Journal of Cell Science 108, 1843-1848 (1995)
Printed in Great Britain © The Company of Biologists Limited 1995

Coupling between gamma irradiation, p53 induction and the apoptotic response depends upon cell type in vivo

Carol A. Midgley[1], Bronwyn Owens[2], Cecilie V. Briscoe[3], David Brynmor Thomas[3], David P. Lane[1] and Peter A. Hall[2,*]

[1]Department of Biochemistry, University of Dundee, Dundee, DD1 4HN, UK
[2]Department of Pathology, Ninewells Hospital and Medical School, University of Dundee, Dundee, DD1 9SY, UK
[3]School of Biological and Medical Sciences, University of St Andrews, KY16 9TS, UK

*Author for correspondence

SUMMARY

The accumulation of p53 protein following whole body irradiation of adult mice was studied using a new polyclonal antibody to mouse p53. While dramatic accumulation of the protein was apparent in splenocytes, thymocytes and osteocytes no p53 protein accumulation was detected in the hepatocytes of the irradiated mouse. Thus the upstream initiating signals that control the induction of p53 are controlled in a tissue specific manner. While massive apoptosis accompanies p53 induction in thymocytes and splenocytes it is not seen in the osteocytes. Thus the downstream consequences of p53 induction are also tightly controlled. These results have profound significance for an understanding of the role of the p53 tumour suppression pathway in different tissues.

Key words: p53, immunohistochemistry, heterogeneity, antibody, genotoxicity, apoptosis

INTRODUCTION

In tissue culture systems, levels of p53 protein rise in response to a variety of DNA damaging agents (Maltzman and Czyzyk, 1984; Kastan et al., 1991; Kuerbitz et al., 1992; Lu and Lane, 1993; Fritsche et al., 1993). This induction is mediated by a post-translational mechanism and is associated with transcriptional activation of p53 responsive genes (Kern et al., 1991; Kastan et al., 1992). The purpose of this response is to initiate and coordinate a set of cellular responses that include cell cycle arrest and apoptotic cell death (Lane, 1992, 1993; Hall and Lane 1994). By activating these processes p53 exerts a profound tumour suppressing effect.Evidence for the in vivo relevance of these phenomena comes from analysis of systems in which p53 function is abrogated (Symonds et al., 1994; Donehower et al., 1992; Harvey et al., 1993; Kemp et al., 1993).

Further compelling support for the role of p53 as a link between genotoxic insult and cellular responses comes from studies of mammalian tissues exposed to genotoxic insult. For example, studies in whole tissues, both in man and mouse, have indicated that environmentally relevant levels of UV irradiation of human skin results in a dramatic and rapid induction of p53 protein in many, but not all, cells of the dermis and epidermis (Hall et al., 1993; Campbell et al., 1993). This is temporally and spatially associated with DNA damage as evidenced by PCNA induction and in situ end labelling methods (Hall et al., 1993; Coates et al., 1995). In the mouse intestinal epithelium, experiments comparing the radiation induced apoptotic and growth arrest response with sites of accumulation of p53 protein have shown that these two phenomena are temporally and spatially coordinated (Merritt et al., 1994; Clarke et al., 1994). In order to further characterise the temporal and micro-anatomical features of this biologically important response to DNA damage in radio-resistant and -sensitive mouse tissues we developed and applied a new polyclonal antiserum to recombinant mouse p53 that can be effectively used on routinely fixed and processed material.

MATERIALS AND METHODS

Expression of mouse p53 in *E. coli* and generation of poyclonal serum CM5

A cDNA encoding mouse p53 (Jenkins et al., 1984) was inserted into plasmid pT7-7 (Tabor and Richardson, 1985) to create pT7-7Mop53. Mouse p53 protein expression in *E. coli* was induced by isopropyl-β-D-thiogalactopyranoside (IPTG) as previously described (Midgley et al., 1992). Under these conditions most of the p53 protein recovered was insoluble and could be purified by the procedure described (Midgley et al., 1992). A polyclonal anti-serum CM-5 was produced in a rabbit by immunisation with the resolubilised mouse p53 protein. The first injection of 0.5 mg of protein was given in Freund's complete adjuvant subcutaneously. Three booster injections of the same dose were given at one month intervals in incomplete Freund's adjuvant.

Affinity purificaton of CM5

5 mls of murine p53 (0.3 mg/ml) was dialysed for 48 hours with 500 ml of carbonate coupling buffer (0.1 M $NaCO_3$, O.1 M Na_2HCO_3, 0.4

M NaCl, pH 9-9.8) with 4 buffer changes. 10 ml of Reacti-gel slurry (Pierce) was removed from the storage buffer by centrifugation for 2 minutes at 900 ***g***. The matrix was then washed 3 times with ice-cold Milli-Q water. Following the final wash, the Reacti-gel was mixed with dialysed protein solution and incubated at room temperature for 60 hours. The Reacti-gel was pelleted as above, the supernatant removed and the gel washed once with carbonate coupling buffer. The gel was mixed with blocking buffer (1.5 M glycine, pH 8.5) for 4 hours at room temperature. The Reacti-gel was removed from blocking buffer as above and washed 3 times with carbonate coupling buffer and twice with ice-cold Milli-Q distilled water. The matrix was stored at 4°C in 10 ml of carbonate coupling buffer with 0.02% sodium azide. For affinity purification of CM5 the coupling buffer was removed and the gel washed in phosphate buffered saline (PBS) twice as above. 10 ml of PBS and 5 ml of CM5 serum were mixed with the gel overnight at 4°C. The gel was pelleted as above, the supernatant removed and the gel resuspended in 30 ml of PBS and transferred to a column. The column was washed with 10 volumes of PBS, 0.1% NP-40, followed by 10 column volumes of PBS. The antibody was eluted in six 5 ml aliquots of 0.1 M glycine, pH 3.0. Each aliquot was collected directly into 0.5 ml of 1 M Tris, pH 8.0, to neutralise pH.

Characterisation of CM5

Cell lines and antibodies

Cell lines were cultured at 37°C in DMEM with 10% FCS. T3T3 cells, a spontaneously transformed 3T3 mouse fibroblast line, were isolated in this laboratory. C6 cells are rat embryo fibroblasts transformed by temperature sensitive mouse p53 and activated *ras*. The mouse null line (–/–) has a homozygous deletion of the p53 gene and does not express p53 protein (Harvey et al., 1993). The monoclonal antibodies PAb421 (Harlow et al., 1981) and PAb240 (Gannon et al., 1990) recognise p53. Pure PAb240 was biotinylated using a kit from Amersham. Cells were rinsed in PBS and fixed in cold acetone:methanol (1:1) for 8 minutes before incubation overnight at 4°C with CM5 serum diluted 1:2,000 in tissue culture medium. Cells were washed with PBS and incubated with HRP goat anti-rabbit Ig (Jackson) diluted 1:100 for 1 hour at room temperature. HRP was detected with 3,3′-diamino-benzidine tetrahydrochloride (DAB, Sigma).

Immunochemistry

For direct immunoblotting total cell protein lysates were separated by SDS-polyacrylamide gel electrophoresis (PAGE) on a 12% gel and transferred to nitrocellulose membrane by electroblotting (Harlow and Lane, 1988). Prestained molecular mass markers (Bio-Rad) were run in parallel. Blots were blocked in PBS containing 0.1% Tween-20, 5% dried milk protein for 1 hour then incubated overnight at 4°C with monoclonal antibody PAb421 supernatant or with affinity purified CM5 diluted 1:2,000 in PBS containing 10% foetal calf serum (FCS). The blot was washed in PBS, 0.1% NP40 and incubated for 1 hour at room temperature with horseradish peroxidase (HRP)-conjugated rabbit anti-mouse Ig (Dako) or HRP goat anti-rabbit Ig (Jackson), respectively, diluted 1:2,000. HRP labelled protein bands were detected using an ECL kit (Amersham).

Immunoprecipitation of p53 from cells lysed in 150 mM NaCl, 50 mM Tris, pH 8.0, 5 mM EDTA, 1% NP40, 1 mM PMSF was carried out as described previously (Midgley et al., 1992) using affinity purified PAb421 or CM5. Immunoprecipitates were subjected to separation by SDS-PAGE and transferred to nitrocellulose. Immunoprecipitates with PAb421 were detected with affinity purified CM5 diluted 1:2,000 followed by HRP goat anti-rabbit Ig as above. Due to a slight cross-reaction between CM5 and mouse heavy chain the CM5 was preadsorbed by the addition of 10% normal mouse serum before incubation with the blot. Similarly the CM5 immunoprecipitate blot was preblocked with 10% normal mouse serum before incubation for 1 hour at room temperature with biotinylated PAb240 diluted 1:100 and premixed with Extravidin-HRP (Sigma) diluted 1:1,000. In both cases HRP labelled protein bands were detected using ECL as above.

Irradiation of mice and immunohistochemistry

Adult female albino CD1 mice (25-30 g) were given whole body irradiated 5 Gy of γ rays from a CIS BioInternational IBL 437C ^{137}Cs gamma source at a dose rate of 4.66 Gy a minute. Three animals were killed at subsequent time points and from all animals liver, spleen and thymus samples were obtained, weighed and fixed in buffered formalin, processed and embedded in paraffin wax using standard methods. All three tissues from any animal were handled identically and were embedded into the same wax block, thus acting as internal controls for each other. Tissue sections (5 μm) were cut, dewaxed and immunostained using CM5 at a dilution of 1 in 3,000 after microwave antigen retrieval. In brief, slides were immersed in citrate buffer and boiled for 3 periods of 10 minutes using a domestic microwave oven at full power setting (750 Watts), with care being taken to not allow the sections to boil dry. Immuno-histochemistry was then performed as described previously (Midgley et al., 1992). After immunostaining sections were washed in distilled water, counterstained with Meyer's haemotoxylin, dehydrated cleared and mounted. Staining was assessed semi-quantitatively by two observers (B.O. and P.A.H.) and this was found to be highly reproducible.

In situ end labelling (ISEL)

The method was employed as previously described (Ansari et al., 1993; Coates et al., 1995). In brief, dewaxed paraffin sections of mouse tissues were briefly digested with proteinase K (40 μg/ml in 50 mM Tris-HCl buffer with 1 mM EDTA). In situ end labelling was then performed using 50 μl of labelling mix. This contains 0.01 mM each of digoxigenin-14-dATP, dCTP, dGTP and dTTP in 50 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 0.005% BSA and the Klenow fragment of DNA polymerase I. Incubation is carried out for 1 hour at 37°C. The reaction was terminated by washing sections in distilled water and the incorporated dNTP detected by an immunohistological method using anti-digoxigenin antibodies (Boehringer). This was then detected by standard immunohistochemical methods. Controls consisted of omission of the polymerase from the labelling mix and other relevant immunohistological controls. Apoptosis was quantified by enumerating the number of ISEL labelled apoptoses in ten high powered fields (×40) in which the number of cells was also counted.

RESULTS

Characterisation of CM5

Fig. 1 demonstrates specific nuclear staining of T3T3 cells (Milner et al., 1993) with CM5 (Fig. 1) whereas p53 –/– cells (Donehower et al., 1992) show no staining (data not shown). Western blotting of C6 and T3T3 cell lysates demonstrates the same pattern with either CM5 (Fig. 2a, lanes 2 and 3) or PAb421 (Fig. 2a, lanes 5 and 6). Immunoprecipitates also show similar patterns for CM5 (Fig. 2b, lanes 2 and 3) and PAb421 (Fig. 2b, lanes 5 and 6) although PAb421 seems to more efficiently precipitate a slower migrating form of p53 which may have increased phosphorylation. The degradation product seen on direct blots of C6 cells (Fig. 2a, lanes 2 and 5; Gannon and Lane, 1991) is not immunoprecipitated efficiently by CM5 or PAb421. These data demonstrate the sensitive and specific detection of mouse p53 by CM5. This reagent can be employed in formalin fixed and wax embedded sections of rodent tissues as illustrated below.

Morphological response of tissues to irradiation and labelling with the ISEL method

Both the spleen and thymus showed the expected massive increase in apoptosis identifiable by the increase in apoptotic bodies stained with haemotoxylin or by the ISEL method. The induction of apoptosis was rapid (Table 1) and as expected from previous studies spleen weight fell coordinately with the appearance of apoptotic bodies (Fig. 3). At later time points restoration of normal splenic weight and splenic and thymic architecture occurred as expected from the sub-lethal dose of irradiation employed. However, both in untreated animals and in the recovery phase (day 7 and 14) a basal level of apoptosis is identifiable. While profound morphological alterations occurred in the bone marrow, the osteocytes showed no morphological abnormalities. Similarly, in the liver, hepatocytes showed no morphological alteration at any time point during the experiment. Small numbers of inflammatory cells are identifiable in the parenchyma and in the portal tracts 6 to 48 hours after irradiation and occasional Kupffer cells show morphological features of apoptosis and were also labelled by the ISEL method. The ISEL method has also been employed to demonstrate some forms of genotoxic DNA damage after UV irradiation and treatment with drugs such as etoposide (Coates et al., 1995). We were, however, unable to see induction of ISEL labelling in non-apoptotic cells after γ irradiation.

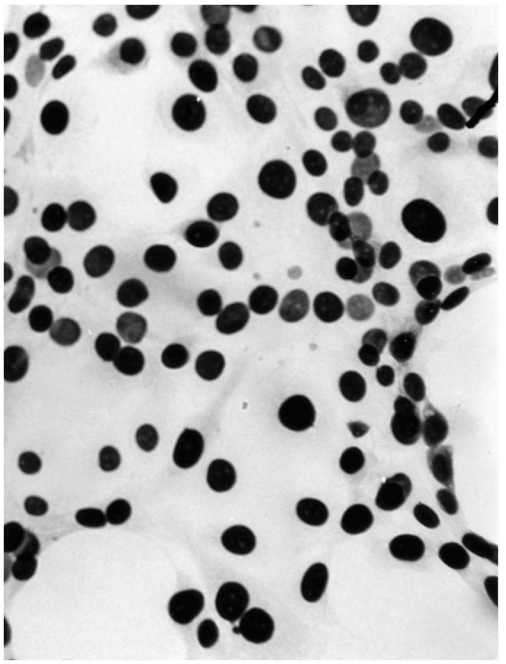

Fig. 1. Immunoperoxidase staining of mouse T3T3 cells.

Expression of p53 and PCNA

Using the CM5 antiserum we examined the expression of p53 protein after γ irradiation. In untreated animals there was no identifiable p53 staining while in the spleen and thymus of irradiated animals there is rapid and massive induction of p53 immunoreactivity in the majority of cells (Fig. 4). The induction of p53 was apparent at three hours and declined after 96 hours to base line levels (Table 1). p53 immunoreactivity is not detectable in clearly apoptotic cells, as has been reported elsewhere (Merritt et al., 1994). The reason for this is unclear but may simply reflect the intense chromatin condensation and

Table 1. The expression of p53 immunoreactivity in normal adult mouse tissues at time points after genotoxic insult

Time	Spleen	Thymus	Bone	Liver
0	–	–	–	–
3	++	++	+	–
6	+++	+++	++	–
12	+++	+++	++	–
24	+++	+++	++	–
48	+++	+++	++	–
96	+	+	–	–
148	–	–	–	–
336	–	–	–	–

–, no p53 immunoreactivity; +, occasional p53 stained cells; ++, moderate numbers of p53 stained cells; +++, extensive p53 stained cells.

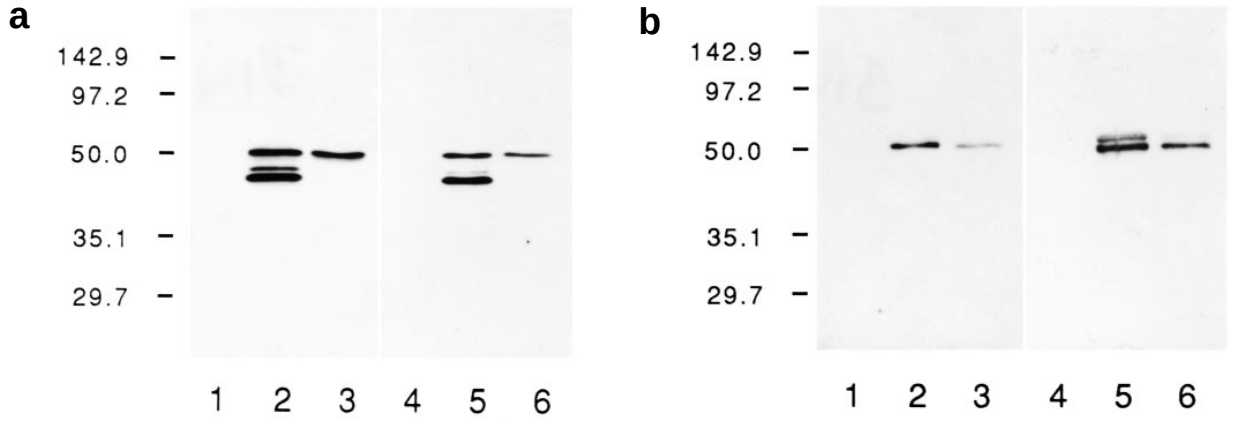

Fig. 2. (a) Direct immunoblot of mouse cell lines with affinity pure CM5 (lanes 1,2,3) and PAb421 supernatant (lanes 4,5,6). Lanes 1 and 4 are mouse p53 null (–/–), lanes 2 and 5 are C6, lanes 3 and 6 are T3T3. (b) Immunoprecipitation with CM5 (lanes 1,2,3) detected with biotinylated PAb240 and immuno-precipitation with PAb421 (lanes 4,5,6) detected with CM5. Lanes 1 and 4 are mouse null (–/–), lanes 2 and 5 are C6, lanes 3 and 6 are T3T3.

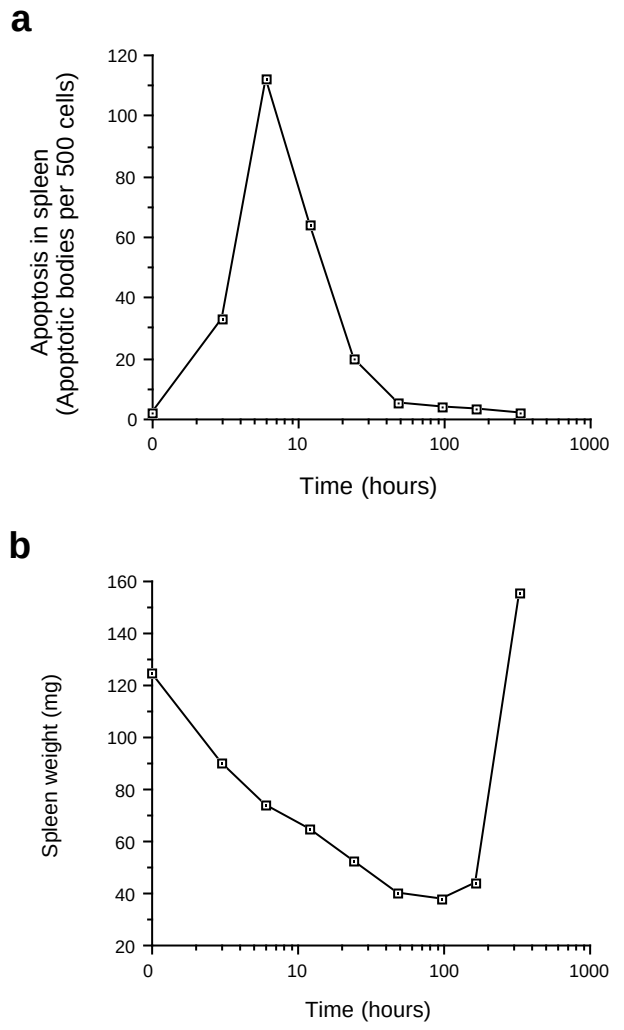

Fig. 3. The induction of apoptosis and loss of splenic mass are co-ordinate. The number of apoptoses and total cells were counted in 10 fields from each of three animals at each time point and the mean number of apoptoses expressed per 500 viable cells (a). The wet splenic weight was measured in all three animals (b).

protein degradation, with activation of proteases such as interleukin-1β-converting enzyme (ICE: Yuan et al., 1993) and related species (Wang et al., 1994) seen in apoptosis. In the bone many osteocytes showed marked p53 immunoreactivity and this had an identical time course. In contrast, no detectable p53 immunoreactivity could be seen in the liver at any time point. The possibility that this was a technical false negative is excluded since the liver, spleen and thymus from each animal were fixed and processed together and embedded into the same block. Sections thus contained all three tissues and these were stained together. Furthermore, expression of PCNA was examined in all animals using the polyclonal serum 3009 (Cox et al., unpublished). In all tissues, including the liver, PCNA immunoreactivity was identifiable. Thus the absence of p53 expression after γ irradiation in the liver is a real phenomenon. Interestingly, PCNA expression was not induced by γ irradiation as we had expected given previous results in vivo and in vitro with other genotoxic insults (Shivji et al., 1992; Hall et al., 1993).

DISCUSSION

The accumulation of p53 protein in response to genotoxic stress in vitro is now well established and appears to be a means of inducing growth arrest and apoptotic cell death by the transcriptional regulation of other genes (Kern et al., 1991; Kastan et al., 1992; El-Deiry et al., 1993), and possibly other more direct mechanisms (Caelles et al., 1994). Previous in vivo studies of p53 expression after genotoxic insult have shown profound induction in the skin (Hall et al., 1993; Campbell et al., 1993) and in sub-populations of intestinal epithelium (Merritt et al., 1994; Clarke et al., 1994). The dependence of the apoptotic response to irradiation on the presence of functional p53 in thymocytes (Lowe et al., 1993; Clarke et al., 1993) suggested that there would be induction of p53 expression in lymphoid tissues and this was demonstrated by immunoblotting of tissue extracts. However, it is well known that some tissues are much more resistant to the effects of irradiation and our previous observations on intestinal epithelia pointed to heterogeneity within tissues. The availability of CM5 and its reliable application to histological material has allowed us to examine these issues.

By studying p53 induction in the tissues of irradiated mice we discovered two striking and unexpected phenomena. The first was that while some tissues show a dramatic accumulation of p53 following irradiation, in other tissues, specifically the liver, no such response is seen. The second was that while in some tissues, specifically the spleen and thymus, induction of p53 is associated with apoptosis, in others, specifically the bone osteocytes, no apoptotic sequelae are seen. These results together with our earlier observations in gut and skin demonstrate that both the induction of p53 and the response to p53 are tightly regulated in a tissue specific manner. This in turn has important implications for the differential sensitivity of tissues to the carcinogenic affects of genotoxic agents and the expected therapeutic response of tumours of different tissue origin. This study has not addressed the possible heterogeneity of p53 dependent growth arrest events, although these may also be of great importance.

Why then should there be such marked tissue differences as reported here, and microanatomical differences as seen in the gut epithelium (Merritt et al., 1994; Clarke et al., 1994). Observations made in Bloom's syndrome and ataxia telangiectasia cells in vitro indicate that the p53 response to genotoxic damage is genetically regulated (Lu and Lane, 1993; Kastan et al., 1992). Our current and previous data on the induction of p53 expression in vivo indicate that the expression of the genes that control p53 is highly regulated. This tissue specific variation in response highlights the difficulty in interpretation of results obtained with tissue culture systems. In particular we note that the time course of induction of p53 seen in vivo in reponse to γ irradiation is very sustained compared to that seen in cell culture (Lu and Lane, 1993; Kastan et al., 1992). It is also notable that in this system the induction of PCNA is not apparent in contrast to the dramatic induction seen following other genotoxic insults (Hall et al., 1993). This may relate to the precise nature of the lesion induced, further support for this idea comes from the absence of detectable DNA damage using the ISEL system after γ irradiation in contrast to its induction by other genotoxic agents (Coates et al., 1995). The explanation for this remains unclear. It may relate to the chemical

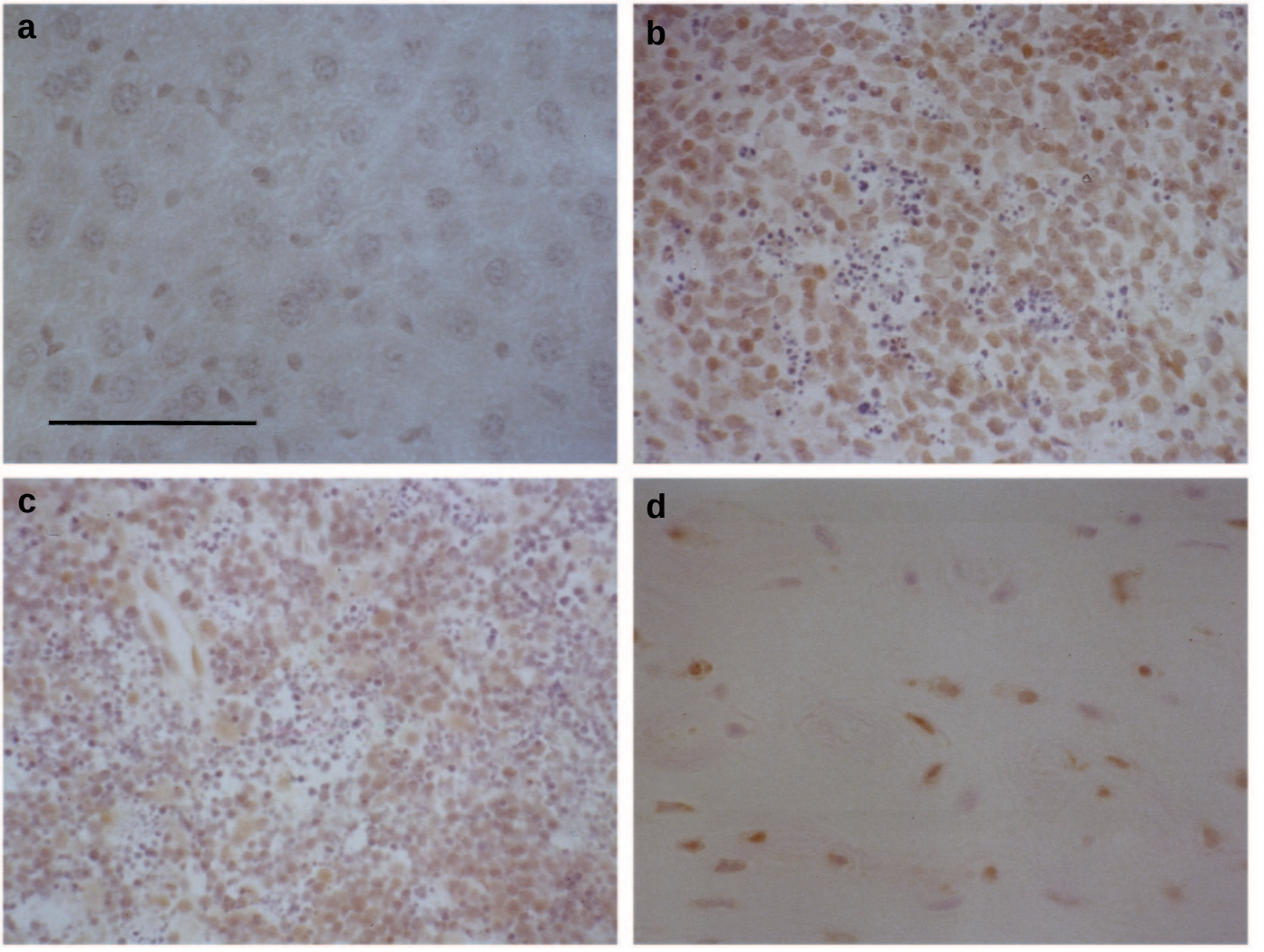

Fig. 4. Expression of p53 protein in liver (a), spleen (b) thymus (c) and bone (d) six hours after γ irradiation. There is clear p53 immunoreactivity in thymus and spleen associated with marked apoptosis. In the liver there is no morphological abnormality and no evidence of p53 expression. The bone shows p53 immunoreactivity in the osteocytes. Bar, 100 μm.

nature of the damage or the kinetics of the damage or repair events.

The absence of a radiation induced apoptotic response in the intestinal epithelia and thymocytes of p53 knockout mice has established the critical role of p53 in this process (Merritt et al., 1994; Clarke et al., 1993, 1994; Lowe et al., 1993). Studies of p53 mutations in tumour models have supported the concept that this p53 dependent apoptotic response may play a critical rate limiting role in the development of neoplasia (Symonds et al., 1994). It is thus of considerable interest that in some model systems of experimental carcinogenesis in rodents p53 appears to play a critical role. For example, this critical function of p53 is readily seen in rodent models of skin carcinogenesis (Kemp et al., 1993). Surprisingly, no such involvement of p53 has so far been seen in rodent hepatocarcinogenesis models. The absence of p53 mutations in rodent hepatocarcinogenesis models may then reflect the absence of any selective advantage since adult mouse hepatocytes do not appear to invoke the p53 pathway after genotoxic stress (Greenblatt et al., 1994).

In this paper we have defined a polyclonal serum generated against recombinant mouse p53 which efficiently recognises p53 protein in immunochemical and immunohistochemical assays, including formalin fixed wax embedded histological material. This reagent is shown to be valuable in the analysis of p53 expression in experimentally manipulated mouse tissues. Our observations indicate a profound anatomically defined heterogeneity of both the upstream induction of p53 and its downstream consequences. This phenomenon may be of considerable biological significance and indicate the importance of whole body experiments that compliment biochemical and in vitro studies. Indeed, the differential sensitivity of a wide range of normal tissues and of tumours with different origins to chemotherapy and radiotherapy may be, in part, determined by these phenomena. Finally, our new reagent may prove of great value in the analysis of rodent models of tumorigenesis and genotoxic stress including transgenic systems.

We thank the Cancer Research Campaign and the Steiner Foundation for their continued financial support. D.P.L. is a CRC Gibb Fellow. The CIS BioInternational IBL 437C ^{137}Cs gamma source was

purchased with the assistance of the Scottish Hospitals Endowment Research Trust.

REFERENCES

Ansari, B., Coates, P. J., Greenstein, B. and Hall, P. A. (1993) In situ end labelling detects DNA strand breaks in apoptosis and other physiological and pathological states. *J. Pathol.* **170**, 1-8.

Caelles, C., Helmberg, A. and Karin, M. (1994). p53-dependent apoptosis in the abscence of transcriptional activation of p53 target genes. *Nature* **370**, 220-224

Campbell, C., Quinn, A. G., Angus, B., Farr, P. M. and Rees, J. L. (1993). Wavelength specific patterns of p53 induction in human skin following exposure to UV radiation. *Cancer Res.* **53**, 2697-9.

Clarke, A. R., Purdie, C. A., Harrison, D. J., Morris, R. G., Bird, C. C., Hooper, L. and Wyllie, A. H. (1993). Thymocyte apoptosis is induced by p53-dependent and independent pathways. *Nature* **362**, 849-852

Clarke, A. R., Gledhill, S., Hooper, M. L., Bird, C. C. and Wyllie, A. H. (1994). p53 dependence of early apoptotic and proliferative responses within the mouse small intestinal epithelium following γ irradiation. *Oncogene* **9**, 1767-1773.

Coates, P. J., Save, V., Ansari, B. and Hall, P. A. (1995). Demonstration of DNA damage/repair in individual cells using in situ end labelling: association of p53 with sites of DNA damage. *J. Pathol.* (in press).

Donehower, L. A., Harvey, M., Slagle, B. L., McArthur, M. J., Montgomery, C. A., Butel, J. S. and Bradley, A. (1992). Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature* **356**, 215-221.

El-Deiry, W. S., Tokino, T., Velculescu, V. E., Levy, D. B., Parsons, R., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W. and Vogelstein, B. (1993). WAF1, a potential mediator of p53 tumor suppression. *Cell* **75**, 817-825.

Fritsche, M., Haessler, C. and Brandner, G. (1993). Induction of nuclear accumulation of the tumor-suppressor protein p53 by DNA-damaging agents. *Oncogene* **8**, 307-18.

Gannon, J. V., Greaves, R., Iggo, R. and Lane, D. P. (1990). Activating mutations in p53 produce a common conformational effect. A monoclonal antibody specific for the mutant form. *EMBO J.* **9**, 1595-1602.

Gannon, J. V. and Lane, D. P. (1991). Protein synthesis required to anchor a mutant p53 protein which is temperature-sensitive for nuclear transport. *Nature* **349**, 802-806.

Greenblatt, M., Bennett, W., Hollstein, M. and Harris, C. (1994). Mutations in the p53 tumour suppressor gene: clues to cancer aetiology and cancer pathogenesis. *Cancer Res.* **54**, 4855-4878.

Hall, P. A., McKee, P. H., Menage, H. d., Dover, R. and Lane, D. P. (1993). High levels of p53 protein in UV irradiated human skin. *Oncogene* **8**, 203-208

Hall, P. A. and Lane, D. P. (1994). Genetics of growth arrest and cell death: key determinants of tissue homeostasis. *Eur. J. Cancer* **13**, 2001-2012.

Harlow, E., Crawford, L. V., Pim, D. C. and Williamson, N. M. (1981). Monoclonal antibodies specific for simian virus 40 tumor antigens. *J. Virol.* **39**, 861-869.

Harlow, E. E. and Lane, D. P. (1988). *Antibodies: A Laboratory Manual*. New York: Cold Spring Harbor Laboratory Press.

Harvey, M., McArthur, M. J., Montgomery, C. A., Butel, J. S., Bradley, A. and Donehower, L. A. (1993). Spontaneous and carcinogen induced tumorigenesis in p53-deficient mice. *Nature Genet.* **5**, 225-229.

Jenkins, J. R., Rudge, K. and Currie, G. A. (1984). Cellular immortalization by a cDNA clone encoding the transformation-associated phosphoprotein p53. *Nature* **312**, 651-654.

Kastan, M. B., Onyekwere, P., Sidransky, D., Vogelstein, B. and Craig, R. W. (1991). Participation of p53 protein in the cellular response to DNA damage. *Cancer Res.* **51**, 6304-6311.

Kastan, M. B., Zhan, Q., el, D. W., Carrier, F., Jacks, T., Walsh, W. V., Plunkett, B. S., Vogelstein, B. and Fornace, A. J. (1992). A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. *Cell* **71**, 587-597.

Kemp, C. J., Donehower, L. A., Bradley, A. and Balmain, A. (1993). Reduction of p53 gene dosage does not increase initiation or promotion but enhances malignant progression of chemically induced skin tumors. *Cell* **74**, 813-822.

Kern, S. E., Kinzler, K. W., Bruskin, A., Jarosz, D., Friedman, P., Proves, C. and Vogelstein, B. (1991). Identification of p53 as a sequence-specific DNA-binding protein. *Science* **252**, 1708-1711.

Kuerbitz, S. J., Plunkett, B. S., Walsh, W. V. and Kastan, M. B. (1992). Wild-type p53 is a cell cycle checkpoint determinant following irradiation. *Proc. Nat. Acad. Sci. USA* **89**, 7491-7495.

Lane, D. P. (1992). p53, guardian of the genome. *Nature* **358**, 15-16.

Lane, D. P. (1993). A death in the life of p53. *Nature* **362**, 786-787.

Lowe, S. W., Schmitt, E. M., Smith, S. W., Osborne, B. A. and Jacks, T. (1993). p53 is required for radiation-induced apoptosis in mouse thymocytes. *Nature* **362**, 847-9.

Lu, X. and Lane, D. P. (1993). Differential induction of transcriptionally active p53 following UV or ionising radiation; defects in chromosome instability syndromes. *Cell* **75**, 765-778.

Maltzman, W. and Czyzyk, L. (1984). UV irradiation stimulates levels of p53 cellular tumor anitgen in nontransformed mouse cells. *Mol. Cell. Biol.* **4**, 1689-1694.

Merritt, A. J., Potten, C. S., Kemp, C. J., Hickman, J. A., Balmain, A., Lane, D. P. and Hall, P. A. (1994). The role of p53 in spontaneous and radiation-induced apoptosis in the gastrintestinal tract of normal and p53 deficient mice. *Cancer Res.* **54**, 614-617.

Midgley, C. A., Fisher, C. J., Bartek, J., Vojtesek, B., Lane, D. P. and Barnes, D. M. (1992). Analysis of p53 expression in human tumours: an antibody raised against human p53 expressed in *E. coli*. *J. Cell Sci.* **101**, 183-189.

Milner, J., Chan, Y. S., Medcalf, E. A., Wang, Y. and Eckhart, W. (1993). Partially transformed T3T3 cells express high levels of mutant p53 in the 'wild-type' immunoreactive form with defective oligomerization. *Oncogene* **8**, 2001-8.

Shivji, M. K. K., Kenny, M. K. and Wood, R. D. (1992). Proliferating cell nuclear antigen is required for DNA excision repair. *Cell* **69**, 367-374.

Symonds, H., Krail, L., Remmington, L., Saenz-Robles, M., Lowe, S., Jacks, T. and VanDyke, T. (1994). p53 dependent apoptosis suppresses tumor growth and progression in vivo. *Cell* **78**, 703-712.

Tabor, S. and Richardson, C. C. (1985). A bacteriophage T7 polymerase/promoter system for controlled exclusive expression of specific genes. *Proc. Nat. Acad. Sci. USA* **82**, 1074-1078.

Wang, L., Miura, M., Bergeron, L., Zhu, H., Yuan, J. (1994)
Ich-1, an Ice/ced-3-related gene, encodes both positive and negative regulators of programmed cell death. *Cell* **78**, 739-750.

Yuan, J., Shaham, S., Ledoux, S., Ellis, H. M. and Horwitz, H. R. (1993). The C. elegans cell death gene ced-3 encodes a protein similar to mammalian interleukin-1β-converting enzyme. *Cell* **75**, 641-652.

(Received 14 November 1994 - Accepted 23 January 1995)