

Reactivity of monoclonal antibodies against intermediate filament proteins during embryonic development

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SUMMARY

Monoclonal antibodies (mAbs) against a preparation of intermediate filaments from trophoblastoma cells were studied for their reactivity pattern during embryonic development and on adult tissue cells. Up to day 12 of embryonic development, epithelial cells of the three germ layers reacted with these mAbs. Later during development and in adult tissues, positive reactions could be observed only with epithelial cells derived from mesoderm and endoderm. Because of their tissue distribution, the proteins reacting with these mAbs might belong to the keratin family of intermediate filaments or they might represent a new group of intermediate filaments.

INTRODUCTION

Attempts to follow the distribution of specific antigenic markers in the course of embryonic development have been hitherto made difficult for two main reasons. The first one is the heterogeneity of the antibody populations when conventional antisera are used to study the distribution of specific markers. The second difficulty comes from possible changes in the antigenic determinants which can either be present on several molecular species, or be altered during development.

The latter is difficult to deal with and requires the use of several independent techniques. The former difficulty can be overcome with the use of affinity-column-purified antibodies, when the antigen can be isolated, or with the help of monoclonal antibodies (mAbs). There are only a few cellular markers known for early mouse embryonic cells and those have not yet been well purified. Instead of making a great effort to purify these markers, it was first attempted to obtain very specific probes against them.

In this laboratory, mAbs against preparations of intermediate filaments from

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trophoblastoma cells have recently been prepared (Brûlet, Babinet, Kender & Jacob, 1980). One of these mAbs reacts with trophoblast, but not with inner cell mass (ICM) cells in the blastocyst. In this paper, we report further properties of this mAb as well as of other ones, and their use to study the distribution of specific antigenic markers on sections of embryos taken at various stages of development.

MATERIALS AND METHODS

Cells

Teratocarcinoma cell lines were used under standard culture conditions and are listed in Table 1. PSA-1-NG-2 cells were cultured under conditions allowing endodermal differentiation, according to Martin, Wiley & Damjanov (1977). Embryonal carcinoma (EC) cells, PCC3/A/1 and F9-41 were treated with hexamethylenediacetamide (Jakob, Dubois, Eisen & Jacob, 1978*b*) or retinoic acid (Strickland & Mahdavi, 1978).

3T3 and Hela cells were cultured in Eagle's medium (Dulbecco's modification, DMEM) supplemented with 15% fetal calf serum (FCS).

Embryos

Preimplantation embryos were collected from virgin superovulated C57BL/6 × CBA females as already described (Kemler, Babinet, Eisen & Jacob, 1977). ICM cells were isolated by immunosurgery (Solter & Knowles, 1975) and blastocysts or ICM were cultured in DMEM, 10% FCS for 24 to 48 h. Females (C57BL/6 × CBA or NCS) were mated with 129/Sv males and the day of vaginal plug was taken as day one of embryonic development.

Fetal liver cells from 14-day-old embryos were gently pipetted and cultured for 24 h in DMEM, 10% FCS on cover slips for subsequent immunofluorescence tests.

Hybridomas: production

A preparation of intermediate filaments from trophoblastoma cells was used to immunize rats. The biochemical extraction of intermediate filaments and the immunization procedure are the same as described by Brûlet *et al.* (1980).

Spleen cells were fused with myeloma cells SP-2-0 (an hypoxanthin-phosphoribosyl-transferase negative and IgG non-producing cell line) using polyethylene-glycol (PEG, MW: 4000, Roth KG, Karlsruhe, West Germany) as a fusion agent (Kemler, Morello & Jacobs, 1979) following basically the technique described by Köhler & Milstein (1975). After fusion, cells were seeded in tissue culture clusters (Costar, 24 wells, 10⁶ splenocytes per well) and selective HAT-medium (hypoxanthin-aminopterin-thymidin) was added 24 h later. The medium was changed twice a week and hybrid cell growth was observed 10–20 days after fusion.

Positive culture supernatants were selected against the preparation of intermediate filaments using an indirect radioactive-binding assay.

For this the preparation of intermediate filaments was covalently coupled on 0.5 cm diazobenzylloxymethyl (DBM) paper discs and bound antibodies were detected with ^{125}I -labelled F(ab)_2 fragments ($5\ \mu\text{Ci}/\mu\text{g}$ protein) of a rabbit anti-rat IgG serum purified by affinity column.

Hybrid cells were subcloned twice as described by Kemler *et al.* (1979). Hybridomas were characterized by immunoprecipitation: $100\ \mu\text{l}$ of [^{14}C]lysine-labelled hybridoma supernatant was incubated for 2 h at $4\ ^\circ\text{C}$ with $5\ \mu\text{l}$ of a rabbit anti-rat serum (Miles). Sepharose-4B-Prot A (Pharmacia), $100\ \mu\text{l}$ of a 1/4 diluted stock solution in $0.1\ \text{M-PO}_4$, pH 8.0 was added for 30 min at $4\ ^\circ\text{C}$. The sepharose-4B-Prot A was then washed three times in $0.1\ \text{M-PO}_4$, pH 8.0 and bound proteins were resuspended in $50\ \mu\text{l}$ of sample buffer, containing 1% NP40, 2% SDS and 5% mercapto-ethanol. Examination of labelled products was done by SDS-polyacrylamide electrophoresis (Laemmli, 1970).

Biochemical identification of proteins

The transfer of proteins from two-dimensional polyacrylamide gels to DBM paper and their subsequent immunological detection with monoclonal antibodies has been described by Brûlet *et al.* (1980).

Immunolabelling of cells and embryos

Indirect immunofluorescence tests were done with supernatants of hybridoma cultures as a first sandwich layer. Antibody binding was revealed with affinity-column-purified rabbit-anti-rat IgG antibodies ($15\ \mu\text{g}/\text{ml}$) conjugated with FITC (Clark & Shepard, 1963). Incubations were usually done for 40 min in a wet chamber at room temperature, followed by a 15 min washing in PBS on a gently agitating magnetic stirrer. For all tests, controls were the culture supernatant of a negative hybrid cell line or the culture supernatant of a hybridoma secreting a monoclonal antibody with an unrelated specificity.

For immunofluorescence tests, cells were grown on cover slips, washed twice in cold PBS and fixed in cold methanol for 15 min.

Preimplantation embryos were air-dried on albumin-coated slides, fixed in cold methanol and subsequently immunohistochemically stained using peroxidase-labelled antibodies as described by Brûlet *et al.* (1980).

To study post-implantation embryos, 8–10 μm -thick cryostat sections (Slee, London) were prepared. Embryos, 6 to 9 days old, were cut within their maternal decidua, while 12- to 14-day-old embryos were dissected and immediately frozen on cryostat holders. Organs from adult mice were cut in small pieces and immediately frozen for cryostat sectioning. Cryostat sections were transferred to slides coated with egg albumin, air dried and fixed for 15 min in acetone at $-20\ ^\circ\text{C}$.

In some cases, embryos or organs were prefixed in 4% paraformaldehyde,

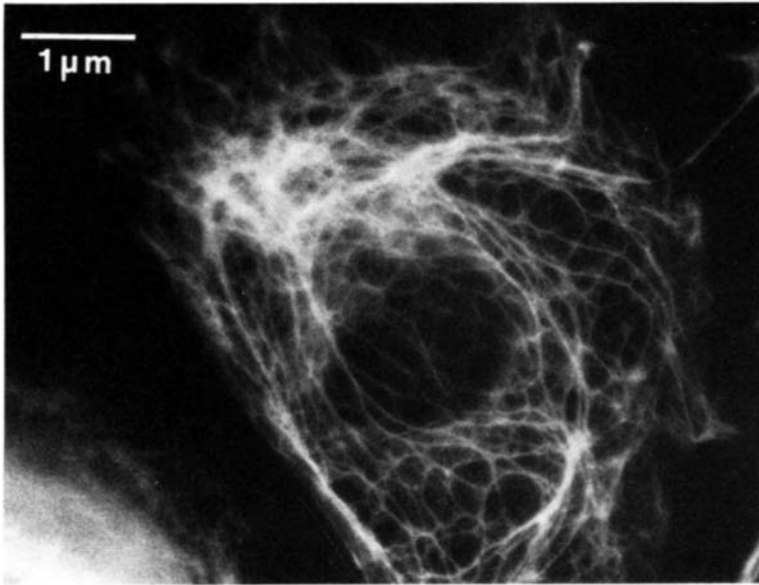


Fig. 1. TROMA 2 decorates an intracellular network on trophoblastoma cells fixed with methanol.

PBS, pH 7.4 for 2 h at 4 °C, washed subsequently with PBS and PBS containing 10% sucrose and incubated overnight in PBS plus 0.8 M-sucrose before sectioning. No major difference in the results between the two types of preparation was observed.

RESULTS

Production and characterization of mAbs

Splenocytes from a rat immunized with the preparation of intermediate filaments from trophoblastoma cells were fused with mouse myeloma cell, SP-2-0. Hybrid growth was observed in about 80% of the wells. Antibody activity against the intermediate filament preparation was found in culture supernatants of 92 wells out of 192.

The original aim of this set of experiments was to obtain a mAb against a marker specific of trophodermal cells in the blastocyst. The trophoderm specific marker as defined by two-dimensional gel analysis can be detected in a teratocarcinoma-derived trophoblastoma cell line and in PYS-2 cells. In contrast, it cannot be detected in ICM, nor in EC cells. The supernatants from hybrid cells were therefore screened on trophoblastoma and EC cells, by an indirect immunofluorescence test. Two major types of reactions could be distinguished.

(1) A series of supernatants decorates an intracellular network in trophoblastoma and not in EC cells (Fig. 1).

(2) Another series gives a more diffused intracellular staining in tropho-

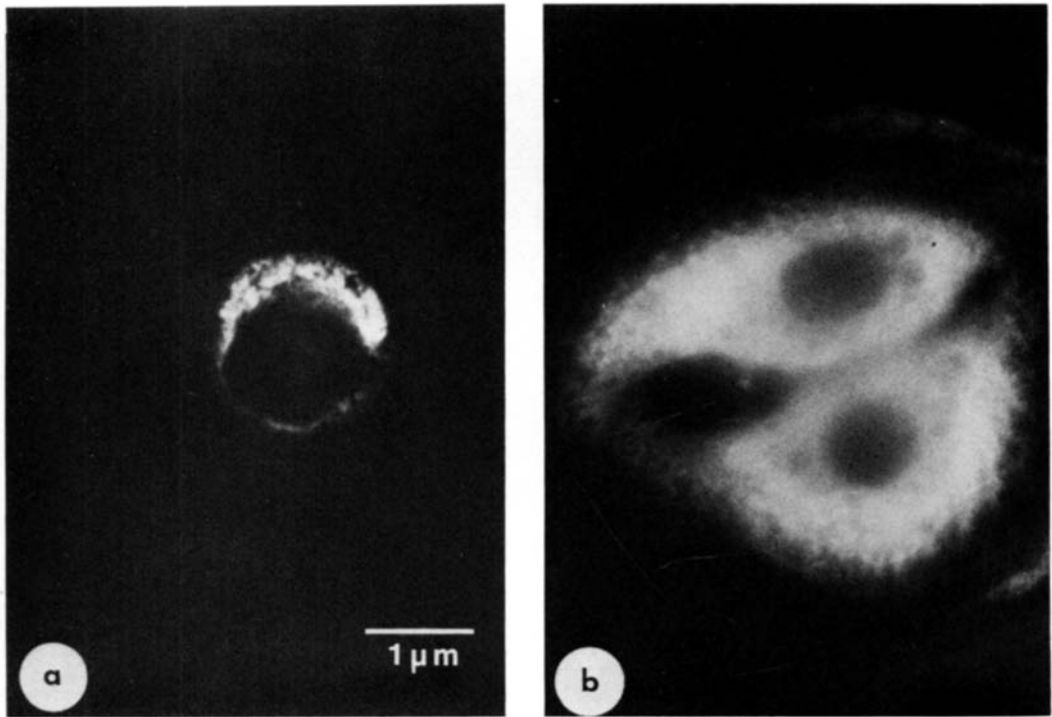
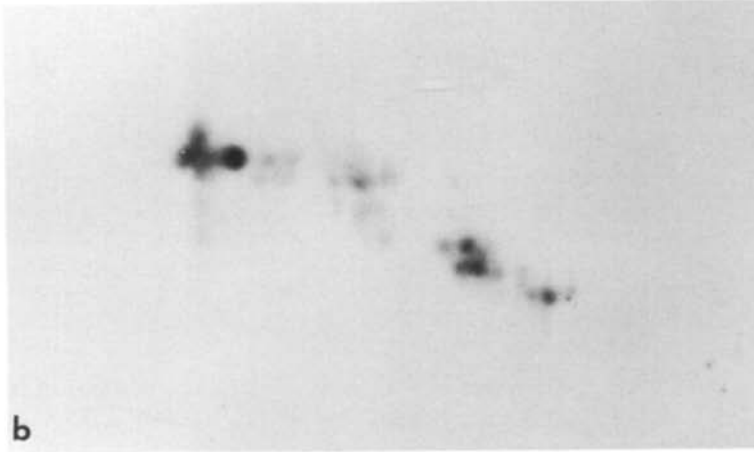
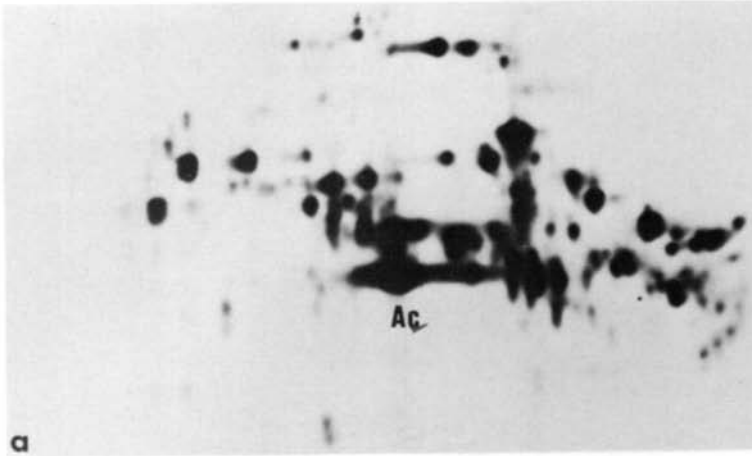


Fig. 2. Two examples of mAbs (prepared by immunization with a preparation of intermediate filaments) which give a diffuse intracellular staining in trophoblastoma cells. The perinuclear reaction like (a) was observed rather frequently.

blastoma and EC cells, which is often restricted to the perinuclear area (Fig. 2a and b).

Culture supernatants of six independent hybrid cell lines of the first type did react with trophectodermal but not with ICM cells in an indirect immunoperoxidase test. They were chosen for subcloning and subsequent investigations.

Attempts were then made to detect, by the protein-transfer technique, which protein from the intermediate filament preparation reacts with each mAb. It turned out that, although subcloned, each of the six mAbs which decorate an intracellular network in trophoblastoma cells detects more than one protein. Five out of six detect an identical pattern while the sixth one detects another pattern (Fig. 3). One of the five similar mAbs, called TROMA 1, and the latter one, called TROMA 2 (TROphectodermal Monoclonal Antibody) were then chosen for further investigation. The reason why TROMA 1 and 2 react with different spots is not yet understood. Some of the peptides might represent products of proteolytic degradation. This, however, seems unlikely since identical spots were found in several independent preparations of intermediate filaments from trophoblastoma cells. Rather, the same antigenic determinant might be present on several proteins.



Further attempts to characterize the target proteins and compare them with known intermediate filament proteins are in progress and will be reported elsewhere.

When fluorograms 3b or 3c of Fig. 3 are superposed on 3a, it is possible to identify most of the spots reacting with TROMA 1 or 2. Fluorograms 3b and 3c have also been compared in order to determine whether or not some of the spots are common. Actually, while the majority of the spots are clearly different, it cannot be clearly ruled out that a few of them are identical. It is nevertheless clear that the antigenic determinants reacting with TROMA 1 and 2 are not identical. Some properties of TROMA 1 and its use to detect a trophoctoderm specific marker have been previously reported (Brület *et al.* 1980). TROMA 1 and 2 are IgG antibodies.

Reactivity pattern of TROMA 1 and 2 on cells in culture

The reactivity of TROMA 1 and 2 on a series of cell lines was tested by indirect immunofluorescence. The results are given in Table 1. As can be seen, only two teratocarcinoma-derived cell lines, namely trophoblastoma and PYS-2 cells, react with these mAbs and decorate an intracellular network. The two mAbs, however, can be distinguished by the fact that human HeLa cells react with TROMA 1 but not with 2. Immunofluorescence staining of EC cells by TROMA 1 and 2 is similar to that obtained with control mAbs. The presence of a small amount of non-organized antigenic molecules in EC cells cannot be ruled out. The immunofluorescence results, however, are in agreement with two-dimensional gel electrophoretic analysis, where the trophoctoderm-specific proteins are not found in preparations of intermediate filaments of EC cells. The usefulness of these mAbs as specific reagents of a cell marker for differentiation can be shown on *in vitro* differentiation of EC cells. As can be seen in Table 1, none of the EC cell lines reacts with these mAbs. This applies in particular to PSA-NG-2 which does not react with the mAbs when grown under conditions preventing differentiation. In contrast, when these cells are grown under

Fig. 3(a) Fluorograms from a two-dimensional gel of a preparation of intermediate filaments extracted from trophoblastoma cells labelled for 15 h with [³⁵S]methionine. 200000 cpm were loaded on to the isoelectrofocusing gel. The pH-range was estimated to be between 4.75 and 6.75. For the second dimension a 10% acrylamide/NaDodSO₄ gel was used. Ac indicate the position of actin. (b) and (c) Corresponding regions of fluorograms after immunological detection of antigens recognized by TROMA 1 (b) and by TROMA 2 (c). 30 µg of unlabelled preparation of intermediate filaments from trophoblastoma cells were fractionated as in (a) and transferred onto DBM-paper. The sheet (17 × 18 cm) was successively incubated with 40 ml of hybridoma supernatant and with iodinated rabbit F(ab)² anti-rat Ig (10⁷ cpm specific activity of 7.5 µCi/g) and exposed at -70 °C for 4-12 h. Fig. 3a and b have already been published in Brület *et al.* (1980). Further experimental details are in the same reference.

Table 1. *Indirect immunofluorescence test with TROMA 1 and 2 on EC cells and differentiated derivatives*

Cell line	Cell type	References	Results with TROMA 1 and 2
PCC3/A/1	Embryonal carcinoma	Nicolas <i>et al.</i> 1976	Negative
PCC4Aza 1	Embryonal carcinoma	Nicolas <i>et al.</i> 1976	Negative
PSA-1-NG-2	Embryonal carcinoma	Martin and Evans, 1975	Negative
3/TM-1	Trophoblastoma	Nicolas <i>et al.</i> 1975	Positive*
PYS-2	Parietal-yolk-sac	Lehman <i>et al.</i> 1974	Positive
3/A/1-D3	Embryonic fibroblast	Nicolas <i>et al.</i> 1976	Negative
3/A/1-D-1	Osteogenic mesenchyme	Nicolas <i>et al.</i> 1976	Negative
C-17-S1-D-1 984	Myoblastic cell	Jakob <i>et al.</i> 1978a	Negative

* Intracellular network in cells grown on cover slips.

conditions allowing endodermal differentiation, the outer layer of endodermal cells became stainable by the two mAbs (Fig. 4).

However, endodermal cells are labelled more intensively by TROMA 1 than by TROMA 2. This difference in intensity does not seem to be due to a difference in the reactivity of the mAbs themselves, since at a similar dilution both produce similar staining on PYS or trophoblastoma cells. Furthermore, a 20-times increase in concentration of TROMA 2 culture supernatant does not increase the intensity of staining on the endoderm produced by differentiation of PSA-NG-2 cells. On cryostat sections of cell aggregates, the fraction of aggregates reacting with both TROMAs increases as a function of time and therefore of differentiation. On day 4, only 15–18% of the aggregates showed a fully positive (complete ring) staining, 60–65% a partially positive (spotted reaction), while the remaining aggregates were completely negative. On day 6, the fraction of fully positive aggregates raised to 50–55% while only 10% remained negative. When both TROMAs were studied in parallel on serial sections of the same aggregate, not only did TROMA 2 produce a weaker reaction than TROMA 1, but the reaction came at a later time with the former than with the latter. It is likely that, in these endodermal cells, the antigenic determinant detected by TROMA 2 is less abundant than that detected by TROMA 1.

When EC cells F9-41 or PCC3/A/1 were treated for five days with retinoic acid or hexamethylenebisacetamide to induce differentiation, almost all cells became positive for TROMA 1 and 2.

Reactivity pattern of TROMA 1 and 2 on the embryo

The two mAbs have been selected to label the trophectoderm but not the ICM in the blastocyst. Their reactivity was followed on sections of embryos taken at various stages of their development. To study embryonic endoderm,

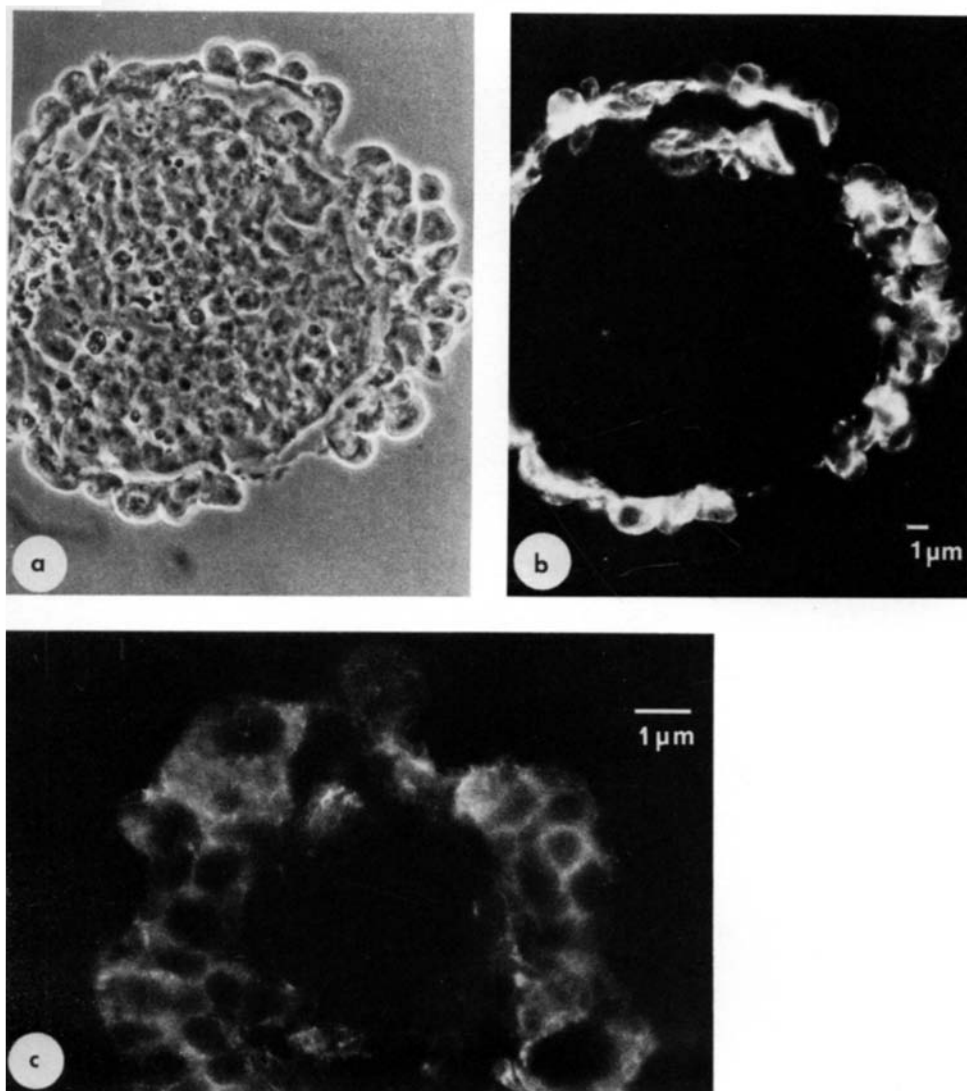


Fig. 4. Indirect immunofluorescence test with TROMA 1 on cryostat sections of PSA-1/NG-2 cells grown in aggregates allowing endodermal differentiation (*a* and *b*) and of cultured ICM (*c*).

ICMs were isolated by immunosurgery and put in culture. Under these conditions, an endodermal layer differentiates which reacts with both TROMA 1 and 2 (Fig. 4). Here again, however, the staining by the two mAbs was different: TROMA 1 stained endoderm as strongly as trophectodermal cells, while the reaction of endoderm with TROMA 2 was much weaker.

Studies on later stages of development were performed by indirect immunofluorescence on frozen sections of implanted embryos. At all these stages,

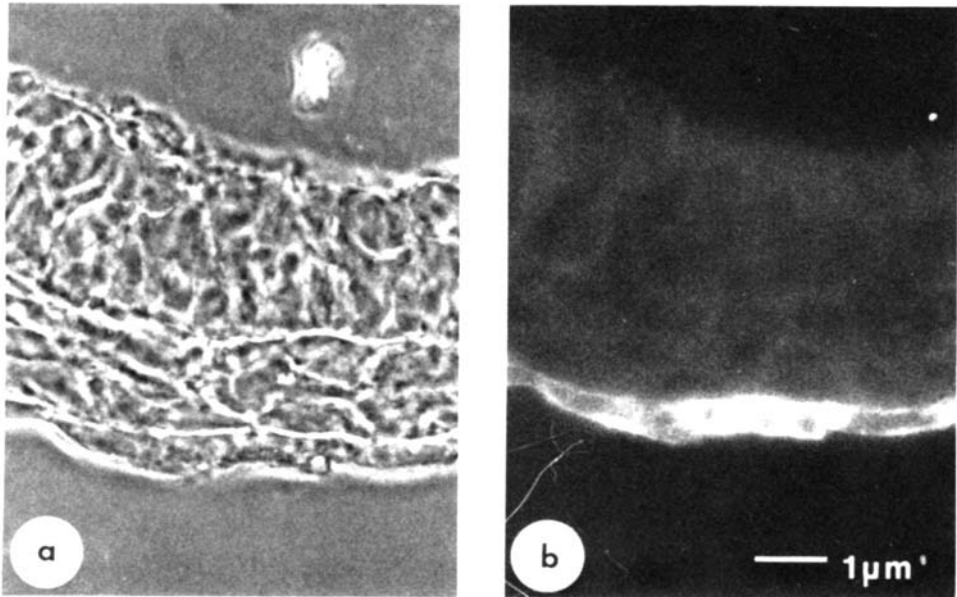


Fig. 5. Indirect immunofluorescence test with TROMA 2 on cryostat sections of 8-day-old embryos. (a) Phase, embryonic part of the egg cylinder. top: proamniotic cavity; bottom: yolk cavity. (b) Immunofluorescence.

TROMA 1 and 2 gave similar reactions, although somewhat weaker for TROMA 2. Very little background was in general observed except for the area between trophoblast and maternal decidua where the two tissues are intercalated, non-specific labelling prevented fine analysis.

From day 7 to day 9, only embryonic and extraembryonic visceral endoderm and parietal endoderm were stained (Fig. 5). At day 12, both mAbs strongly stained various epithelial cells derived from different germ layers: epithelium of the skin, of oral and nasal cavity, Rathke's pouch, thymus, trachea and lung anlage, epithelium of the digestive tube, of the metanephros, pancreas and uterus, as well as liver cells and the notochord. In contrast, neuroepithelial cells were not stained. Examples of the reaction of TROMA 1 and 2 are given in Figs. 6 and 7. Primary cultures of 12-day liver cells were also treated with TROMA 1 and 2. Both mAbs decorated an intracellular network in a large fraction of the cells. After day 14, the pattern of reactivity remained essentially similar with one important exception: skin epithelium became almost completely negative (Fig. 7c and d) as were already neuroepithelial cells. In the study of ectoderm-derived epithelial cells, a reaction was observed only in the oral cavity, the outer layer of the optic cup and the choroid plexus.

Reactivity pattern of TROMA 1 and 2 on adult tissues

The reactivity of the two mAb's was investigated on cryostat sections of various organs of adult mice. Both mAbs reacted with epithelial cells mainly of

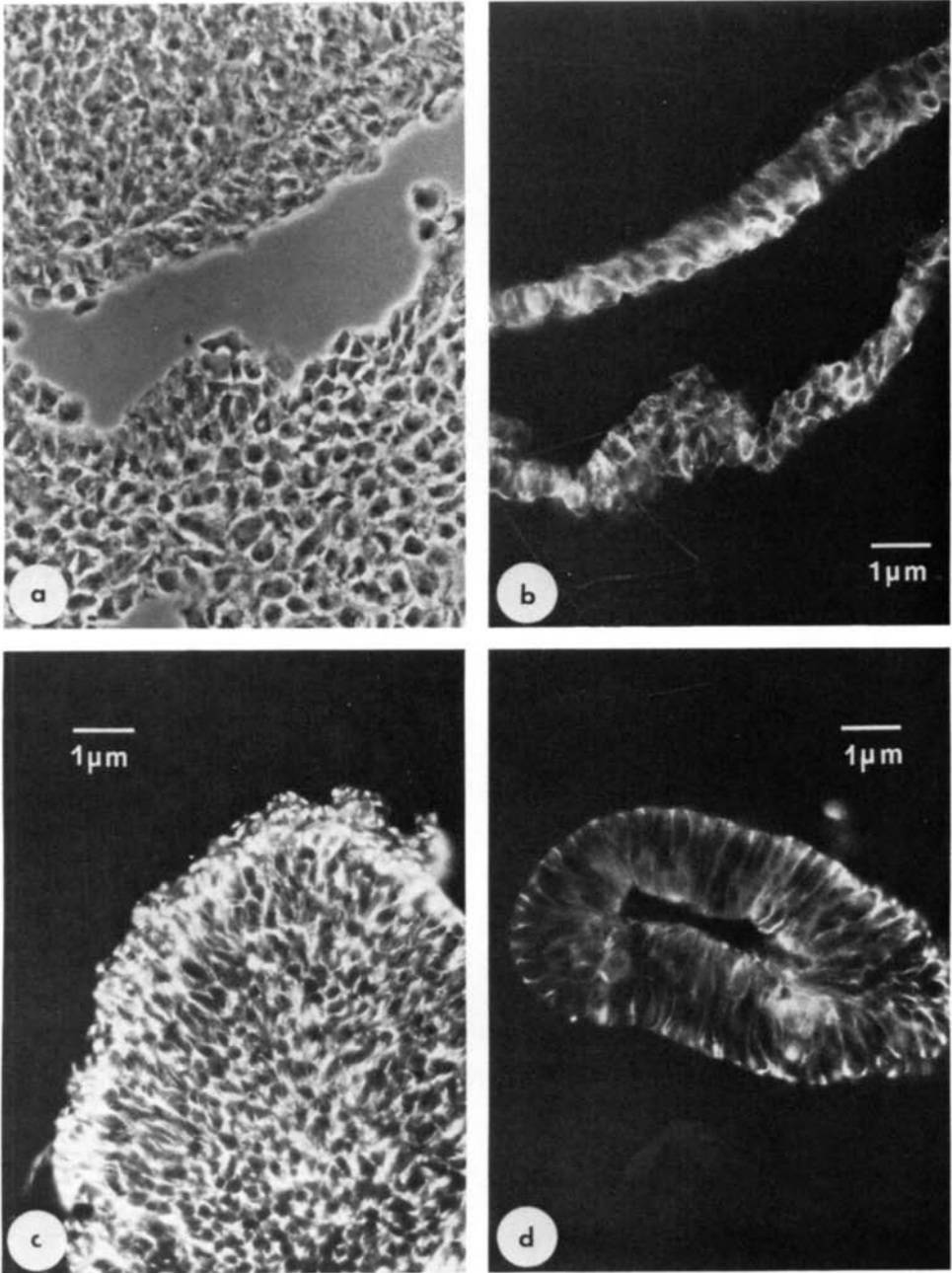


Fig. 6. Indirect immunofluorescence test with TROMA 2 on cryostat sections of 13-day-old embryos, mouth cavity (*a* and *b*), nasal epithelium (*c*) and gut epithelium (*d*).

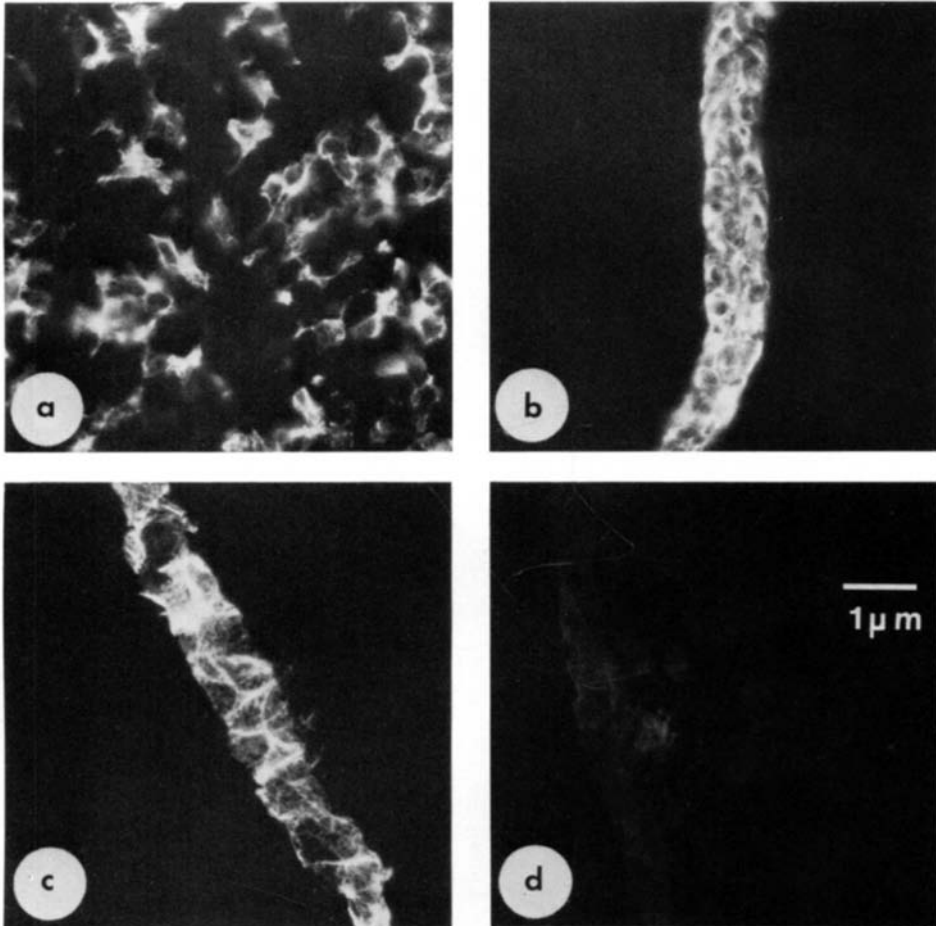


Fig. 7. Indirect immunofluorescence test with TROMA 1 on cryostat sections of 14-day-old embryos, liver (a), notochord (b). Skin epithelial cells of 12-day (c) and 15-day (d) old embryos.

endodermal and mesodermal origin. In contrast, ectoderm-derived epithelial cells were negative. Among the organs studied, the following were found positive: uterus, kidney, vas deferens, epididymis, bladder, gut, pancreas, trachea and salivary gland (Figs. 8 and 9), while skin from ear, abdomen or tail, epithelial cells of tongue, oesophagus or vagina (seminiferous tubules, muscle, connective tissue, adrenal gland and blood vessel endothelia were negative. Small differences in the expression of the antigens detected by TROMA 1 and 2 could be observed on duodenal sections: TROMA 1 uniformly labelled all epithelial cells of villi and crypts of Lieberkuhn, while TROMA 2 did not label these cells but heavily stained goblet cells (Fig. 9a and b).

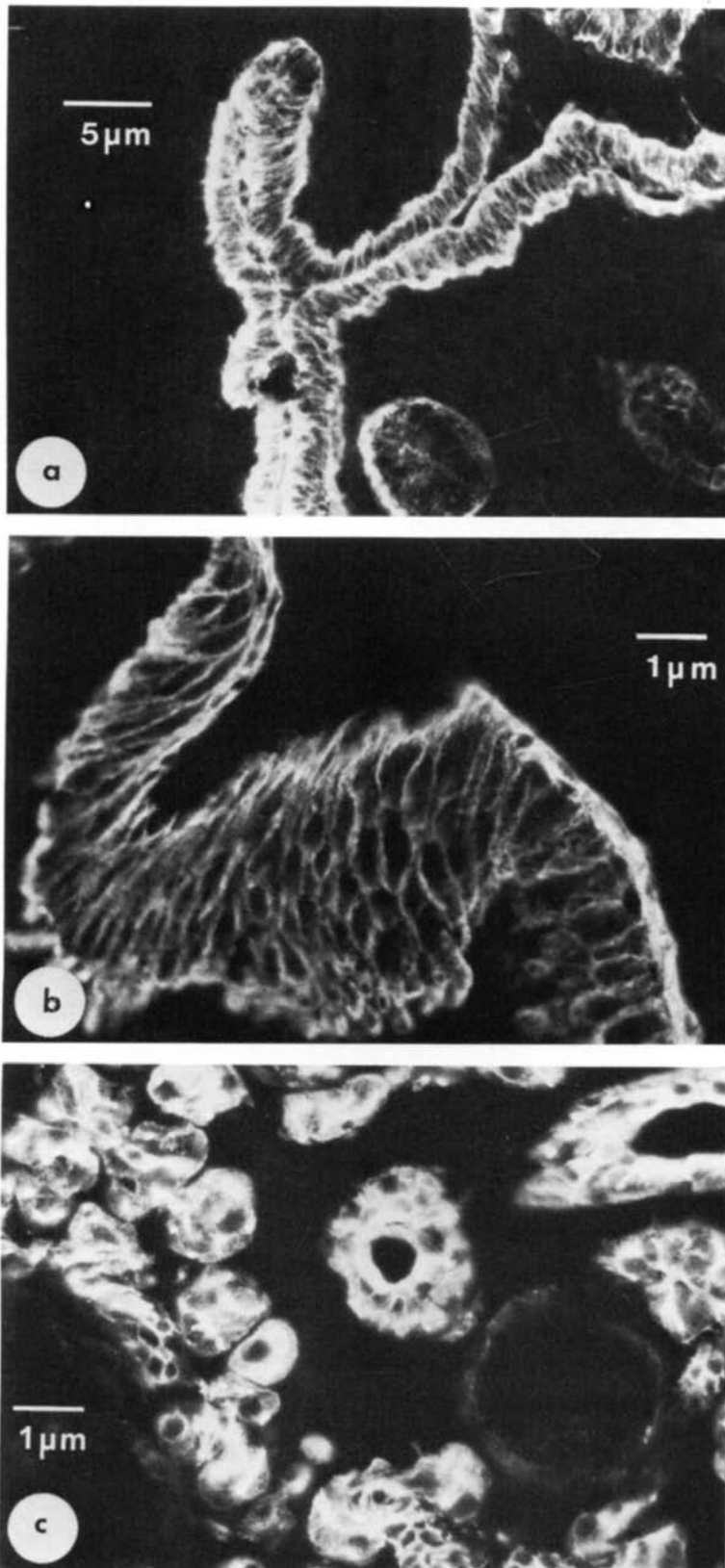


Fig. 8. Indirect immunofluorescence test with TROMA 1 on cryostat sections of adult tissue, uterus (a), vas deferens (b) and salivary gland (c).

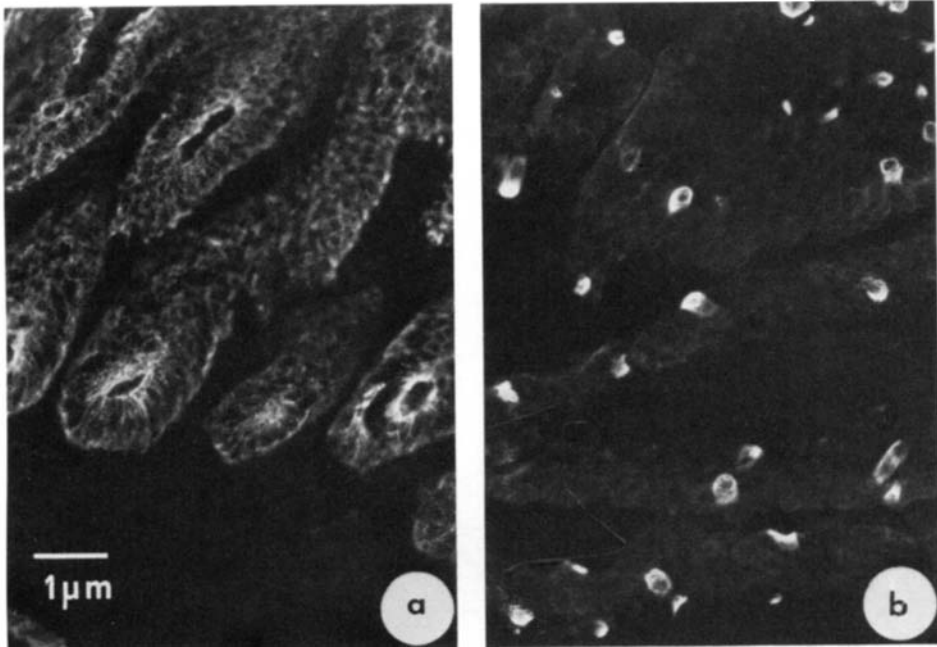


Fig. 9. Indirect immunofluorescence test with TROMA 1 (a) and 2 (b) on cryostat sections of adult duodenum.

DISCUSSION

The expression of the antigenic determinants recognized by the two mAbs, TROMA 1 and 2, has been studied in two ways: (1) *in vitro*, on EC cells and their differentiated derivatives and (2) *in vivo*, on embryonic and adult tissues.

In vitro, the two mAbs do not react with any of the EC cell lines tested. They react, however, with some of the epithelial cell lines derived from EC cells, such as trophoblastoma and PYS-2. They react also with the major part of EC cell populations induced by retinoic acid to differentiate. This result is in agreement with the suggestion by Strickland, Smith & Marotti (1980) that, upon treatment of EC cells with retinoic acid, the differentiated derivatives share phenotypic similarities with parietal endoderm.

In 4- to 9-day-old embryos, visceral and parietal endoderms are, in addition to trophoderm, the only cells reacting with TROMA 1 and 2.

In 12- and 13-day-old embryos, the two mAbs react with epithelial cells whatever their origin, ecto-, meso- or endodermal. Between day 12 and 15 of embryonic development, the pattern of reactivity remains similar with one striking difference: skin epithelial cells become negative.

The antigenic determinants detected by the two mAbs at day 12 are still associated with intermediate filaments, since on primary cultures of liver cells the two mAbs decorate the same network, as observed earlier.

Intermediate filament proteins are known to be heterogeneous. Five groups have been described, each specific for a type of tissue (Lazarides, 1980). From their tissue distribution, the antigenic structures detected by TROMA 1 and 2 seem to belong to the keratin group. If this is correct, TROMA 1 and 2 would react with only some proteins of the keratin family. But the possibility still exists that the proteins recognized by TROMA 1 and 2 belong to another, unknown group of intermediate filaments. Further biochemical analysis is therefore needed to compare the proteins described here with the group of intermediate filaments previously described. To our knowledge, this is the first time that a cell marker expressed on mouse preimplantation stages could be followed all along embryonic development up to adult tissues.

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REFERENCES

- BRÛLET, P., BABINET, C., KEMLER, R. & JACOB, F. (1980). Monoclonal antibodies against trophoblast specific markers during mouse blastocyst formation. *Proc. natn. Acad. Sci., U.S.A.* **77**, 4113–4117.
- CLARK, H. F. & SHEPARD, C. C. (1963). A dialysis technique for preparing fluorescent antibody. *Virology* **20**, 642–644.
- ERLICH, H. A., CAHEN, S. N. & McDEVITT, H. O. (1979). Immunological detection and characterization of products translated from cloned DNA fragments. *Methods in Enzymology* **68**, 443–453.
- JAKOB, H., BUCKINGHAM, M. E., COHEN, A., DUPONT, L., FISZMAN & JACOB, F. (1978*a*). A skeletal muscle cell line isolated from a mouse teratocarcinoma undergoes apparently normal terminal differentiation *in vitro*. *Expl Cell Res.* **114**, 403–408.
- JAKOB, H., DUBOIS, P., EISEN, H. & JACOB, F. (1978*b*). Effects de l'hexaméthylènebisacétamide sur la différenciation de cellules de carcinome embryonnaire. *C. r. hebd. Séanc. Acad. Sci., Paris* **286**, 109–111.
- KEMLER, R., BABINET, C., EISEN, H. & JACOB, F. (1977). Surface antigen in early differentiation. *Proc. natn. Acad. Sci., U.S.A.* **74**, 4449–4452.
- KEMLER, R., MORELLO, D. & JACOB, F. (1979). Properties of some monoclonal antibodies raised against mouse embryonal carcinoma cells. In *Cell lineage, Stem Cell and Cell Differentiation* (ed. N. Le Douarin), pp. 101–113. Inserm Symp. no. 10. Elsevier–North-Holland Biomedical Press.
- KÖHLER, G. & MILSTEIN, C. (1975). Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature, Lond.* **256**, 495–497.
- LAEMMLI, U. K. (1970). Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature, Lond.*, **227**, 680–685.
- LAZARIDES, E. (1980). Intermediate filaments as mechanical integrators of cellular space. *Nature, Lond.* **283**, 249–256.
- LEHMAN, J. M., SPEERS, W. C., SWARTZENDRUBER, D. E. & PIERCE, G. B. (1974). Neoplastic differentiation: characteristics of cell lines derived from a murine teratocarcinoma. *J. cell. Physiol.* **84**, 13–28.
- MARTIN, G. & EVANS, M. J. (1975). Differentiation of clonal lines of teratocarcinoma cells: Formation of embryoid bodies *in vitro*. *Proc. natn. Acad. Sci., U.S.A.* **72**, 1441–1445.

- MARTIN, G. R., WILEY, L. M. & DAMJANOV, I. (1977). The development of cystic embryoid bodies *in vitro* from clonal teratocarcinoma stem cells. *Devl Biol.* **61**, 230–244.
- NICOLAS, J. F., AVNER, P., GAILLARD, J., GUENET, J. L., JAKOB, F. (1976). Cell lines derived from teratocarcinoma. *Cancer Res.* **36**, 4224–4231.
- NICOLAS, J. F., DUBOIS, P., JAKOB, H., GAILLARD, J. & JACOB, F. (1975). Tératocarcinome de la souris: différenciation en culture d'une lignée de cellules primitives à potentialités multiples. *Annls Microbiol. (Inst. Pasteur)* **126A**, 3–22.
- SOLTER, D. & KNOWLES, B. B. (1975). Immunosurgery of mouse blastocyst. *Proc. natn. Acad. Sci., U.S.A.* **72**, 5099–5102.
- STRICKLAND, S. & MAHDAVI, V. (1978). The induction of differentiation in teratocarcinoma stem cells by retinoic acid. *Cell* **15**, 393–403.
- STRICKLAND, S., SMITH, K. K. & MAROTTI, K. R. (1980). Hormonal induction of differentiation in teratocarcinoma stem cells: generation of partietal endoderm by retinoic acid and dibutyryl cAMP. *Cell* **21**, 347–355.

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Note added in proof

Similar results concerning intermediate filament proteins in preimplantation stage embryos have been reported by Jackson *et al.* (1980) (*Differentiation* **17**, 161–179).