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Tissue polypeptide antigen (TPA) is related to the non-epidermal keratins 8, 18 and 19 typical of simple and non-squamous epithelia: re-evaluation of a human tumor marker

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Because of the broad clinical interest which tissue polypeptide antigen (TPA) has attracted as a tumor marker, human cell lines and human tissues have been analyzed for TPA expression using immunofluorescence microscopy. Epithelial cell lines including HeLa, MCF-7, and A-431 are recognized by TPA antibodies whereas human lines of non-epithelial origin are not. The positive staining patterns coincide with keratin-type intermediate filaments of the cytoskeleton. On tissue sections a subset of epithelial cells including uterine epithelium, bile duct cells in liver and tumor cells in breast carcinoma are strongly positive; cells of the squamous epithelia of skin and tongue as well as cells of non-epithelial origin are negative. In immunoblots of human epidermis, human tongue mucosa, human hair follicles, Detroit 562 cells, HeLa cells, MCF-7 and RT-4 cells, only keratins 8, 18 and 19 show TPA antigenicity. Conversely a TPA preparation is recognized by various antibodies known to react with keratins, including α-IFA, K_G 8.13.2 and two antibodies which recognize keratins 18 (CK_2) and 19, respectively. Our results thus relate TPA to human keratins 8, 18 and 19 which are known cytoskeletal components in both normal and malignant epithelial cells of simple and non-squamous origin. We speculate that the elevated levels of circulating TPA antigenicity present in the sera of patients with carcinoma, which are often used to monitor tumor progression, correspond to soluble proteolytic fragments originating from this particular keratin subgroup.

Key words: cancer antigens/intermediate filaments/keratins/radioimmunoassay/tissue polypeptide antigen/TPA

Introduction

Tissue polypeptide antigen (TPA) is a protein antigen identified immunologically by Björklund and Björklund (1957) in tumors using horse sera raised originally against the insoluble residues remaining after successive extractions of pooled human tumors. TPA antibodies can be raised using material from a single tumor, or from a mixture of different carcinomas, or using material from HeLa cells (Björklund *et al.*, 1973). TPA antigenicity can be identified in sera, in body fluids and in tissue extracts using radioimmunoassay (Wiklund *et al.*, 1979), and kits are available commercially. In the last decade TPA has been studied extensively in serological testing as a general tumor-associated antigen (Björklund *et al.*, 1973; Björklund, 1981; Skryten *et al.*, 1981; Lütgens and Schlegel, 1983) even though the current literature raises some questions as to the validity of this claim. Thus, for instance TPA can be isolated from normal human placenta (Björklund *et al.*, 1973), and using immunofluorescence microscopy TPA has been clearly demonstrated in certain epithelial cells in normal human tissues (Björklund *et al.*, 1982; Löning *et al.*, 1983).

Although the experiments just cited did not relate TPA staining to the cytoskeleton, three indirect arguments point to a possible relation to TPA and keratins. (i) HeLa cells contain keratin-type intermediate filaments (Franke *et al.*, 1979) in line with their derivation from a cervical carcinoma, and these filaments remain insoluble in material similar to that used as the original TPA antigen. (ii) The five subgroups of intermediate filaments show a well characterized cell- and tissue-specific expression pattern (Osborn *et al.*, 1982; Franke *et al.*, 1982; Holtzer *et al.*, 1982) which is essentially kept after malignant transformation (Moll *et al.*, 1982b; Osborn and Weber, 1983). Although keratins occur only in epithelial cells the 19 currently known human keratin components segregate in morphologically distinct epithelia (Moll *et al.*, 1982b; Wu *et al.*, 1982) thus allowing a distinction to be made by biochemical as well as by immunological criteria. (iii) A variety of properties of certain purified TPA preparations (Lüning *et al.*, 1980; Redelius *et al.*, 1980; Wiklund *et al.*, 1981), i.e., the high α-helix content, the rod-like morphology of the protein and the nature of the partial amino acid sequences are reminiscent of intermediate filament proteins (Geisler and Weber, 1982; Geisler *et al.*, 1983; Hanukoglu and Fuchs, 1982) and have led to the recent speculation that TPA may be related to the keratin IF family (Lüning and Nilsson, 1983; Weber and Geisler, 1984).

Here we prove the keratin nature of TPA by showing that antibodies to TPA react with some human keratin components, as well as by showing that a standard TPA preparation can be recognized by a variety of polyclonal and monoclonal antibodies that recognize particular keratins. Our results show that TPA is related to keratins 8, 18 and 19 which are typical of simple epithelia and are also found in certain carcinomas. The results exclude TPA as a marker found only in tumor cells and suggest that currently available monoclonal antibodies specific for certain keratins could substitute for polyclonal TPA antibodies in certain applications.

Results

TPA antibodies specifically decorate the keratin IFs of HeLa and other epithelial cells in culture

The TPA antibodies used in this study are well characterized and have been shown to react with TPA:B_1 by a variety of immunochemical methods (see Materials and methods). HeLa was selected for the initial study since TPA is known to be present in this cervical carcinoma cell line (e.g., Björklund *et al.*, 1973). When rabbit antibodies against TPA were used in indirect immunofluorescence microscopy extensive filamentous networks were visualized (Figure 1a). These are essentially identical to those seen using a broad specificity

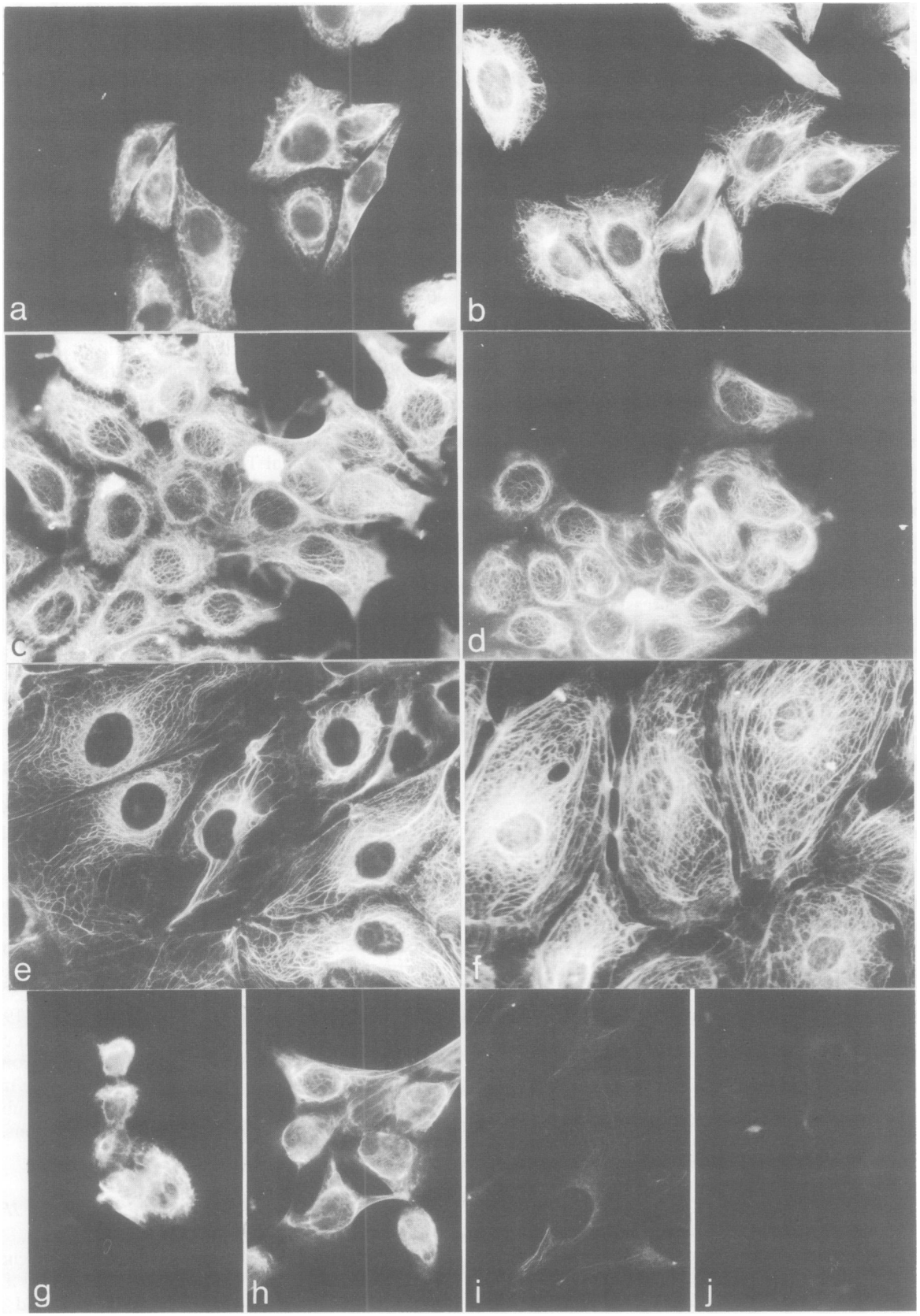

Fig. 1. Comparison of profiles of human and rat kangaroo cell lines (PtK2) by immunofluorescence microscopy using either TPA antibodies, TPA pre-serum, a broad specificity keratin antibody made in guinea pigs (ker_{GP}) or a monoclonal keratin antibody recognizing keratin 18 (CK_2). (**a**) Human HeLa, TPA, (**b**) HeLa, ker_{GP}, (**c**) human MCF-7, TPA, (**d**) MCF-7, CK_2, (**e**) rat kangaroo PtK2, TPA, (**f**) PtK2, ker_{GP}, (**g**) human A-431, TPA, (**h**) A-431, CK_2, (**i**) PtK2, TPA pre-serum, (**j**) human RD cells, TPA. Note the strong staining by the TPA antibodies and by keratin antibodies of all the epithelial lines tested, i.e., HeLa, MCF-7, PtK2 and A-431 and that the TPA antibodies do not stain the non-epithelial rhabdomyosarcoma line RD. Note also that both TPA and the keratin antibodies reveal typical keratin profiles in the epithelial cell lines selected for study (x 400).

keratin antibody (e.g., Figure 1b and Franke *et al.*, 1979). The keratin IFs can be distinguished by their staining patterns from vimentin IFs which are also found in HeLa cells. Other cultured epithelial cell lines were also tested. Very strong staining was also found in the human MCF-7 line, a breast carcinoma derivative which does not express vimentin (Figure 1c). Again the profiles seen with TPA antibodies were indistinguishable from those obtained with a broad specificity keratin antibody (Figure 1d), and were clearly of the keratin IF type (compare Figure 1c and d). In addition we tested dog kidney MDCK cells as well as rat kangaroo PtK2 cells, because these are epithelial cell lines in which keratin IFs have been extensively characterized. Here again the TPA profiles were similar to those obtained with conventional broad specificity keratin antibodies (compare Figure 1e and f). The human epidermoid A-431 cell line showed extensive keratin filaments (Figure 1g and h) when tested with both the TPA antibody and a broad specificity keratin antibody. In each case the TPA antibodies and the pre-sera were tested in parallel at the same dilution. Although in PtK2 cells the pre-serum reveals a very faint staining of keratin filaments (Figure 1i) in line with previous results using a variety of 'normal' rabbit sera (Osborn *et al.*, 1977), the keratin staining demonstrated here is clearly very strongly increased when the TPA antibodies are used (compare Figure 1e and i). Staining patterns such as those shown in Figure 1 for HeLa cells were not seen if cells were fixed with formaldehyde, a characteristic that has also been reported for several keratin antibodies (e.g., Osborn *et al.*, 1977).

In contrast to the results with cultured epithelial cells TPA antibodies did not stain IFs in non-epithelial cells of human origin. Thus no cytoplasmic filamentous staining was seen with the rhabdomyosarcoma line RD, which co-expresses vimentin and desmin IFs (Figure 1j), or with the fibroblast line IMR-90 which expresses only vimentin IFs. In the latter case the particular TPA antibody we have available generates a weak fibronectin-like pattern (data not shown). These results suggest strongly that TPA is related to keratin(s) or to keratin-associated protein(s).

TPA antibodies decorate a subset of epithelial cells of normal human tissues and of carcinomas

A variety of normal human tissues was re-examined using the TPA antiserum and the pre-serum in parallel at the same dilution. Some representative results are shown in Figure 2. When human uterus was used, the glandular epithelium was stained strongly by the TPA antibody, but not by the pre-serum (compare Figure 2a and i). The profile obtained with the TPA serum is indistinguishable from that seen with a broad specificity keratin antibody (Figure 2b). Hepatocytes in human liver appeared weakly positive (Figure 2c). In both rat and human liver, epithelial cells of the hepatic ducts were strongly stained (Figure 2c), a feature also seen with the ker_{19} antibody, an antibody reported as specific for ker_{19} (Wu *et al.*, 1982) (Figure 2d). Rat and human intestinal epithelium were stained by the TPA antibodies and not by the pre-serum (data not shown). Two normal squamous epithelia were tested, i.e., monkey tongue (Figure 2e) and human skin (Figure 2f, g and h). When tongue was used no staining of stratified squamous epithelium was seen, although occasional groups of epithelial cells which stained strongly were detected. These cells may correspond to the taste-bud cells (Figure 2e) (cf. Lane, 1982). When skin was used the stratified squamous epithelium was not stained either by the TPA antibody or by the pre-serum (Figure 2f and h). However, both stratified squamous epithelia could be stained by the broad specificity keratin antibody (e.g., Figure 2g).

In none of the tissues that we examined were cell types other than epithelial cells stained by the TPA antibodies. Thus the TPA antibodies appear not to recognize non-epithelial cell types such as fibroblasts, skeletal and smooth muscle cells, and neurones. We also examined the staining patterns of the TPA antibodies on certain tumors, and in particular on three cases of breast carcinoma. In the latter cases the tumor cells were positive with the TPA antibody but not with the pre-serum (Figure 2j and k), and the profiles resembled those obtained with the broad specificity ker_{GP} antibody (Altmannsberger *et al.*, 1981).

TPA antibodies react only with certain cytokeratins

The immunofluorescence microscopical data taken together with the known distribution of the different keratins in human epithelia (Moll *et al.*, 1982b) indicated that TPA recognizes only a limited number of the 19 human cytokeratins. These were identified by immunoblotting experiments. In one-dimensional blots of MCF-7 the TPA serum reacted with all three keratins (components 8, 18 and 19) present in this cell type whereas the pre-serum showed no reaction (slots 3, 4, Figure 3b). The positions of keratins 8, 18 and 19 were identified by immunoblots using three keratin antibodies for which the specificities are known. Thus ker_{19} identifies component 19 (slot 5, Figure 3b), the $K_G8.13.2$ monoclonal recognizes only 8 and 18 of the keratins present in MCF-7 (slot 6) and CK_2 monoclonal recognizes only component 18 (slot 7). One-dimensional blots were also performed using material purified from mammary epidermis, from human tongue mucosa and from human hair follicles, as well as from Detroit 562 cells (for cytoskeletal composition see Moll *et al.*, 1983) and from HeLa cells. The left part of Figure 3c identifies the keratin polypeptides seen on a Coomassie blue-stained gel for material purified from each of these sources, and on the right the corresponding immunoblots are shown. Inspection of Figure 3c shows that the TPA antibody does not recognize any of the keratin polypeptides present in mammary epidermis, in the tongue mucosa or in hair follicles, but does recognize keratins present in Detroit 562 cells and in HeLa cells. A careful comparison of the two portions of Figure 3c shows that the TPA antibody does not recognize keratins 1, 2, 4, 5, 6, 10, 11, 13, 14, 15, 16 or 17; in contrast it reacts strongly with keratins 8, 18 and 19. To exclude a possible reaction of this TPA antibody with keratin 7 a two-dimensional immunoblot was made using a cytoskeletal preparation from RT-4 cells. As shown in Figure 3d although keratins 8, 18 and 19 again react with the TPA antibody, keratins 7 and 17 do not. Thus we conclude that the TPA antibody is specific for keratins 8, 18 and 19. Note also that the TPA antibody does not cross-react with the vimentin polypeptide present in HeLa cells (slot 5, Figure 3c).

Characterization of TPA protein by keratin antibodies

As a further control we studied the reactivity of TPA purified from pooled carcinomas with various keratin antibodies whose component specificity is known (Figure 3a). In the dot blot test TPA reacted strongly with the murine monoclonal antibody 8.13.2 which recognizes components 1, 5, 6, 7, 8, 10, 11 and 18 (Gigi *et al.*, 1982). The murine monoclonal antibody CK_2 which recognizes only component 18 (Debus *et al.*, 1982, 1984) was also strongly positive on TPA, and so was a

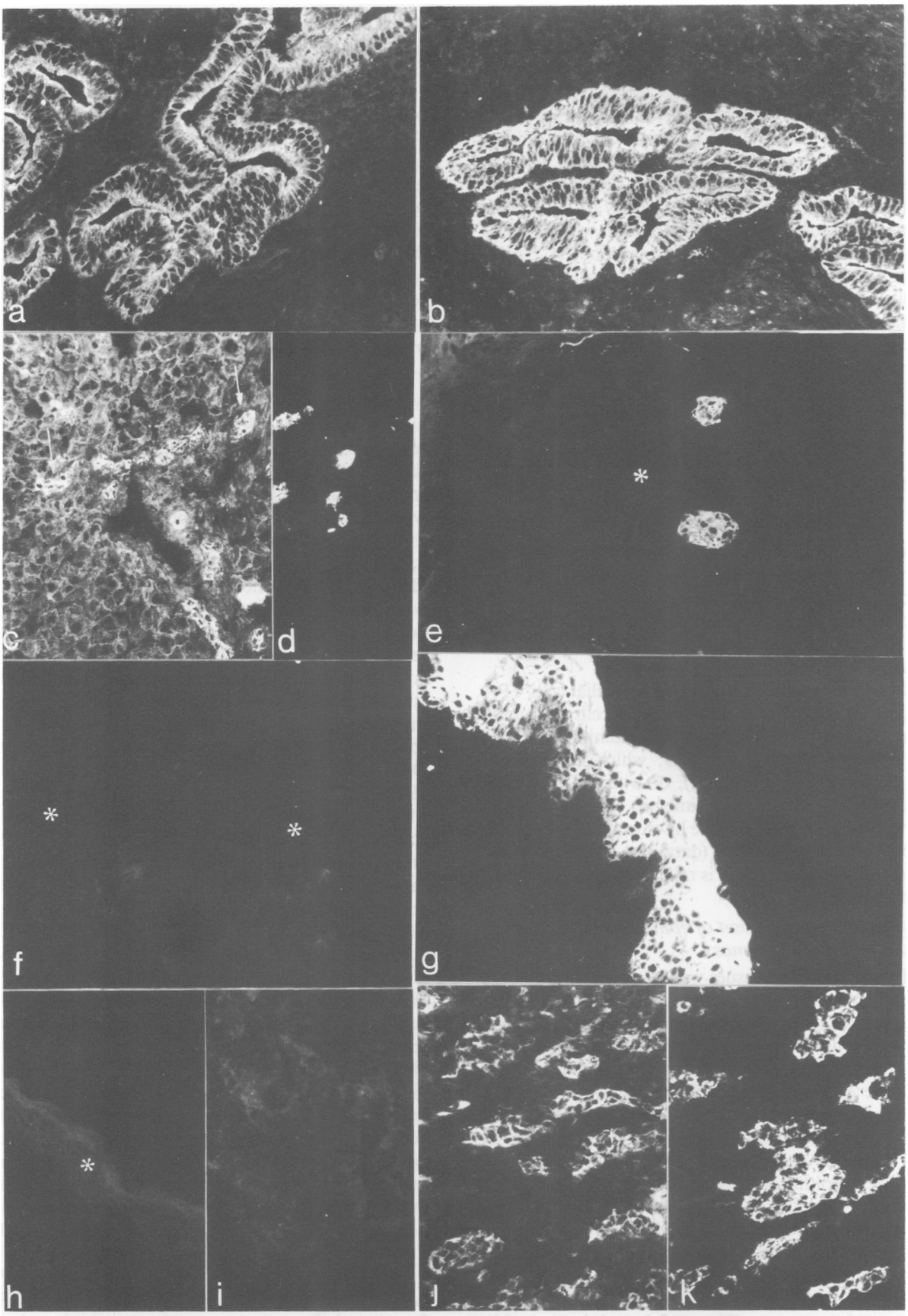

Fig. 2. Comparison of staining patterns on human (all except **e**) and monkey tissues (**e**) by immunofluorescence microscopy using either TPA antibodies, TPA pre-serum, a broad specificity keratin antibody made in guinea pig (ker_{GP}), or a keratin antibody made in rabbits specific for component 19 (ker_{19}). (**a**) Uterine tissue, TPA, (**b**) uterine tissue, ker_{GP}, (**c**) liver, TPA, (**d**) liver, ker_{19}, (**e**) tongue, TPA, (**f**) skin, TPA, (**g**) skin, ker_{GP} (**h**) skin, TPA pre-serum, (**i**) uterus, TPA pre-serum, (**j**) mammary carcinoma, TPA, (**k**) mammary carcinoma, ker_{GP}. Asterisks in **e**, **f** and **h** indicate the position of the unstained squamous epithelia. Note that TPA antibodies and ker_{GP} antibodies give equivalent staining patterns on uterine epithelium (**a**,**b**) and on mammary carcinoma (**j**,**k**), but that TPA antibodies do not stain the squamous epithelia of skin (**f**) or of tongue (**e**) while the ker_{GP} antibodies do (**g**). The groups of cells stained in **e** probably correspond to taste-bud cells. TPA antibodies stain hepatocytes and also stain very strongly cells of the bile ducts (arrows in **c**). In contrast the ker_{19} antibody (**d**) appears to stain only the bile duct cells (x 160).

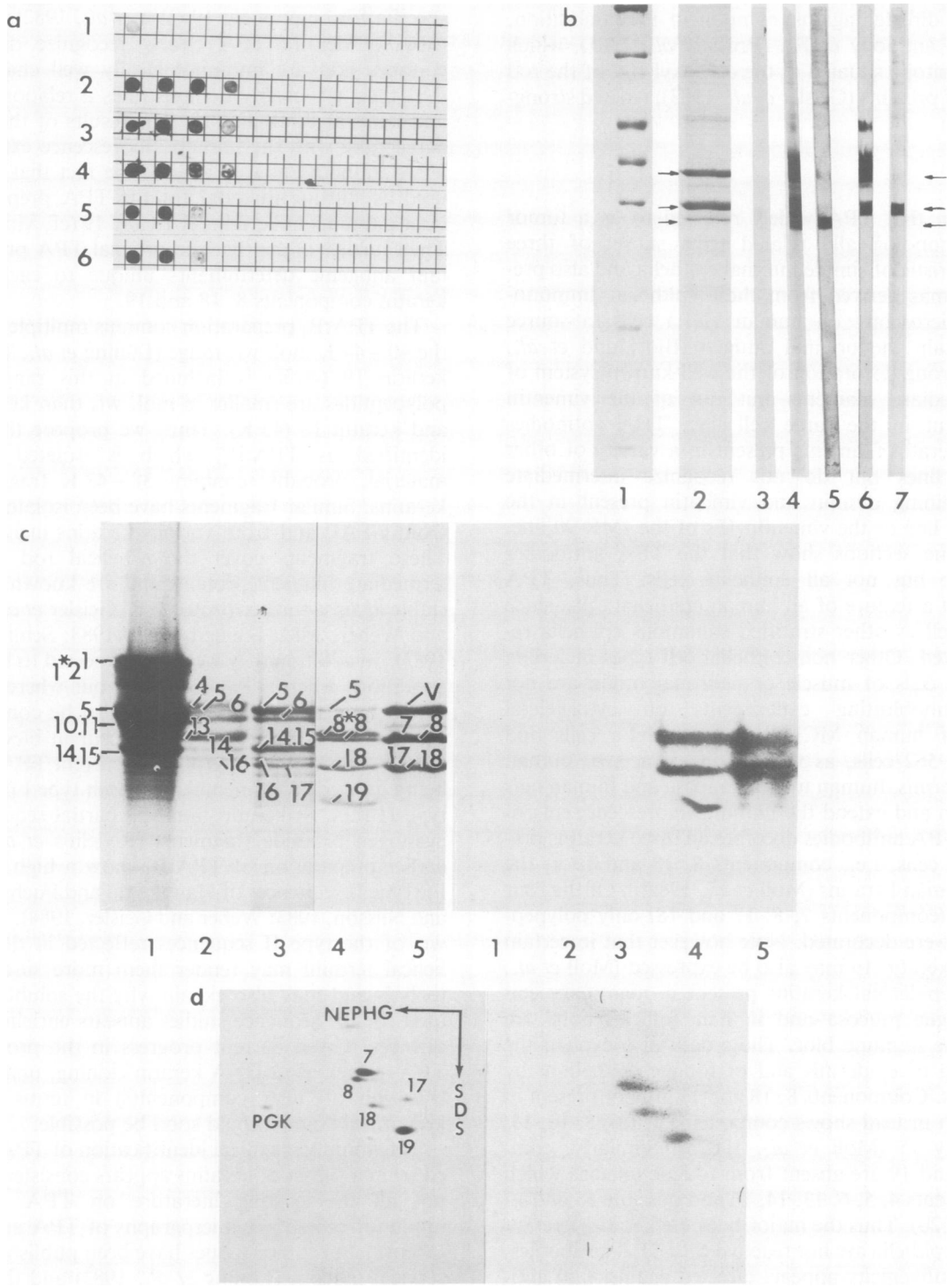

Fig. 3. (**a**) Dot blots of purified TPA fractions tested against a variety of antibodies previously shown to recognize keratins. Amounts spotted are from left 800, 160, 32, 6.4, 1.5 and 0.26 ng. Antibodies tested are (**1**) TPA pre-serum, 1 + 100; (**2**) TPA serum, 1 + 100; (**3**) k_G8.13.2, a broad specificity monoclonal antibody, supernatant 1 + 20; (**4**) CK_2, a monoclonal antibody directed against keratin 18, supernatant 1 + 20; (**5**) ker_{19}, a rabbit antibody directed against keratin 19, 1 + 100; and (**6**) IFA, a monoclonal antibody which recognizes all intermediate filament proteins so far tested, ascites 1 + 100. Note that the TPA antigen reacts not only as expected with the TPA antibody but also with the keratin antibodies k_G8.13.2, CK_2, ker_{19} and with the IFA antibody. (**b**) Coomassie blue-stained gel (**slot 2**) and immunoblots (**slots 3–6**) of MCF-7 cytoskeletons after transfer to nitrocellulose paper. **Slot 1**, mol. wt. standards (Coomassie blue); **slot 2**, MCF-7 cytoskeletons (Coomassie blue); **slots 3–6**, immunoblots with **slot 3** TPA pre-serum, 1 + 100; **slot 4**, TPA serum, 1 + 100; **slot 5**, ker_{19} antibody, 1 + 300; **slot 6**, k_G8.13.2 supernatant, 1 + 10; and **slot 7**, CK_4, 1 + 100. The arrows on **slots 2** and **6** show from the top the positions of ker_8, ker_{18} and ker_{19}. Note that TPA antibodies but not the pre-serum react with the keratin components 8, 18, 19. K_G8.13.2 reacts with 8, 18, ker_{19} with ker_{19} only, and CK_4 with ker_{18}. (**c**) One-dimensional Coomassie blue-stained gels (left) and corresponding immunoblots (right) of cytoskeletal proteins from different sources. **Slot 1** shows the keratins of human epidermis; **slot 2** keratins of human tongue mucosa; **slot 3** keratins of human hair follicles; **slot 4** keratins from Detroit 562 cells; **slot 5** vimentin and keratins of HeLa cells. After transfer the blot has been reacted with TPA antibodies. Note the reaction of the TPA antibodies with keratins 8, 18, 19 in Detroit 562 cells, and 8 and 18 in HeLa cells. Note that the TPA antibodies do not react with vimentin (V, **slot 5**) or with the keratin components present in epidermis, tongue mucosa, and human hair follicle which are indicated by numbers on the left hand gel, i.e., keratins 1, 2, 4, 5, 6, 7, 10, 11, 13, 14, 15, 16, 17. (**d**) Two-dimensional Coomassie blue-stained gel (left) and corresponding immunoblot (right) of keratins from the bladder carcinoma line RT-4. Note the reaction of the TPA antibodies with keratins 8, 18 and 19 but not with 7 and 17.

rabbit antibody directed against component 19. In addition, the monoclonal antibody α-IFA (Pruss *et al.*, 1981), which recognizes an epitope situated at the carboxyl end of the rod domain in all IF proteins (Geisler *et al.*, 1983), reacted strongly with TPA.

Discussion

We have shown that TPA widely referred to as a tumor marker is immunologically related to a subset of three keratins chracteristic of simple internal epithelia and also present in carcinomas derived from these epithelia. Immunofluorescence microscopy on human HeLa cells, a source known to contain the original antigen (Björklund *et al.*, 1973), shows strong decoration of the well-known system of keratin intermediate filaments but not of the vimentin filaments present in the same cell line. TPA antibodies recognize the keratin filaments present in a variety of other epithelial cell lines but do not recognize intermediate filaments containing desmin and vimentin present in the human RD cell line or the vimentin IFs of the IMR-90 line. Studies on tissue sections show that the TPA antibodies recognize some but not all epithelial cells. Thus, TPA reacitivity is not a feature of all human epithelial cells, since epidermis as well as other stratified squamous epithelia remain undecorated. Other non-epithelial cell types including fibroblasts and cells of muscle or neuronal origin are not stained. Immunoblotting experiments on cytoskeletal preparations of human MCF-7, human HeLa cells and human Detroit 562 cells, as well as on extracts of human mammary epidermis, human tongue mucosa and human hair follicles confirm and extend the immunofluorescence microscopical data. TPA antibodies decorate all three keratins present in MCF-7 cells, i.e., components 8, 18 and 19 in the catalogue of human keratins (Moll *et al.*, 1982b). Of the four HeLa keratins (components 7, 8, 17 and 18) only polypeptides 8 and 18 were decorated. Note however that in certain HeLa sublines keratin 19 may also be expressed (Moll *et al.*, 1983). Of the epidermal keratins present in mammary epidermis, in tongue mucosa and in hair follicles none are decorated in the immune blot. These data also explain the lack of staining of epidermis and of tongue epithelium by TPA antibodies. Components 8, 18 and 19 are not present in epidermis which instead shows components 1, (2), 5, 10, 11, 14 and possibly 15 (Moll *et al.*, 1982b). Similarly, components 8, 18 and 19 are absent from tongue mucosa which reveals components 4, 5, 6, 13, 14, 16 and possibly 15 and 17 (Moll *et al.*, 1982b). Thus the majority of the keratins present outside simple epithelia are not reactive with TPA antibodies, and the TPA antigenicity appears directed against (an) antigenic determinant(s) present on three of those keratins typically found in various simple epithelia (i.e., 8, 18 and 19). Interestingly these same three keratins are typical of adenocarcinomas, and are also present in certain other carcinomas (Moll *et al.*, 1982b, 1983; Debus *et al.*, 1984).

TPA protein reacts strongly with the monoclonal antibody α-IFA which recognizes all intermediate filament proteins so far tested because of an epitope residing in a consensus sequence at the carboxyl end of the α-helical domain (Geisler *et al.*, 1983). It also reacts strongly with a broad specificity monoclonal antibody k_G8.13.2 which reacts with a variety of keratins including 8 and 18 (Gigi *et al.*, 1982). TPA also reacts with the monoclonal antibody CK_2 specific for keratin 18 (Debus *et al.*, 1982) and with a polyclonal antibody specific for component 19 (Wu *et al.*, 1982). The antibodies used to identify TPA clearly recognize different keratin epitopes and are immunologically well characterized, and thus their use clearly demonstrates a relation between TPA and keratins 8, 18 and 19. The results of these experiments agree fully with the immunofluorescence experiments using TPA antibody, as well as with the fact that the carcinomas used as starting material for the TPA preparation contain large amounts of keratins 8, 18 and 19 (cf. Moll *et al.*, 1982b). They further argue that the original TPA preparations contain antigenic determinants unique to each of the three keratin components 8, 18 and 19.

The TPA:B_1 preparation contains multiple polypeptides in the 40–44 K mol. wt. range (Lüning *et al.*, 1980). Although keratin 19 (40 K) is included in this range the TPA:B_1 polypeptides are smaller in mol. wt. than keratin 8 (52.5 K) and keratin 18 (45 K). Thus, we propose that the material identified as TPA:B_1, which is isolated from autopsy material, probably contains 38–43 K fragments of these keratins. Similar fragments have been isolated from desmin, from GFAP and from various keratins including keratin 8. These fragments cover the α-helical rod domain of intermediate filament proteins and are known to be relatively stable against further proteolysis (Geisler *et al.*, 1982; Geisler and Weber, 1982; Steinert *et al.*, 1983; Schiller and Franke, 1983). In addition they would be expected to be soluble under conditions where TPA is soluble but where intact keratins would be insoluble. This would also be consistent with the fact that TPA antigenicity can occur in blood and in body fluids. Although we interpret the current TPA preparation as being derived from members of both type I (i.e., 18, 19) and type II (i.e., 8) keratin families, partial sequence studies of cyanogen bromide fragments (Redelius *et al.*, 1980) of an earlier preparation of TPA:B_1 show a high homology with the type II sequences of Hanukoglu and Fuchs (1982) (Lüning and Nilsson, 1983; Weber and Geisler, 1984). The higher mol. wt. of the type II sequences reflected in their longer nonhelical termini may render them more susceptible to proteolysis and may also expain why the soluble TPA material used for the sequence studies appears enriched in type II sequences. Given current progress in the protein seqence of TPA as well as in DNA keratin cloning, unambiguous identification of TPA component(s) in terms of the human keratin catalogue should soon be possible.

The immunochemical identification of TPA as being derived from a subset of keratins appears consistent with most but not all the existing literature on TPA. Relatively few immunofluorescence micrographs of TPA antibody staining of normal or tumor tissues have been published, but those of cervical mucosa (Löning *et al.*, 1983) and the trophoblastic layer of placenta (Björklund, 1981) as well as of breast carcinomas (Björklund *et al.*, 1982) are consistent with our interpretation, particularly if it is remembered that the profiles obtained with conventional keratin antibodies depend both on the keratin antibody selected and on the method of fixation of the tissue. We do not, however, understand why such profiles and other data have been interpreted by some authors as showing plasma membrane staining (e.g., Björklund *et al.*, 1982) or staining of the endoplasmic reticulum. In retrospect it is astounding that an antigen first described in 1954, and shown in 1973 to be present in HeLa cells, was not located for 10 years in immunofluorescence microscopy on HeLa. Micrographs of TPA antibodies on cultured cells appear restricted to the report of perinuclear staining on HeLa cells

which the authors interpret as of microtubular origin (Kirsch *et al.*, 1983). A comparison of their profiles with previously published pictures of microtubules in HeLa cells (e.g., Figure 9 in Osborn and Weber, 1977) excludes this interpretation.

Most TPA identification has been by means of radioimmunoassays on sera or on body fluids, or on tissue or tumor extracts. Interestingly, by this assay the epithelial cell types HeLa, Hep2 and Detroit 6 (the latter two cells lines have been shown to share characteristics peculiar to HeLa cells) have been reported as TPA-positive while the human fibroblast line WI-38 which contains no keratin filaments is TPA-negative (Björklund *et al.*, 1973). These data are therefore consistent with our finding by immunofluorescence microscopy that epithelial cell lines are TPA-positive while other cell lines are not. Perhaps the biggest difficulty, however, is in understanding the data derived from serological analyses of tumor patients, and again perhaps it is important to distinguish between the fluorescence microscopical results and the results obtained by direct assay of patient sera. Thus, taking into account the published catalogues of human cytokeratins (Moll *et al.*, 1982b; Wu *et al.*, 1982) most carcinomas, with the exception of certain tumors derived from stratified epithelial cells (such as basal cell epithelioma and squamous cell carcinoma of the skin and tongue), might be expected to be positive for TPA when assayed directly on frozen sections. Correspondingly, from our results with normal tissues we would expect non-epithelial tumors such as lymphoma, solid leukemia, non-muscle sarcoma, muscle sarcomas and tumors of the glial and sympathetic nervous system to be negative for TPA when assayed on frozen sections. Unfortunately very few such data exist, although the finding that 32 breast carcinomas were TPA-positive while the one case of neurofibroma of the breast was TPA-negative (Björklund *et al.*, 1982) is in line with our interpretation. The major clinical interest in TPA has been as a tumor marker, and therefore levels in serum, or in urine or other body fluids have usually been assayed. Here we can only note that the percentage of patients showing elevated TPA levels is highest for carcinomas of different sites usually ranging between 60 and 100% (e.g., Björklund *et al.*, 1973; Skryten *et al.*, 1981; Lütgens and Schlegel, 1983; Oehr *et al.*, 1981), depending on the tumor site and on the study. For example 92% of those with urinary bladder carcinoma show elevated levels of TPA in a radioimmunoassay of plasma (Oehr *et al.*, 1981) while in 65–95% of the cases elevated levels of TPA can be detected in the urine (Oehr *et al.* 1981; Kumar *et al.*, 1981). When malignant disease other than carcinoma is considered, the data are unfortunately more confusing. In a series of 1483 persons, 23 individuals with malignant disease other than carcinoma were studied. The percentage of individuals showing elevated TPA levels is listed as 9% (Björklund *et al.*, 1973) or 26% (Björklund, 1978) which are in the same studies not too far different from values obtained for individuals not known to have cancer. Another study has concluded that TPA is not a valid marker for lymphoma (Rasmusson *et al.*, 1983) which is confirmed by recent data showing that in a series of 200 lymphomas $< 10\%$ showed elevated TPA levels (personal communication Dr. Å. Silen, AB Sangtec Medical, Sweden). Two further studies find much higher percentages of elevated TPA for non-carcinomas and cite, for instance, numbers of 70–85% for sarcoma, melanoma and lymphoma (Lütgens and Schlegel, 1983; Menendez-Botet *et al.*, 1978). How to reconcile the latter reports is currently not clear; nor is it clear how to interpret individual assays which have detected up to ten times the 'normal limit' of 70 U in normal sera (Rasmusson *et al.*, 1983). We note, however, that although we would expect all TPA antibody preparations to react with the epitope(s) present on keratins 8, 18 and 19, it is at least theoretically possible, in view of the fact that keratins and other IF types share related but not identical sequences (Geisler and Weber, 1982; Hanukoglu and Fuchs, 1982; Steinert *et al.*, 1983), that one or other TPA antibody (but not those used here) could show cross-reactivity with one or more non-keratin IF proteins (cf., the α-IFA antibody described by Pruss *et al.*, 1981).

Levels of TPA antibodies rather than the TPA antigen have also been assayed in a set of 957 sera (Björklund *et al.*, 1973). These data are of interest because they show that 50% of healthy adults have circulating TPA antibodies with the percentages for cancer patients being decreased. Whether in the future any relation can be demonstrated between these TPA antibodies and keratin auto-antibodies present in some normal human serum (Kurki *et al.*, 1983; M. Osborn, unpublished data) or in rabbit sera (Osborn *et al.*, 1977) remains to be demonstrated.

The relation of TPA to a subset of keratins raises several interesting questions concerning the future use of TPA and of monoclonal antibodies to keratins in human tumor diagnosis. First, as argued above, TPA cannot be considered as a tumor-specific antigen, since TPA reactivity coincides with the presence of keratins 8, 18 and 19 characteristic of the various simple and non-squamous epithelia of normal and malignant human tissues. However, TPA antibodies would be expected to be a marker of histogenetic origin, identifying tumors which originate from epithelia containing keratins 8, 18 and 19, in a manner analogous to that shown previously for intermediate filaments in general, and for keratins or subsets of keratins in particular. Second, if our interpretation of the nature of TPA is correct, TPA antibodies, because of their high biological specificity, can complement the emerging bank of monoclonal antibodies to different keratins. Thus, for instance monoclonal antibodies specific for component 18 have been raised in several laboratories (Debus *et al.*, 1982; Lane, 1982), and their use in differential carcinoma diagnosis is documented (Debus *et al.*, 1984). Polyclonal antibodies specific for keratin 19 (Wu *et al.*, 1982), as well as different monoclonal antibodies recognizing well-characterized subsets of different keratins (Tseng *et al.*, 1982) or with wide specificity (Gigi *et al.*, 1982) have been reported. However, given the cumbersome purification schemes used for TPA in the literature, the use of well-defined mono- and polyclonal antibodies to the different keratins in serological analyses should be explored. Third, clearly it would be of interest to investigate further the staining patterns in immunofluorescence microscopy of the different human tumor groups and of carcinomas with TPA antibodies. Conversely it would be interesting to know what the levels of cytokeratins 8, 18 and 9 are in sera from cancer patients and from controls.

In summary, TPA cannot be considered as a tumor-specific antigen but is identified as being derived from keratins 8, 18 and 19 which are restricted to simple and non-squamous epithelia. TPA is therefore a component of the cytoskeleton of certain normal and malignant epithelial cells. It remains to be seen whether other tumor markers for which the titre is increased in patient sera arise by a similar mechanism, i.e., they are not restricted to tumor cells and their increased presence in the circulation reflects cell destruction, shedding, etc.

Materials and methods

TPA and antibodies to TPA

TPA:B_1 was purified from pooled human carcinomas (from colon, pancreas, lung, rectum, mammary tissue, and liver) obtained at autopsy and stored at −24°C, using the procedure described in Björklund *et al.*, 1973 and Lüning *et al.*, 1980. Antibodies to TPA:B_1 were raised in rabbits (Wiklund *et al.*, 1981). The specificity of rabbit antibodies has been shown by (i) double diffusion analysis against TPA:B_1 (Wiklund *et al.*, 1981); (ii) anti-TPA:B_1 agglutinates TPA-labelled sheep red blood cells in a hemagglutination assay where TPA:B_1 completely inhibits the reaction as a dose response; (iii) anti-TPA:B_1 shows up to 85−90% bound/total with ^{125}I-labelled TPA:B_1 in a double antibody radiommunoassay where TPA:B_1 completely inhibits the reaction as a dose response. Kits to measure TPA are available commercially (AB Sangtec Medical, Bromma, Sweden).

Other antibodies

The broad specificity keratin antibody, ker_{GP}, was raised in guinea pigs against keratin purified from cow snout (Osborn *et al.*, 1982). The rabbit antibody ker_{19} reported to be specific for the 40 kd keratin, i.e., keratin 19 (Wu *et al.*, 1982), was very kindly provided by Dr. J. Rheinwald (Harvard Medical School, Boston, MA, USA). K_G8.13.2, a mouse monoclonal antibody recognizing a wide variety of keratins (Gigi *et al.*, 1982) was provided by Dr. B. Geiger, The Weizmann Institute. The CK monoclonal antibodies recognizing cytokeratin 18 have been described (Debus *et al.*, 1982). CK_2 was used in this study. The α-IFA antibody which recognizes all IF proteins has been described (Pruss *et al.*, 1981). Martin Raff, University College, London, kindly provided this hybridoma line.

Immunofluorescence studies

Cell lines were obtained from the American Type Culture Collection. Cells on 12 mm glass coverslips were fixed in methanol for 6 min. The first antibody was then applied for 45 min at 37°C, the cells washed with phosphate-buffered saline (PBS) and then the second antibody, FITC goat anti-rabbit IgG was applied again for 45 min at 37°C. After washing with PBS, cells were mounted in Mowiol 4-88.

Sections of rat and human tissue were from material frozen in isopentane or liquid nitrogen and stored at −70°C until use. Sections nominally 5 μm in thickness were cut on a cryostat, and dried for 30 min before being fixed in acetone at −10°C for 10 min. Standard immunofluorescence procedures (see above) were then used.

Immunoblots

In the dot blot, TPA antigen was spotted onto nitrocellulose paper at the concentrations indicated in Figure 3a. The blots were blocked by incubation with a buffer containing 4% bovine serum albumin, and then reacted with the appropriate first antibody. The second antibody was 20 nm gold-labelled goat anti-rabbit, or goat anti-mouse antibody (Janssen Pharmaceutica, Beerse, Belgium).

For the immunoblotting experiments on gels shown in Figure 3b cytoskeletons of MCF-7 cells were prepared using Triton X-100 (e.g. Osborn *et al.*, 1982). After separation of the polypeptides by SDS gel electrophoresis the bands were blotted by diffusion onto nitrocellulose paper. Reaction of the transferred polypeptides with antibodies was as described above for the dot blots.

For the one-dimensional immunoblotting experiments shown in Figure 3c epidermis was prepared from non-mammilar breast epidermis by taking thin slices parallel to the surface (Moll *et al.*, 1982a). Tongue epithelium was prepared in the same manner. Hair follicles were prepared from plucked scalp hairs (Moll *et al.*, 1982a). Cytoskeletal residues from Detroit 562 cells and from HeLa cells were prepared using Triton X-100 (Franke *et al.*, 1982). One-dimensional SDS gel electrophoresis was on 12% polyacrylamide gels and polypeptides were electrophoretically transferred onto nitrocellulose paper according to Towbin *et al.* (1979). Immunoblotting experiments were performed with a 1:600 dilution of the TPA serum. For the two-dimensional electrophoresis shown in Figure 3d NEPHG electrophoresis was used in the first dimension followed by SDS gel electrophoresis in the second dimension. After electrophoresis the gel was incubated for 2 h in 4 M urea, 10 mM Tris-HCl, pH 7.5, 2 mM EDTA, 1 mM dithiothreitol, to re-nature the polypeptides. They were then electrophoretically transferred and reacted with a 1:600 dilution of the TPA antibody in 0.1% BSA in PBS. The position of reactive keratin polypeptides was revealed using [^{125}I]protein A.

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