed on the surface of the molecules (1). Monoclonal antibodies specific for large T antigen are better defined than tumor sera, but for a protein of the structural and functional complexity of the large T antigen (13), many monoclonal antibodies may be needed to identify all the epitopes. About 70 anti-T monoclonal antibodies have been reported (2, 5–7, 10), but the number of epitopes represented is not yet known. We report here the isolation and characterization of 16 more monoclonal antibodies specific for the SV40 large T antigen. Three of these antibodies cross-react with the large T antigen of BKV, a human papovavirus with extensive homology to SV40 (15).

We produced 16 anti-T monoclonal-antibody-secreting hybridoma cell lines with the method of Köhler and Milstein (9). Hybrid cells were selected in a hypoxanthine-aminopterin-thymidine medium after fusion of NS1 myeloma cells with splenocytes from mice immunized with the SV40-transformed mouse line B4 (6). Anti-T hybridoma clones were identified by three screening methods. One was an enzyme-linked immunosorbent assay (ELISA) with methanol-fixed, SV40-transformed human SV80 cells (6). Method 2 was immunofluorescence with cells fixed by acetone-ethanol, using human SV80 and BKV-infected MG178 cells and mouse SVT2 (SV40-transformed) and BALB/3T3 (uninfected) cells (6). Method 3 was immunoprecipitation and gel electrophoresis of radiolabeled large T antigen from extracts of SV80 cells and of SV40-infected TC-7 cells (6). The immunoglobulin class and subclass of the monoclonal antibodies were determined by an ELISA (6) with mouse class- and subclass-specific sera (Litton Bionetics). Culture medium harvested from dense cultures of the hybridoma clones, grown as described previously (6), was used as antibody source in all the experiments reported here.

Approximate locations of the binding sites on large T antigen were identified by adenovirus type 2 (Ad2)-SV40 hybrid virus mapping (3). Three nondefective hybrid viruses ($Ad2^+ND1$, $Ad2^+ND2$, and $Ad2^+ND4$) encode overlapping carboxyl-terminal fragments of the SV40 large T antigen (12). HeLa cells infected with Ad2 or a hybrid virus were used for immunofluorescence assays and immunoprecipitation analysis (3). Our mapping divided large T antigens into four regions (Fig. 1). The SV40 inserts in Ad2-ND1, Ad2-ND2, and Ad2-ND4 defined three regions (IV, III, and II, respectively, in Fig. 1). Region I was defined as the amino-terminal portion of the large T antigen shared by the small t antigen but lacking in the $Ad2^+ND4$ large T polypeptides. We have considered only the largest SV40-specific polypeptides in cells infected with $Ad2^+ND2$ and $Ad2^+ND4$, although smaller proteins are also present (see reference 17 for a review). We think that the map is more reliable in regions I and IV than it is in regions II and III, because the last two depend on negative results.

Monoclonal antibodies were tested for recognition of denatured large T antigen by immunoblotting. Total cellular proteins were extracted from SV80 cells in 2% sodium dodecyl sulfate (SDS), 100 mM dithiothreitol, 80 mM Tris (pH 6.8), 10% glycerol, and 0.02% bromphenol blue for 30 min at 20 to 25°C, heated for 3 min at 91°C, passed 5 to 10 times through a 30-gauge needle, and separated by SDS-polyacrylamide gel electrophoresis. The proteins were transferred to a nitrocellulose membrane, exposed to monoclonal antibodies and then to horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin G (IgG) (Cooper Biomedical, Inc.), and revealed with *O*-dianisidine (Sigma) as described by Towbin et al. (18). A brown-stained band at 94,000 daltons denoted recognition of large T antigen. No additional bands were seen with the anti-T monoclonal antibodies.

Mapping of epitopes in three regions of SV40 large T antigen. The 16 anti-T monoclonal antibodies were designated PAb 102 through 117. All 16 precipitated large T antigen from extracts of SV40-infected cells (Fig. 2) and SV40-transformed cells (data not shown). All the antibodies gave nuclear staining in immunofluorescence assays with SV40-transformed mouse and human cells but were negative with BALB/3T3 cells (data not shown). Nine monoclonal antibodies reacted with determinants located in region I, because the antibodies precipitated small t as well as large T antigens from extracts of SV40-transformed or -infected cells but did not stain HeLa cells infected by $Ad2^+ND1$, $Ad2^+ND2$, or $Ad2^+ND4$. Five antibodies mapped in region

* Corresponding author.

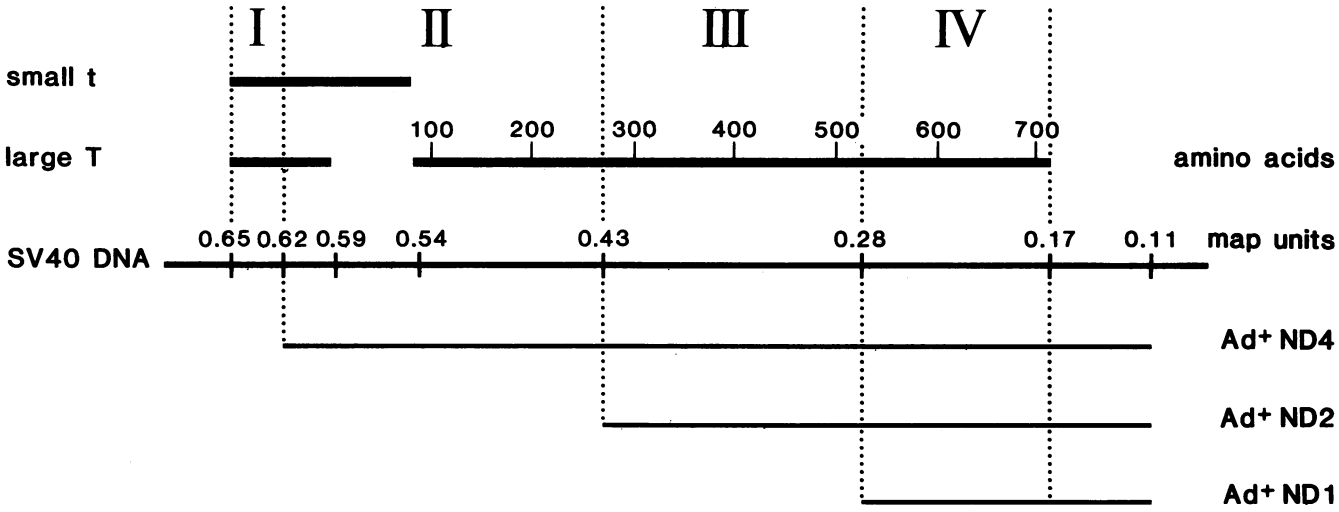

FIG. 1. Regions of SV40 large T polypeptide for mapping monoclonal antibody epitopes. The map positions for small t and large T antigens and the ends of the SV40 DNA inserts in the Ad2-SV40 hybrid viruses have been summarized by Tooze (17).

III of the large T antigen, because they stained HeLa cells infected with Ad2$^+$ND2 and Ad2$^+$ND4 (but not Ad2$^+$ND1) and did not precipitate small t antigen. Of the 16 monoclonal antibodies, 2 mapped in region IV of the SV40 large T antigen, because they stained HeLa cells infected with Ad2-ND1, Ad2-ND2, or Ad2-ND4 but did not precipitate small t antigens. Most of the antibodies were also tested by immunoprecipitation with extracts of hybrid virus-infected HeLa cells; the results are consistent with the immunofluorescence data summarized above. Among the 16 antibodies reported here and the 2 reported previously (6), 9 recognized a determinant in region I, none in region II, 6 in region III, and 3 in region IV (Table 1).

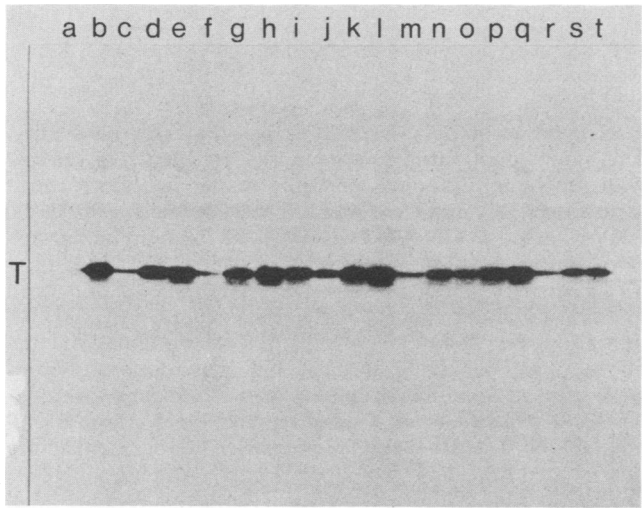

FIG. 2. Comparative reactivity of monoclonal antibodies with SV40 large T antigen. TC-7 cells infected with SV40 were labeled with [^{35}S]methionine (560 μCi/60-mm plate) for 2 h, beginning 40 h after infection. Soluble proteins were extracted with 1% Nonidet P-40, immunoprecipitated with fixed staphylococci, and analyzed on a 7.5 to 15% gradient polyacrylamide gel with fluorography (6). Rabbit anti-mouse IgG was included with all the samples. The antibodies used were (a) normal mouse serum and (b) mouse SV40 tumor serum, as well as medium from: (c) PAb 100, (d) 101, (e) 102, (f) 103, (g) 104, (h) 105, (i) 106, (j) 107, (k) 108, (l) 109, (m) 110, (n) 111, (o) 112, (p) 113, (q) 114, (r) 115, (s) 116, and (t) 117. The large T antigen was present as a triplet, presumably due to loss of the carboxyl terminus from the smaller molecules (16).

All antibodies identified as mouse IgG molecules. The monoclonal antibodies PAb 101, 102, 108, and 109 were found to be mouse IgG2a molecules that bound tightly to staphylococcal protein A. The other 14 antibodies were identified as mouse IgG1 molecules and, therefore, were

TABLE 1. Mapping and characterization of anti-T monoclonal antibodies

SV40 large T region	PAb no.	IgG subclass	Recognition of antigen			
			Native[a]		Denatured T[b]	BKV T[c]
			t	T		
I	105	1	+	+	+	−
	108	2a	+	+	+	+
	109	2a	+	+	+	−
	110	1	+	+	+	−
	111	1	+	+	+	−
	112	1	+	+	+	−
	113	1	+	+	+	−
	116	1	+	+	+	−
	117	1	+	+	+	−
II	N[d]	N	N	N	N	N
III	100[e]	1	−	+	−	−
	102	2a	−	+	−	−
	103	1	−	+	−	−
	104	1	−	+	−	−
	106	1	−	+	−	+
	114	1	−	+	−	−
IV	101[f]	2a	−	+	+	−
	107	1	−	+	+	−
	115	1	−	+	+	+

[a] Determined by immunoprecipitation of extracts of SV80 cells and of SV40-infected TC-7 cells followed by gel electrophoresis.
[b] Determined by immunoblotting of SV80 proteins.
[c] Determined by immunofluorescence of BKV-infected MG178 cells.
[d] N, None.
[e] Originally known as clone 7 (6).
[f] Originally known as clone 412 (6).

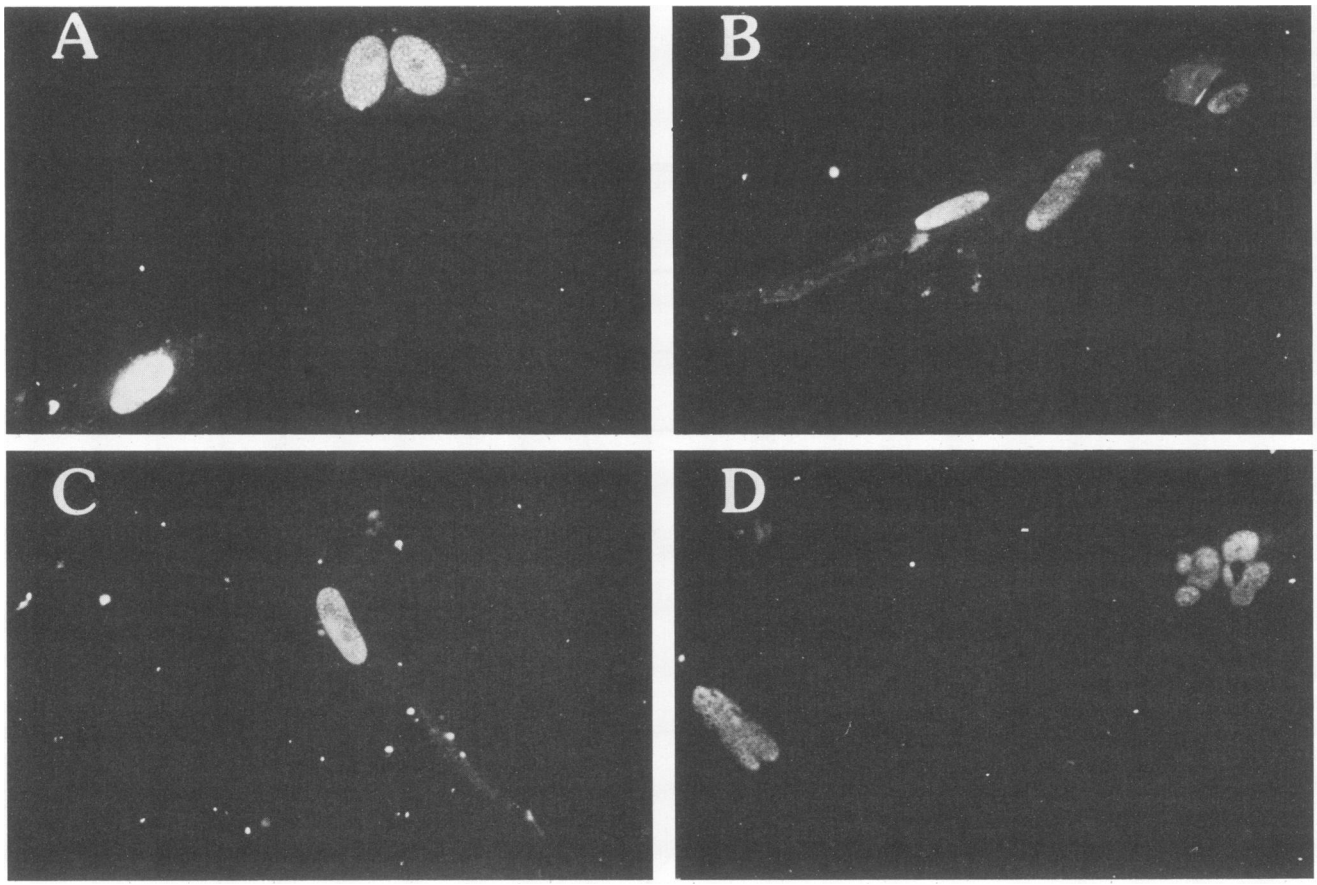

FIG. 3. Immunofluorescence analysis of monoclonal antibodies PAb 106, 108, and 115 with BKV-infected cells. WI38 cells were infected with BKV, fixed 72 h after infection, and analyzed for expression of BKV large T antigen by immunofluorescence as described previously for analysis of SV40 large T antigen (4). The antibodies used were: (A) rabbit anti-SDS-T serum, (B) PAb 106, (C) 108, and (D) 115.

used with rabbit anti-mouse IgG antibodies to ensure precipitation by fixed staphylococci or protein A Sepharose (8).

Only antibodies that map near ends of large T antigen react with denatured large T antigen. The nine monoclonal antibodies of region I and the three antibodies of region IV reacted with denatured large T antigen in immunoblots, but the six antibodies assigned to region III did not (Table 1). Antibodies PAb 101, 102, 105 to 114, and 116 were tested for recognition of denatured large T antigens by two additional methods: the radioimmunoassay of Lane and Robbins (11) and immunoprecipitation of SDS-treated extracts of SV80 cells (data not shown). In all cases, the results confirmed the immunoblot results. Harlow et al. also found that antibodies that bind near the ends of large T antigen are denaturation resistant, whereas others are denaturation sensitive (7). This pattern suggests either that epitopes in the amino- and carboxyl-terminal regions of large T antigen are sequence specific rather than conformation specific or, alternatively, that the native conformation of large T antigen in these areas can be readily restored after denaturation.

In radioimmunoassay data reported previously (6), PAb 101 did not react with denatured large T antigen, but here PAb 101 did in all three types of assays. The carboxyl terminus of large T antigen is readily lost in vitro (16). Therefore, the PAb 101 epitope may have been missing in the large T antigen used for the earlier radioimmunoassays.

Recognition of BKV large T antigen by three antibodies. The monoclonal antibodies PAb 106, 108, and 115 reacted with the large T antigen of BKV in immunofluorescence assays of BKV-infected human MG178 cells (Table 1). BKV-infected WI38 cells were stained for immunofluorescence with an antiserum against gel-purified, SDS-denatured SV40 large T antigen (anti-SDS-T serum [4]) and with monoclonal antibodies PAb 106, 108, and 115 (Fig. 3). All four antibodies show the pattern of nuclear staining nucleoplasm typical of large T antigens. The same four antibodies were used to immunoprecipitate large T antigens from ^{35}S-labeled extracts of BKV-infected WI38 cells and SV40-infected TC-7 cells. Only the anti-SDS-T serum efficiently precipitated BKV large T antigen (Fig. 4). Although PAb 108 precipitated all of the SV40 large T antigens, in sequential precipitations (data not shown) and in the precipitations shown here (compare lanes b and k in Fig. 2 and lanes a and c in Fig. 4A), it reacted with only a small fraction of BKV large T antigen (compare lanes a and c in Fig. 4B). The results suggest that either the PAb 108 binding sites are not identical on BKV and SV40 large T antigens or that only a small fraction of the BKV large T molecules have the epitope recognized by PAb 108. Immunoprecipitation of BKV large T antigen by monoclonal antibodies PAb 106 and 115 (Fig. 4B, lanes b and d, respectively) was just detectable, though weak. Since the three antibodies that cross-reacted with BKV large T antigen mapped in three different regions along

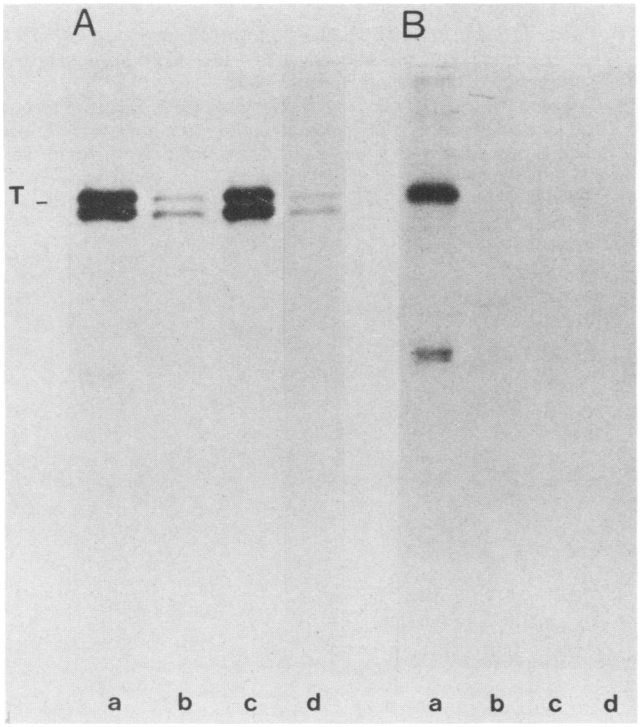

FIG. 4. Immunoprecipitation analysis of monoclonal antibodies PAb 106, 108 and 115 with SV40- or BKV-infected cells: (A) analysis of TC-7 cells infected with SV40; (B) analysis of WI38 cells infected with BKV. The large T antigen is present as a doublet in A, presumably due to loss of the carboxyl terminus from the smaller molecules (16). TC-7 cells infected with SV40 or WI38 cells infected with BKV were labeled at 36 or 72 h after infection, respectively, with [^{35}S]methionine (50 μCi/100-mm plate) for 1 h. Soluble proteins were extracted with 1% Nonidet P-40 and immunoprecipitated with rabbit anti-SDS-T serum (lanes a) or with purified monoclonal antibodies of PAb 106 (lanes b), 108 (lanes c), or 115 (lanes d), using protein A-Sepharose (14). Immunoprecipitations with PAb 106 and 115 included rabbit anti-mouse IgG. Immunoprecipitated proteins were analyzed on a 11.5% polyacrylamide gel followed by fluorography (3).

with antibodies that did not cross-react, we conclude that the 18 monoclonal antibodies recognize a minimum of six different epitopes.

The cross-reaction of PAb 106, 108, and 115 with BKV large T antigen was detected by immunofluorescence analysis of BKV-infected human cells. We probably would have missed the cross-reactivity of PAb 106 and 115 with BKV large T antigen if we had analyzed these antibodies only by immunoprecipitation. It is also possible to miss such cross-reactivity by immunofluorescence analysis, since PAb 105 precipitates BKV large T antigen from extracts of BKV-infected human embryo kidney cells (K. Rundell, personal communication), although PAb 105 was negative in all our immunofluorescence assays.

Two technical comments are in order for the use of monoclonal antibodies for biological or biochemical analysis of large T antigen. As might be expected, the ability of a monoclonal antibody to react with SDS-denatured large T antigen in radioimmunoassays and immunoprecipitation experiments correlates with its utility in immunoblots. Also, of our 18 monoclonal antibodies, only PAb 108 completely precipitated SV40 large T antigen in sequential precipitation experiments. Several other antibodies brought down more than 90% of the large T antigen in sequential and single precipitations such as those shown in Fig. 2. However, for many experiments it is preferable to use an antibody that reacts with all SV40 large T molecules.

We thank Theodore Gurney, Jr., and Judy Goddard for critical reading of the manuscript and Nikolaus Mueller-Lantzsch for gifts of WI38 cells and BKV. E.G.G. thanks Pierre and Evelyne May for their hospitality at the Institut de Recherches Scientifiques sur le Cancer in Villejuif, France.

This study was supported by Public Health Service research grant CA-21797 from the National Cancer Institute, by grant De212/5-3 from the Deutsche Forschungsgemeinschaft, and by NATO grant RG65(80).

LITERATURE CITED

1. **Atassi, M. Z.** 1978. Precise determination of the entire antigenic structure of lysozyme: molecular features of protein antigenic structures and potential of "surface-simulation" synthesis—a powerful new concept for protein binding sites. Immunochemistry **15**:909–936.
2. **Ball, R. K., B. Siegl, S. Quellhorst, G. Brandner, and D. G. Braun.** 1984. Monoclonal antibodies against simian virus 40 nuclear large T tumour antigen: epitope mapping, papova virus cross-reaction and cell surface staining. EMBO J. **3**:1485–1491.
3. **Deppert, W., E. G. Gurney, and R. O. Harrison.** 1981. Monoclonal antibodies against simian virus 40 tumor antigens: analysis of antigenic binding sites, using adenovirus type 2-simian virus 40 hybrid viruses. J. Virol. **37**:478–482.
4. **Deppert, W., and R. Pates.** 1979. Cell surface location of simian virus 40-specific proteins on HeLa cells infected with adenovirus type 2-simian virus 40 hybrid viruses Ad2$^+$ND1 and Ad2$^+$ND2. J. Virol. **31**:522–536.
5. **Gooding, L. R., R. W. Geib, K. A. O'Connell, and E. Harlow.** 1984. Antibody and cellular detection of SV40 T-antigenic determinants on the surface of transformed cells, p. 263–269. *In* A. J. Levine, G. F. Vande Woude, W. C. Topp, and J. D. Watson (ed.), Cancer cells 1: the transformed phenotype. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
6. **Gurney, E. G., R. O. Harrison, and J. Fenno.** 1980. Monoclonal antibodies against simian virus 40 T antigens: evidence for distinct subclasses of large T antigen and for similarities among nonviral T antigens. J. Virol. **34**:752–763.
7. **Harlow, E., L. V. Crawford, D. C. Pim, and N. M. Williamson.** 1981. Monoclonal antibodies specific for simian virus 40 tumor antigens. J. Virol. **39**:861–869.
8. **Kessler, S. W.** 1975. Rapid isolation of antigens from cells with a staphylococcal protein A-antibody adsorbent: parameters of the interaction of antibody-antigen complexes with protein A. J. Immunol. **115**:1617–1624.
9. **Köhler, G., and C. Milstein.** 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature (London) **256**:495–497.
10. **Lane, D. P., and W. K. Hoeffler.** 1980. SV40 large T shares an antigenic determinant with a cellular protein of molecular weight 68,000. Nature (London) **288**:167–170.
11. **Lane, D. P., and A. K. Robbins.** 1978. An immunochemical investigation of SV40 T antigens. I. Production, properties and specificity of a rabbit antibody to purified simian virus 40 large-T antigen. Virology **87**:182–193.
12. **Mann, K., T. Hunter, G. Walter, and H. Linke.** 1977. Evidence for simian virus 40 (SV40) coding of SV40 T-antigen and the SV40-specific proteins in HeLa cells infected with nondefective adenovirus type 2-SV40 hybrid viruses. J. Virol. **24**:151–169.
13. **Rigby, P. W. J., and D. P. Lane.** 1983. The structure and function of the simian virus 40 large T-antigen, p. 31–57. *In* G. Klein (ed.), Advances in viral oncology. Raven Press, Publishers, New York.
14. **Schwyzer, M.** 1977. Purification of SV40 T-antigen by immuno-

affinity chromatograpy on staphylococcal protein A-sepharose. Colloq. INSERM **69**:63–68.
15. **Seif, I., G. Khoury, and R. Dhar.** 1979. The genome of human papovavirus BKV. Cell **18**:963–977.
16. **Stitt, D. T., R. B. Carroll, J. A. Melero, and W. F. Mangel.** 1981. Analysis of the 84K, 55K, and 48K proteins immunoprecipitable by SV40 T antibody from SV40-infected and -transformed cells by tryptic peptide mapping on cation-exchange columns. Virology **111**:283–288.
17. **Tooze, J. (ed.).** 1981. Molecular biology of tumor viruses. DNA tumor viruses, 2nd ed., revised, p. 584–609. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.