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# Monoclonal antibodies displaying a novel species specificity for the primate transformation-related protein, p53

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**SV40 large T antigen associates with a cellular phosphoprotein, p53, in virus-transformed cells. We have raised three new monoclonal antibodies, PAb1101, PAb1102 and PAb1103, to this cellular protein, derived from SV40-transformed human fibroblasts. These define at least two non-overlapping determinants on human p53 that are in different areas of the molecule from those recognised by previously available antibodies. Unlike those antibodies, PAb1102 and PAb1103 do not react with rodent p53. PAb1101 reacts far more weakly with rodent p53 than with primate p53. All three antibodies show a preference for binding to the large T-associated form of p53, an effect that is particularly marked with PAb1102. The novel specificity of these antibodies allows further probing of the nature and function of the large T/p53 complex in human cells.**

*Key words:* p53/anti-p53 antibodies/SV40 large T antigen/transformation

## Introduction

In recent years considerable effort has been put into analysing the structure, function and expression of a cellular protein, p53. This protein was detected by virtue of its association with the SV40 large T antigen in SV40-transformed mouse cell lines (Lane and Crawford, 1979; Linzer and Levine, 1979; McCormick and Harlow, 1980). It was subsequently found in a similar complex in SV40-transformed human cell lines (Gurney *et al.*, 1980) and in SV40-infected monkey kidney cells (Harlow *et al.*, 1981a), and has recently been shown to associate with the adenovirus E1b protein, 58K (Sarnow *et al.*, 1982a, 1982b). Cell lines transformed in a variety of ways, or isolated from spontaneous tumours, have been found by comparision of metabolic labelling with normal cell cultures to show various alterations in the metabolism of p53 (Linzer and Levine, 1979; DeLeo *et al.*, 1979; Rotter *et al.*, 1980; Crawford *et al.*, 1981; Oren *et al.*, 1982) and this can be manifested as a high steady-state level of p53 in some transformed cell lines (Benchimol *et al.*, 1982). The functions of p53 in normal and transformed cells are not known, but experiments by two groups (Milner and Milner, 1981; Mercer *et al.*, 1982) suggest that p53 has a role in cell division, probably early in the sequence of events committing a cell to enter S phase.

In some instances the presence of a tumour in an animal has correlated with the development of an immune response by the animal to its p53 protein (Lane and Crawford, 1979; Linzer and Levine, 1979; DeLeo *et al.*, 1979; Rotter *et al.*, 1980; Crawford *et al.*, 1982); in no case have such antibodies been found in sera from normal animals. There must, therefore, be some alteration in the state or amount of p53 in these tumours that renders it immunogenic and much of the work referred to above has used either these antitumour sera, or else monoclonal antibodies isolated using various protocols.

The anti-p53 monoclonal antibodies so far described (Gurney *et al.*, 1980; Harlow *et al.*, 1981b; Coffman and Weissman, 1981; Rotter *et al.*, 1980; Dippold *et al.*, 1981) have activity against the mouse protein and provide probes for several areas on the p53 molecule as judged by competition assays (Crawford, 1982), but only PAb122 (Gurney *et al.*, 1980), PAb410 and PAb421 (Harlow *et al.*, 1981b) react with the primate p53 molecule. Antibody 200-47, isolated by Dippold *et al.* (1981) was reported to have activity against the human p53 but in our hands has no activity to the human protein from any cell line tested (L. Crawford, unpublished data). Since the three antibodies with anti-human p53 activity in pairwise combinations block each other's binding to p53 (Crawford, 1982), they clearly react with determinants that are topographically close on the p53 molecule. One can therefore probe four areas on mouse p53 but only one area on human p53 with the available antibodies. To allow an extension of our analyses of the expression, molecular structure and association of p53 in various human cell lines, we have isolated further monoclonal antibodies and describe here the properties of three antibodies that define new determinants on p53 which are primate-specific. All three show preferential binding to the SV40 large T-p53 complex rather than to 'free' p53.

## Results

### *Isolation of monoclonal antibodies*

Antigen for immunisation of mice was prepared from the human SV40-transformed fibroblast line, SV80, grown on bioassay trays, each 440 $cm^2$ in area. Extracts of 12 such monolayers, from $1 \times 10^9$ to $2 \times 10^9$ cells were pooled with extracts from three 90 mm dishes of cells that had been $^{32}P$-labelled as described. This provided a radiolabelled tracer for p53 during subsequent immunoprecipitation and gel electrophoresis. Large T and p53 were immunoprecipitated from the supernatant by a 30 min room temperature incubation with a monoclonal antibody cocktail comprising 7.5 $\mu g$ each of PAb405, 406, 413, 416, 419, 423 and 440 with 22.5 $\mu g$ of PAb421. Precipitated proteins were separated by gel electrophoresis, the labelled species and markers were detected by autoradiography of the wet gel, and the p53-containing slice cut from the gel and homogenised in phosphate-buffered saline (PBS) for i.p. injection into BALB/c female mice. Four weeks later, the mice received an immunisation boost *via* the tail vein with an eluate of a similar gel slice in PBS. Each immunisation comprised $\sim 5$ $\mu g$ of p53, (the product of $\sim 5 \times 10^8$ cells) estimated from Coomassie staining.

Spleens were taken, and fusions performed, 4 days after the second immunisation. Procedures for the fusion of splenocytes with non-producing plasmacytoma cells and the subsequent selection and growth of hybrids were as described by Harlow *et al.* (1981b) except that initial fusion products

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were cultured in 24 well plates, giving a total of 400–500 wells from each of three fusions. Supernatants from wells bearing hybrid clones were screened for the presence of antibody able to immunoprecipitate the large T/p53 complex from a $^{32}$P-labelled SV80 cell extract using rabbit anti-mouse Ig antiserum to allow detection of hybridomas secreting immunoglobulins that do not bind to *Staphylococcus aureus* protein A. Twelve wells proved positive on initial screening and of these, five gave rise to cloned lines after three clonings by limiting dilution using this same screening procedure. These were designated PAb1101, 2, 3, 4 and 5 (Crawford and Harlow, 1982). Data on the activities of PAb1104 and PAb1105 will be presented elsewhere and will not be discussed further here.

*Analysis of the basic properties of PAb1101, 2 and 3*

The class of immunoglobulin secreted by each cell clone was determined in a radioimmune blocking assay, the results of which are shown in Figure 1. Each of the three test antibodies blocked the association of known IgM with anti-IgM antiserum but not the association of any IgG class with the appropriate antiserum, and was therefore classified as IgM. This conclusion was confirmed by an experiment in which these antibodies were metabolically labelled with [$^{35}$S]methionine and precipitated either directly on *S. aureus* protein A or *via* rabbit anti-mouse Ig antiserum. Each clone produced a single heavy chain species migrating at 80 K, as expected of a $\mu$ class heavy chain. None of the three antibodies bound to protein A in the absence of rabbit anti-mouse Ig antiserum (data not shown).

A previously applied test of the sensitivity to denaturation of the determinant recognised by an antibody has been to attempt to rebind SDS-denatured, gel purified antigen to the antibody. When PAb1101, 2 and 3 were mixed with [$^{35}$S]methionine-labelled gel-purified SV80 large T or p53 (the two components of the complex against which activity was selected in the screen) in a direct binding assay using the method described by Lane and Robbins (1978) as modified by Harlow *et al.* (1981b), there was no detectable specific rebinding of probe to antibody. In the same experiment, the appropriate positive control antibodies PAb416 and PAb421 rebound 70% and 45% of large T and p53, respectively (data not shown). This could imply either that PAb1101, 2 and 3 recognise denaturation-sensitive determinants on p53 or large T, or that they actually recognise other uncharacterised components of the large T/p53 complex, and have no direct reactivity towards either p53 or large T.

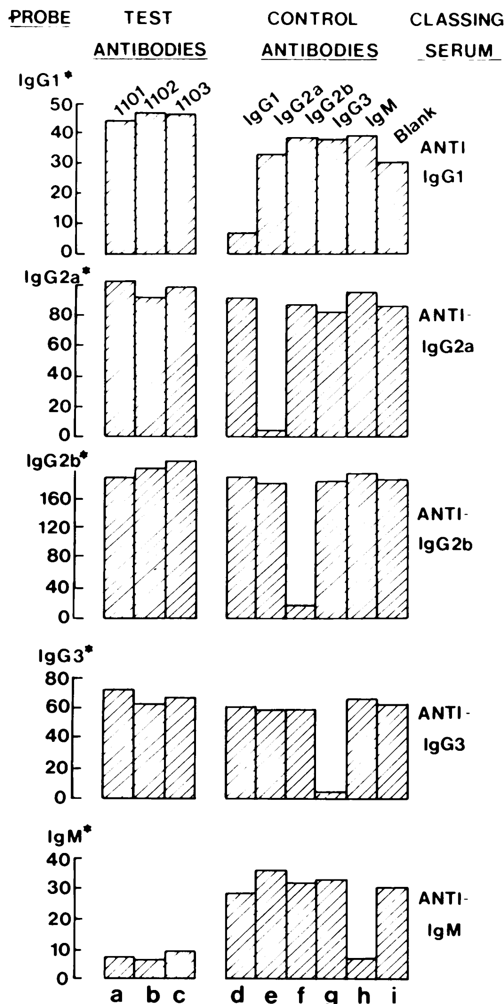


**Fig. 1.** Sepharose-protein A affinity-purified antibodies from rabbit antisera directed against individual mouse IgM and IgG classes and subclasses were bound to PVC microtitre wells in 10 mM phosphate buffer, pH 7.0. $^{125}$I-labelled antibodies of the class appropriate to interact with each classing antiserum (PAb405-IgG1; PAb421-IgG2a; PAb413-IgG2b; PAb430-IgG3; RA3-2C2-IgM) were then added in 10 $\mu$l NET/gel buffer, mixed with 40 $\mu$l of tissue culture supernatant containing either no antibody (**column i**), test antibodies PAb1101, 2, 3 (**a–c**) or control antibodies of known class as indicated (**d–h**). Wells were washed in NET/gel buffer after 4 h at room temperature, dried and counted. All results are expressed as c.p.s. on the ordinate.

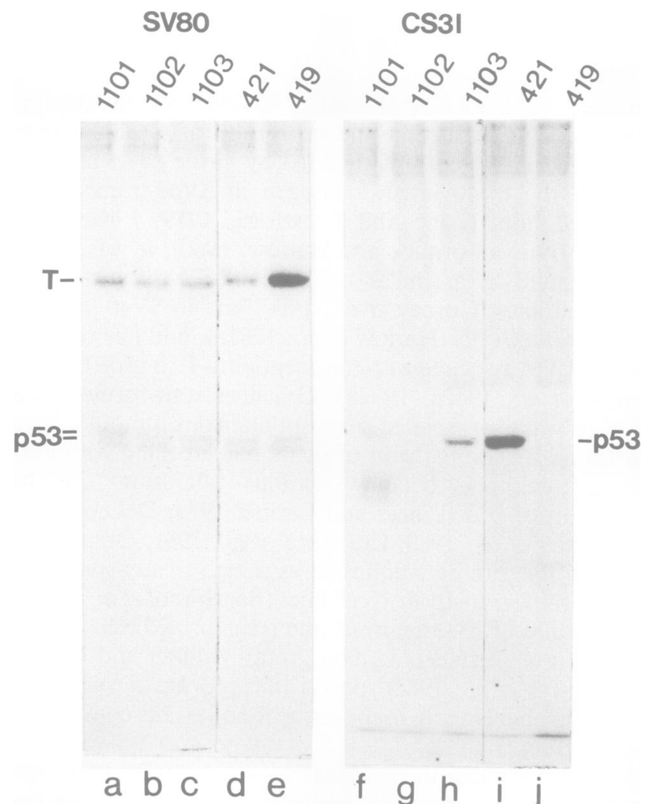


**Fig. 2.** Aliquots of $^{32}$P-labelled SV80 (**lanes a–e**) or C331 (**lanes f–j**) cell extract were immunoprecipitated with 1.0 $\mu$l PAb1101 (**a,f**), 5.0 $\mu$l PAb1102 (**b,g**), 5.0 $\mu$l PAb1103 (**c,h**), 1.5 $\mu$g PAb421 (**d,i**) or 1.5 $\mu$g PAb419 (**e,j**) for a total of 2 h with 0.25 $\mu$l/reaction rabbit anti-mouse Ig antiserum added for the final 30 min. Precipitates were analysed by electrophoresis through 10% polyacrylamide-SDS gels. Large T and p53 migrated to the positions indicated.

*Antibodies PAb1101, 2 and 3 recognise p53*

PAb1101, 2 and 3 were selected for the ability to immunoprecipitate SV80 T/p53 complex. An example of their interaction with this antigen is shown in Figure 2, lanes a – c. They clearly precipitate the same $^{32}P$-labelled species as does the anti-p53 antibody PAb421 (lane d) and do not apparently precipitate the extra uncomplexed large T that is brought down by anti-large T antibody PAb419 (lane e). When the interactions of these antibodies with $^{32}P$-labelled proteins from extracts of C33I human cervical carcinoma cells (Auersperg, 1964), a cell line expressing high levels of p53 (Crawford *et al.*, 1981; Benchimol *et al.*, 1982), were compared (Figure 2, lanes f – j) it was clear that PAb1103 could immunoprecipitate p53 though at a lower level than the control antibody PAb421. PAb1101 and 2 also have detectable activity against p53 (lanes f, g), although at a very low level (see below).

The possibility that one or more of PAb1101, 2 and 3 might have anti-large T activity was examined by first removing all p53 and associated large T from a $^{32}P$-labelled SV80 lysate with PAb421 and then attempting to precipitate the residual T antigen with the various antibodies (Figure 3). Whilst anti-large T antibody PAb419 immunoprecipitated a considerable amount of uncomplexed large T (panel D, lane 5), none of the three antibodies PAb1101, 2 or 3 had any detectable affinity for this antigen (panels A, B, C, lane 5). It is therefore clear that none of these three antibodies have anti-T activity whilst PAb1103, and with low affinity PAb1101 and PAb1102, have activity towards unassociated p53.

*PAb1101, 2 and 3 display a preference for large T-associated p53*

The data presented in Figure 2 shows that whilst the chosen inputs of antibodies PAb1101, 2 and 3 precipitate the same amount of T/p53 from SV80 extract as does an excess of PAb421 (1.5 $\mu$g), all three precipitate less p53, and in the case of PAb1102 very much less p53 from an aliquot of C33I lysate than does PAb421. To investigate this difference further, PAb1101, 2 and 3 were titrated in parallel with PAb421 against labelled extracts of SV80, C33I and a second SV40-transformed human fibroblast line VA13 (Figure 4). The amounts of PAb1101, 2 and 3 needed to achieve the same immunoprecipitation response from C33I extract as 0.08 $\mu$g of PAb421 varied from 25-fold to 125-fold more than that needed to achieve the same result in SV80 or VA13 extracts. The dose-response in VA13 was identical to that in SV80 for all the antibodies. At high antibody inputs, the activity of PAb1101 and PAb1102 to C33I p53 is readily detected.

These data suggest that PAb1101, 2 and 3 have a strong preference for binding to the T-associated form of p53. Possible bases for this effect are discussed below.

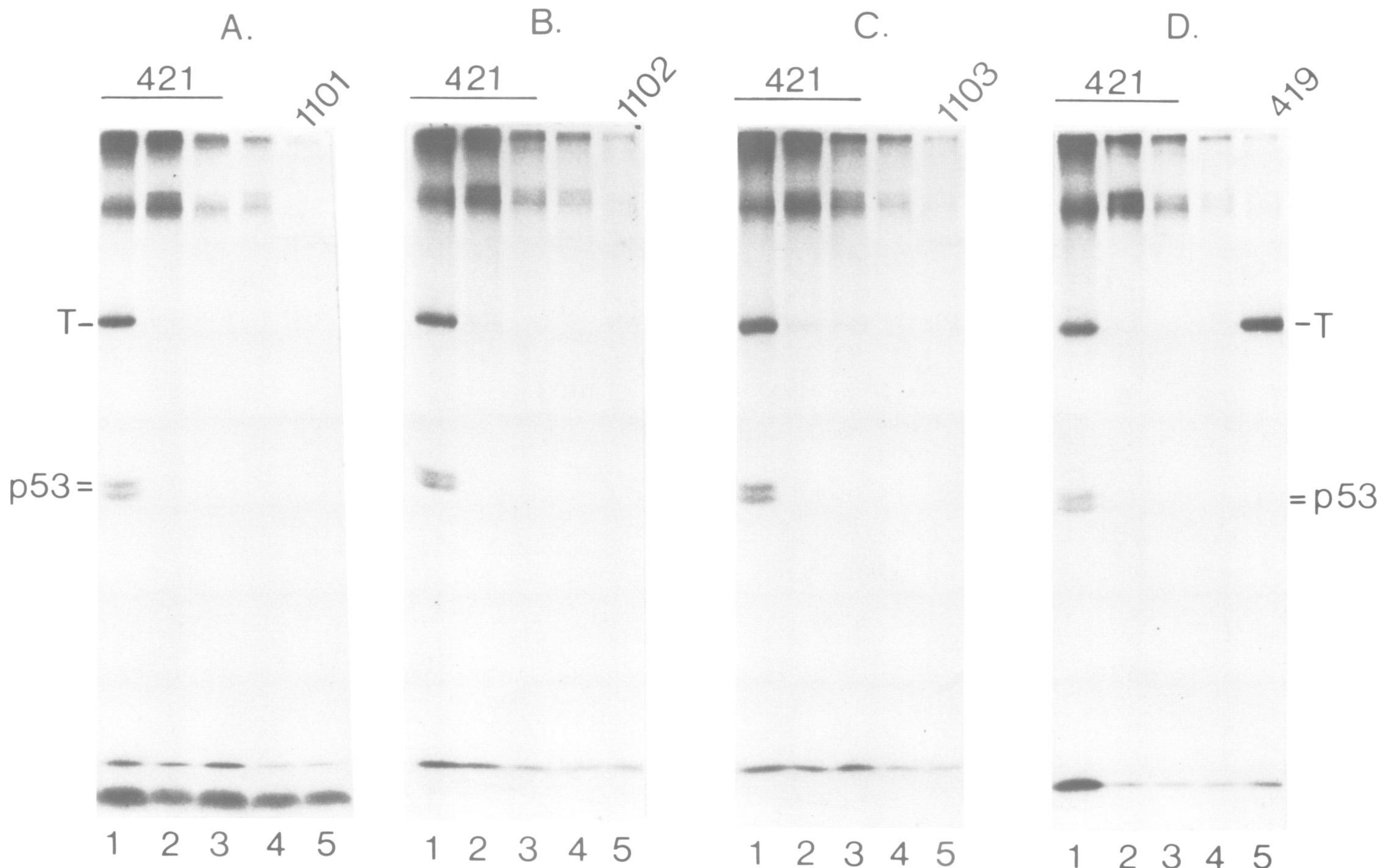


**Fig. 3.** Aliqots of $^{32}P$-phosphate labelled SV80 extract were subjected to three sequential immunoprecipitations with PAb421 (each panel, **lanes 1 – 3**) followed by an additional clearance with SAC (**lanes 4**). Finally, the extracts were immunoprecipitated (**lanes 5**) with PAb1101 (**panel A**), PAb1102 (**panel B**), PAb1103 (**panel C**) or PAb419 (**panel D**). Antibody inputs were 1.5 $\mu$g of PAb421 and 419 or 20 $\mu$l of tissue culture supernatant from PAb1101, 2 and 3. All reactions were for a total of 1 h with 0.25 $\mu$l rabbit anti-mouse Ig added after 30 min. Immune complexes were collected on 20 $\mu$l 10% SAC for 15 min at 4°C, and supernatants immediately transferred to the next reaction. Precipitated proteins were separated on 10% polyacrylamide-SDS gels and detected by autoradiography.

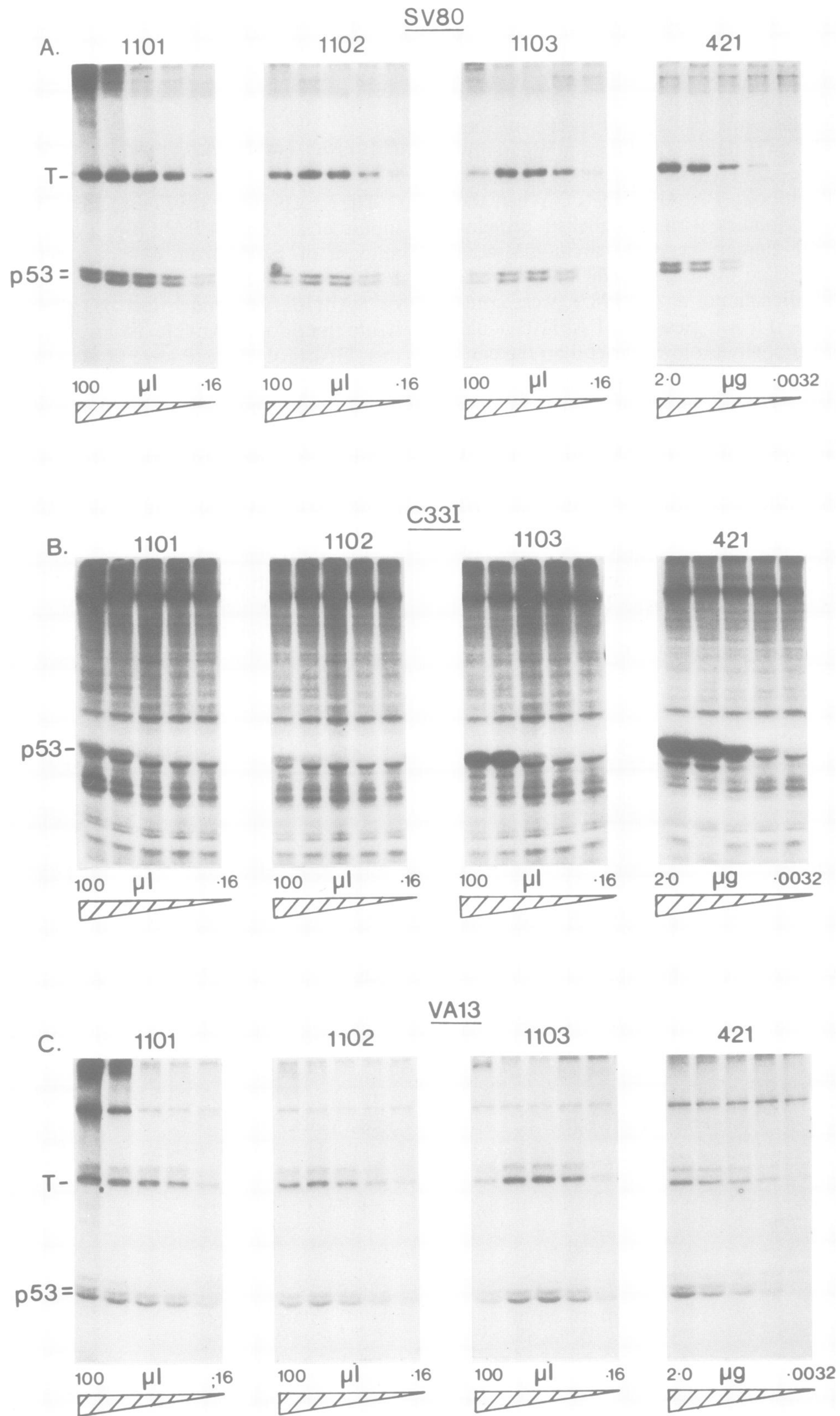


**Fig. 4.** $^{32}$P-phosphate-labelled extracts were prepared from SV80 **(panel A)**, C33I **(panel B)** or VA13 **(panel C)** cells and aliquots immunoprecipitated with the antibodies indicated for 2 h at room temperature with 0.25 $\mu$l rabbit anti-mouse Ig antiserum added for the final 30 min. Proteins were separated on SDS 10% polyacrylamide gels and detected by autoradiography. Each antibody was used at five inputs in 5-fold dilutions from left to right with maximum and minimum inputs as indicated.

### *PAb1101, 2 and 3 have novel species-specificities*

As described above, each of these antibodies has activity towards p53 from human cells. To determine whether they also had activity to p53 from other species, mouse, rat and monkey cell lines were labelled with [$^{32}$P]phosphate and aliquots of extract immunoprecipitated as before. As shown in Figure 5, PAb1101, 2 and 3 had only barely detectable activity to T/p53 complex from the SV40-transformed BALB/c 3T3 line, SVA31E7, lanes a–c, at antibody inputs that show strong immunoprecipitation of SV80 antigen (Figure 2) and no activity against p53 from the C3H 3T12-like line, CQ15 (lanes f–h). PAb1101, 2 and 3 also showed a similar pattern of activity towards p53 from the rat SV40-transformed fibroblast line, 14B (data not shown). High inputs of PAb1101 produced readily detectable precipitation of T/p53 from E7 extract implying that this antibody has reduced affinity for mouse p53 rather than completely lacking anti-mouse p53 activity.

When extracts of CV1 cells labelled 48 h post-infection with SV40 at 7.5 p.f.u./cell or mock-infected were used (Figure 6), it was found that PAb1101 and 2 (lanes a and b) but not PAb1103 (lane c) precipitated two species that comigrated with large T and p53 precipitated by PAb421 (lane d). PAb1101, 2 and 3 had no detectable activity, however, towards the uncomplexed p53 from mock-infected CV1 cells that was readily precipitated by PAb421 (lane i). Even when high inputs of antibody were used in titration experiments similar to those described in Figure 4, no activity could be detected towards the uncomplexed CV1 p53 for any of the three antibodies. In contrast, PAb1101 and PAb1102 bound the complex of monkey p53 with large T as efficiently as they did the SV80 or VA13 complex (data not shown). Each of these antibodies therefore has a discrete and novel species specificity. PAb1101 shows activity towards the human and monkey p53, and, at a much reduced affinity, to rodent p53, whilst PAb1102 is primate-specific and PAb1103 human specific. All show a strong preference for T-associated p53.

### *PAb1101, 2 and 3 recognise new areas of the p53 molecule*

Previously described anti-p53 antibodies react either with rodent and human p53 or with rodent p53 only. Since PAb1101, 2 and 3 have been shown to recognise primate or human-specific determinants on p53, they necessarily define a new class of determinant on p53. To examine the topological distribution of the various determinants on human p53, we performed a series of blocking assays, the principles of which have been described previously (Crawford, 1982). The results of experiments using various labelled and unlabelled anti-p53 antibody pairings competing for fixed SV80 large T/p53 complex are shown in Table I.

The binding of labelled PAb421 to p53 is completely blocked by unlabelled PAb421 or PAb122 but none of the new antibodies PAb1101, 2 or 3 have any effect on the binding of either this probe or labelled PAb122. By this criterion, in confirmation of the data presented above, they therefore recognise new determinants on p53. Furthermore, these determinants fall on previously unrecognised areas of the p53 molecule, since their binding to antigen is unaffected by PAb421 or PAb122. Competition between PAb1101, 2 and 3 in pairwise combinations shows that there is no interaction between PAb1103 and PAb1101 or PAb1102. The PAb1103 determinant is therefore in a different area of p53 from those recognised by PAb1101 and PAb1102. Whilst PAb1102 has little effect on the binding of labelled PAb1101, the reverse combination shows a strong blocking effect. The possible bases of this distinction are discussed below. Clearly, PAb1101, 2 and 3 define at least two new determinants on human p53 to which antibodies have not previously been raised.

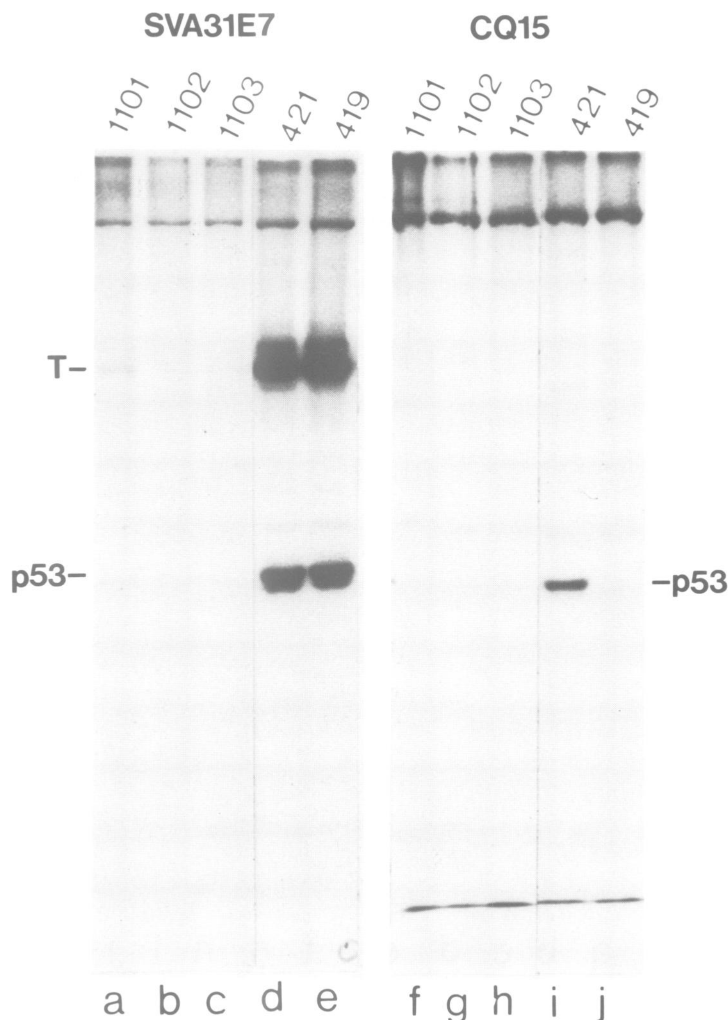


**Fig. 5.** $^{32}$P-phosphate labelled extracts of mouse SVA31E7 (**lanes a–e**) and CQ15 (**lanes f–j**) cells were immunoprecipitated with 1 μl PAb1101 (**a,f**), 5 μl PAb1102 (**b,g**), 5 μl PAb1103 (**c,h**), 1.5 μg PAb421 (**d,i**) or 1.5 μg PAb419 (**e,j**) for 2 h at room temperature with 0.25 μl per reaction of rabbit anti-mouse Ig antiserum added for the final 30 min. Precipitated proteins were separated by electrophoresis through SDS-10% polyacrylamide gels and detected by autoradiography.

## Discussion

A number of monoclonal antibodies have been raised against the p53 protein. All have activity towards the p53 molecule found in rodent cells and three (PAb122, PAb410 and PAb421) also react with primate p53. We have described here the isolation of three monoclonal antibodies, designated PAb1101, PAb1102 and PAb1103, that react with the T/p53 complex from SV80 cells. They do not react with free T antigen from these cells, but do react with p53 from a human tumour cell line, C33I, that contains no SV40 large T antigen. Therefore, although none of the three will rebind gel purified SV80 p53, it appears that they do have direct anti-p53 activity. This implies that the determinants on p53 recognised by

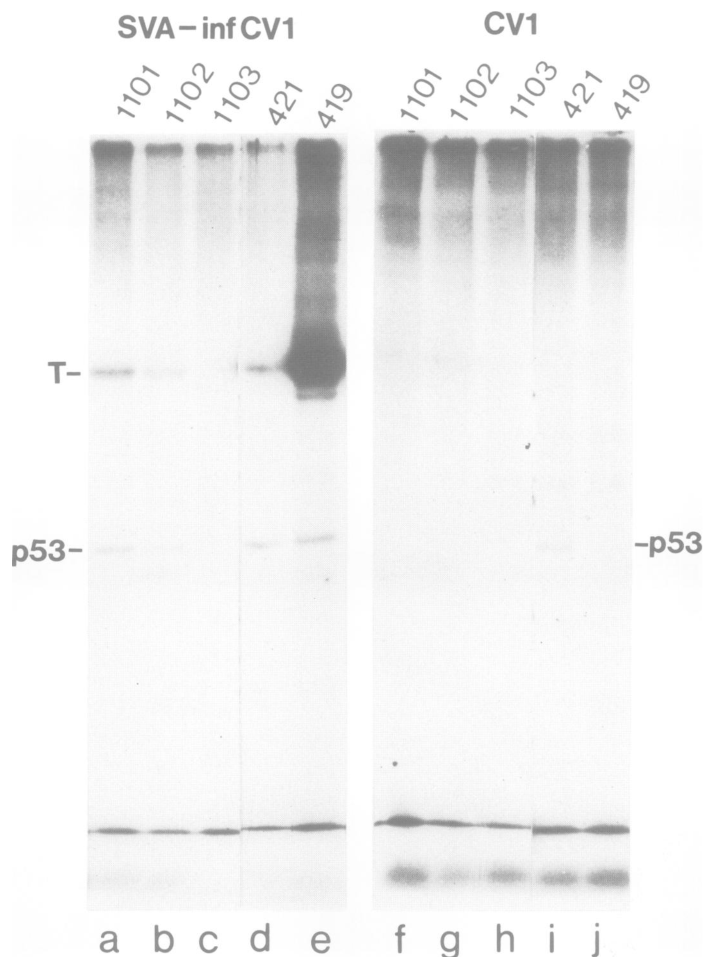


**Fig. 6.** As Figure 5 except extracts were from CV1 monkey cells labelled at 48 h post-infection with 7.5 p.f.u./cell SV40 (**lanes a – e**) or mock-infected CV1 (**lanes f – j**) and antibody inputs were 5 μl PAb1101 (**a,f**), 20 μl PAb1102 (**b,g**) and 20 μl PAb1103 (**c,h**).

**Table I.** Competition between anti-p53 antibodies

| Blocking Ab | Probe | | | | |
|---|---|---|---|---|---|
| | PAb1101 % | PAb1102 % | PAb1103 % | PAb421 % | PAb122 % |
| PAb1101 | 23 | 1.7 | 70 | 87 | 90 |
| PAb1102 | 74 | 9.8 | 99 | 92 | 96 |
| PAb1103 | 97 | 63 | 4.1 | 74 | 95 |
| PAb421 | 96 | 68 | 87 | 0.5 | 59 |
| PAb122 | 84 | 66 | 90 | 3.5 | 31 |
| RA3 2C2 | 98 | 67 | 89 | 99 | 94 |
| PAb416 | 98 | 66 | 93 | 86 | 89 |

Antibodies as indicated in the left hand column, in 25 μl 50 mM Tris pH 7.4, 150 mM NaCl were allowed to compete with $^{125}$I-labelled antibodies as indicated across the table, in 10 μl of the same buffer containing 1.0 mg/ml bovine serum albumin, for binding to large T/p53 complex from SV80 cell extract immobilised via PAb419 on PVC microtitre wells, and the counts remaining bound after 2 h determined. Data are expressed as % of a negative control in which probe was diluted with 25 μl of buffer containing no antibody and are the mean of two determinations. Under conditions of zero blocking, the bound counts from each probe were ~200 c.p.s.

these new antibodies are sensitive to denaturation, though it is still possible that their activity is to a still uncharacterised cellular protein that interacts with p53 and with T/p53 complex.

When tested for the ability to immunoprecipitate p53 from cells of monkey and rodent origin, PAb1102 was able to immunoprecipitate monkey, but not mouse or rat, p53 whilst PAb1103 had no activity against p53 from any of these species. PAb1101 had activity against monkey p53 and, at much lower efficiency, mouse p53. We have, therefore, succeeded in isolating antibodies whose anti-p53 activities are of novel species specificity, being largely or completely primate- or human-specific.

PAb1101, 2 and 3 were reproducibly efficient at immunoprecipitating p53 in complex with SV40 large T from two human SV40-transformed fibroblast lines, and from SV40-infected monkey CV1 cells. They were much less efficient, relative to an internal PAb421 control, at immunoprecipitating p53 from a human cervical carcinoma line C33I and from uninfected CV1 cells. Two human mammary tumour cell lines MB157 and Hs578T showed a similar effect. This could reflect a conformational change in p53 caused by the association with large T that leads to the generation of the preferred conformations for antibody binding in the PAb1101, 2 and 3 determinants.

Alternatively, since the determinants recognised would appear to be absent from the denatured polypeptide chain, it may be that the effect of large T association is to stabilise p53 in the cell and during extraction. Other workers (Linzer *et al.*, 1979; Oren *et al.*, 1981) have shown that SV40 infection of mouse 3T3 cells causes an increase in the incorporation of [$^{35}$S]methionine into p53, dependent on the expression of a functional large T antigen, and that the half-life of p53 in SV40-transformed mouse 3T3 cells is >22 h as compared with only 20–30 min in 3T3 cells. Benchimol *et al.* (1982) have shown that an SV40-transformed 3T3 line, E7, has at least 100-fold more p53 protein per cell than does the untransformed 3T3 parent. If large T does stabilise p53 by its association with it, it would be reasonable to expect this effect to be reflected at an immunological level in the stabilisation of a set of determinants on p53, which can therefore be detected more readily on the large T/p53 complex than on free p53. PAb1101, 2 and 3 may recognise determinants of this type.

A third possibility is that T antigen actually contributes part of the determinants recognised by PAb1101, 2 and 3. If this is the case then the ability of these antibodies to interact with p53 in non-SV40-transformed cells must be attributed to the existence of a cellular protein capable of interacting with p53 and creating a structure that is in some respects equivalent to the T/p53 complex. A theoretical justification for expecting such a T-equivalent cellular protein has been given by Lane and Hoeffler (1980), however, there has not yet been any convincing demonstration of the existence of such a protein. Further experiments to determine the basis of the preference of each antibody for T/p53 complex are in progress.

Competition assays between PAb1101, PAb1102, PAb1103, PAb421 and PAb122 showed that none of the three new antibodies recognised determinants that were close to the known PAb421 and PAb122 determinants. Furthermore, PAb1101, 2 and 3 define determinants that themselves lie in at least two distinct areas of the p53 molecule. They

therefore expand considerably our ability to probe the human p53 molecule. In the assay employed, PAb1101 blocks the association of [$^{125}$I]PAb1102 with SV80 complex but the opposite combination of probe and blocking antibody shows virtually no blocking of probe binding to T/p53 complex. This may be a reflection either of the two antibodies having very different affinities for what are truly competing sites or, alternatively, of some directionality of steric hindrance imposed by fixing the antigen to the PVC wells in one particular orientation. However, the precise basis of this effect has still to be determined.

Levine and his collaborators have recently isolated an antibody, PAb1005, (Thomas *et al.*, in preparation) some of whose properties may be similar to that of PAb1101, PAb1102 and PAb1103 reported here. These antibodies provide a new set of molecular probes for distinct areas of the human p53 molecule and make possible further studies of the interaction of p53 with SV40 large T and with other cellular proteins in normal and transformed human cells which should shed light on the function of this protein.

## Materials and methods

*Cell culture and virus*

VA13 is an SV40-transformant of WI38 human lung fibroblasts and was provided by L. Gooding. Other cell lines and viruses have been previously described (Harlow *et al.*, 1981a; Benchimol *et al.*, 1982). Cells were grown on NUNC 90 mm tissue culture dishes or bioassay trays in Dulbecco's modified Eagles medium (E4) supplemented with 10% fetal calf serum (FCS) for SV80, Hs578T, MB157 and CQ15, with 5% FCS for SVA31E7 or with 10% calf serum for CV1, or in RPMI 1640 supplemented with 10% FCS for C331 and VA13.

The non-producing mouse myeloma line NS-1 was used as the parent cell line in fusions with splenocytes. NS1, fused cell populations and hybridomas during and after cloning were passaged in RPMI 1640 medium supplemented with 10% FCS, 10% NCTC 135 (GIBCO Laboratories), 0.15 mg/ml oxalacetate, 0.05 mg/ml pyruvate and 0.2 U/ml insulin ('growth medium'). Selection was achieved by the addition to this medium of 0.1 mM hypoxanthine, 0.01 mM methotrexate and 0.01 mM thymidine. Hypoxanthine and thymidine were maintained for 2–3 days after withdrawal of methotrexate to avoid cell killing by residual methotrexate. During cloning steps, cells were maintained in a mixture of equal volumes of growth medium and similar medium preconditioned by normal BALB/c splenocytes for 3–4 days at 37°C.

*Antibodies*

Monoclonal antibodies of the PAb400 series were isolated by E. Harlow (Harlow *et al.*, 1981b), PAb122 by E. Gurney (Gurney *et al.*, 1980) and RA3-2C2 by R. Coffman (Coffman and Weissman, 1981). Antibodies PAb122, 410 and 421 interact with rodent and human p53s, RA3-2C2 with rodent p53 only and PAb405, 406, 413, 416, 419, 423 and 440 with SV40 large T antigen. PAb419 also interact with SV40 small T antigen. Monoclonal antibody W6/32 is directed against HLA antigen (Barnstaple *et al.*, 1978) and was provided by M. Crumpton. Lyophilised rabbit antibodies directed at specific mouse immunoglobulin heavy chain classes were obtained from Miles Laboratories, Inc.

*Cell labelling and preparation of extracts*

Monolayers of cells grown on 90 mm dishes were labelled for 3 h at 37°C with [$^{32}$P]phosphate (1 mCi/dish, carrier-free; Amersham International) contained in 2.5 ml of E4 lacking phosphate. $10^7$ hybridoma cells were grown in suspension in 2.0 ml E4 lacking methionine with 1.0 mCi [$^{35}$S]methionine (1000–1300 Ci/mmol; Amersham International) to label secreted proteins. Extracts were prepared from monolayers after washing with Tris-buffered saline (150 mM NaCl, 50 mM Tris pH 7.4) at 0°C by lysing on ice for 30 min with 1.0 ml lysis buffer (1% Nonidet P-40, 150 mM NaCl, 50 mM Tris pH 8.0). Dishes were then scraped dry and the extracts clarified by spinning for 1 min in an Eppendorf microfuge.

*Immunoprecipitation and gel eletrophoresis*

Labelled cell extracts were used immediately for immunoprecipitation. Aliquots of extracts were diluted 5- to 10-fold with NET/gel buffer (150 mM NaCl, 5 mM EDTA, 50 mM Tris pH 7.4, 0.05% Nonidet P40, 0.02% $NaN_3$, 0.25% gelatin) before addition of the appropriate antibody and the reaction allowed to proceed for 2 h at room temperature (or at +4°C overnight for screening reactions). 0.25 $\mu$l of rabbit anti-mouse Ig antiserum was added to all small-scale reactions for 30 min prior to the end of the reaction period. Immune complexes were collected on 20 $\mu$l 10% *S. aureus* strain Cowan 1 (SAC) (Kessler, 1975) per 1.5 $\mu$g purified antibody or 20 $\mu$l tissue culture supernatant for 15 min at +4°C, washed twice in NET/gel buffer (except screening reactions, unwashed) and eluted into 1.25 volumes of sample buffer (2% SDS, 100 mM dithiothreitol, 10% glycerol, 25 mM Tris pH 6.8) per volume of SAC by heating to 70°C for 5 min. Samples were loaded onto 10% polyacrylamide-SDS gels (Laemmli, 1970) and electrophoresed for 3–4 h at 100–200 V. Gels were fixed in 7.5% acetic acid, 25% methanol for at least 30 min before drying and were exposed either to Kodak SB-5 film at room temperature or to pre-flashed Fuji RX film at −70°C with fast tungstate screens.

*Purification of antibodies*

Antibodies of the PAb400 series and PAb122 were purified from tissue-culture supernatant by E. Harlow as previously described (Harlow *et al.*, 1981b), concentrated by ammonium sulphate precipitation and redissolved at high concentration in phosphate-buffered saline. RA3-2C2 was provided as a concentrated solution by R. Coffman. PAb1101, 2 and 3 were partially purified on a small scale by ammonium sulphate precipitation from tissue-culture supernatant. PAb1103 was obtained 90% pure and PAb1101 and PAb1102 ~10–20% pure as judged by Coomassie blue staining of samples after gel electrophoresis. The only major contaminant had the mol. wt. expected of serum albumin.

*Radioimmunoassays*

Iodination of antibodies was by the modification of the chloramine-T method of Syvanen *et al.* (1973) described by Benchimol *et al.* (1982), or by the method of Bolton and Hunter (1973). The determination of antibody class was as described by Harlow *et al.* (1981b) except that all tests used 40 $\mu$l of tissue culture fluid.

Blocking assays, to determine the extent of interference between pairs of anti-p53 antibodies simultaneously interacting with SV80 antigen, were performed as follows. A first antibody was applied to PVC wells overnight at room temperature as a 20 $\mu$g/ml solution in 10 mM phosphate buffer, pH 7.0 (50 $\mu$l, 1.0 $\mu$g per well) and the excess rinsed off with NET/gel. Freshly prepared extract of unlabelled SV80 cells (~$10^7$ cells extracted in a total volume of 400 $\mu$l) was then added at 20 $\mu$l per well for 2 h at room temperature, and rinsed off. A mixture of $^{125}$I-labelled antibody in 10 $\mu$l NET/gel with 25 $\mu$l of antibody-containing supernatant dialysed exhaustively against 50 mM Tris pH 7.4, 150 mM NaCl, 1 mg/ml BSA was then added to each well and the number of counts retained on the well after a 4 h incubation determined.

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