Localization of Tamm–Horsfall-glycoprotein-like immunoreactivity in cultured baby-hamster kidney cells, shown by immunofluorescence and by light- and electron-microscopic immunoperoxidase techniques

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Tamm–Horsfall glycoprotein was isolated from hamster urine, and antiserum against it was produced in rabbits. IgG was isolated from the antiserum. Immunocytochemical methods were used to localize Tamm–Horsfall-like immunoreactivity in three substrains of baby-hamster kidney (BHK) cells. Indirect immunofluorescence techniques showed that, in two substrains (BHK-21/C13/2P and BHK-21/C13/3P), a proportion of the cells fluoresced brilliantly, whereas those of the third substrain (BHK-21/ICRF) were totally negative. Related findings were obtained by the immunoperoxidase optical-microscopic technique. From the results of immunoperoxidase techniques using the electron microscope, it was concluded that the substance was present in association with the plasma membranes of the reacting cells. Our data suggest that the line of baby-hamster kidney cells, BHK-21/C13, may contain cells of renal-tubular epithelial origin, and that the proportion of these may be variable from one subculture to another.

Dunstan et al. (1974) observed that sonicated preparations of an established line of baby-hamster kidney cells, BHK-21/C13/2P (MacPherson & Stoker, 1962; Capstick et al., 1966), contained material that was immunologically cross-reactive with antisera raised against human, rabbit and hamster urinary Tamm–Horsfall glycoprotein (Tamm & Horsfall, 1950, 1952). The presence of the glycoprotein in a major proportion of a sample of the cultured kidney cells was later demonstrated by an indirect immunoperoxidase technique, and it was found that the action of crystalline trypsin, under conditions that did not change the permeability to Trypan Blue, led to a considerable decrease in the quantity of the glycoprotein associated with the cultured cells (Bloomfield et al., 1977).

The precise localization of Tamm–Horsfall glycoprotein, at the subcellular level, in the tubular system of the foetal-, neonatal-(Sikri et al., 1981a) and adult-(Sikri et al., 1979) hamster kidney is known: the glycoprotein is restricted to an association with the whole of the plasma membrane of cells of the thick ascending limb of the loops of Henle, and of those particular distal-tubular cells which are morphologically closely like those of the ascending limb. A similar distribution of the glycoprotein occurs also in the kidney of the rat (Hoyer et al., 1979; Sikri et al., 1982) and of man (Sikri et al., 1981b). The probable implications of this distribution for the function of the kidney have been discussed (Sikri et al., 1979; Foster et al., 1979; Hoyer et al., 1979).

The present work is concerned with identifying the subcellular localization of the related glycoprotein in three different substrains of cultured baby-hamster kidney cells, BHK-21/C13. Immunoelectron-microscopic procedures have been used for identifying the glycoprotein on these cells for the first time.

Experimental

Materials

Eagle's minimum essential growth medium, foetal-calf serum and tryptose/phosphate broth were purchased from Gibco-Biocult, Paisley,
Renfrewshire, Scotland, U.K. DEAE-cellulose (DE-23) was obtained from Whatman Biochemicals, Maidstone, Kent, U.K.; fluorescein-labelled sheep anti-(rabbit IgG) serum was from Wellcome Research Laboratories, Beckenham, Kent, U.K.; sheep anti-(rabbit IgG) serum and horseradish peroxidase-anti-peroxidase complex (PAP) were from Miles Laboratories, Stoke Poges, Slough, Berks., U.K.; and 3,3'-diaminobenzidine was from BDH Chemicals, Poole, Dorset, U.K.

Samples of baby-hamster kidney cells, BHK-21/C13, substrain 2P, adapted to grow in suspension culture (Capstick et al., 1966), were supplied by The Wellcome Foundation, Pirbright, Surrey, U.K., those of sub- strain 3P by The Wellcome Research Laboratory, Beckenham, Kent, U.K., and a third sample (BHK-21/ICRF) by the Imperial Cancer Research Fund Laboratory, Lincoln's Inn Fields, London W.C.2, U.K.

Methods

Hamster Tamm–Horsfall glycoprotein. The glycoprotein was isolated from hamster urine by the method of Dunstan et al. (1974), which includes gel filtration under dissociating conditions. Electrophoretic homogeneity on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was ascertained. Antiserum to Tamm–Horsfall glycoprotein was raised in rabbits (Sikri et al., 1979), and IgG was isolated from the antiserum by a method (Bloomfield et al., 1977) similar to that described by Sober & Peterson (1958). The freeze-dried product was dissolved in a volume of water equal to that of the serum from which it was derived, and the solution was stored in batches at −20°C. For use in the immunohistological experiments, the solution was diluted further (1:10, v/v) with phosphate-buffered saline, pH 7.3 (Dulbecco A buffer; Dulbecco & Vogt, 1954).

Growth and immunohistochemical staining of baby-hamster kidney cells. The cells were grown on glass surfaces, in the form of coverslips, or in 500 ml medical ‘flats’ for immunoperoxidase experiments. In either case the cells were cultured in Eagle’s minimum essential growth medium containing 10% (v/v) foetal-calf serum and 5% tryptose/phosphate broth at 37°C for 18–42h.

Cells grown to confluence on coverslips were washed in 0.05m-sodium phosphate buffer, pH 7.4, followed by 2–3h fixation at 4°C in McLean &
Nakane's (1974) periodate/lysine/(2%, w/v) paraformaldehyde solution. The fixed cells were rinsed thoroughly with several changes of phosphate-buffered saline and processed for indirect fluorescence microscopy as described in detail elsewhere (Bloomfield et al., 1977). The treated cells were examined with a Gillet and Siebert conference microscope fitted with a Zeiss FL epifluorescence attachment.

Cells grown in bottles were released from the glass by treatment with 0.02% neutralized EDTA in phosphate-buffered saline, pH 7.3. The suspension of cells was centrifuged (500g, 5 min, 22°C). After the pelleted cells had been washed with the same buffer, they were fixed in periodate/lysine/paraformaldehyde solution (2–3 h, 4°C) and processed for immunoperoxidase microscopy by using the peroxidase–anti-peroxidase complex (Sternberger et al., 1970; Sikri et al., 1979).

After histochemical staining for peroxidase activity with 3,3-diaminobenzidine and H₂O₂ (10–15 min; Graham & Karnovsky, 1966), the pelleted cells were post-fixed in 2% (w/v) OsO₄ for 1 h and embedded in Taab's resin (Taab Laboratories, Emmey Green, Reading, Berks., U.K.). Sections (1 μm thick) were cut from the embedded material, mounted on glass slides and used for optical-microscopic observations. Ultrathin sections (0.05μm) were cut on a Huxley ultramicrotome (Cambridge Instrument Co., Cambridge, U.K.), collected on uncoated 200-mesh copper grids and viewed, without further heavy-metal staining, with a Hitachi H300 electron microscope operating at 60kV.

In order to check for the specificity of the

Fig. 3. Low-power (magnification ×3500) electron micrograph of pelleted BHK-21/C13/2P cells showing positively and negatively reacting cells

Note strong reactions in the numerous microvilli associated with the upper cell. Immunoperoxidase method.
reaction, control tests were done in which anti-(Tamm–Horsfall glycoprotein) antibody was omitted or was replaced by normal rabbit serum. Further control tests were done in which material was treated with anti-(Tamm–Horsfall glycoprotein) antibody which had been pre-absorbed with excess Tamm–Horsfall glycoprotein.

Results

Use of the immunofluorescence method indicated that, in two of the sublines of cells which were used (BHK-21/C13/3P and BHK-21/C13/2P), a proportion of the cells contained material cross-reactive with antibody raised against urinary Tamm–Horsfall glycoprotein (Fig. 1). The observations were similar to those reported previously (Bloomfield et al., 1977).

Some of the cells, after use of the horseradish peroxidase technique using the optical microscope (Fig. 2), showed a strong cytoplasmic reaction, but none in the nuclei. These findings by the two different methods are consistent in two respects, namely that only a relatively small proportion (5–10%) of the total cells was reactive, and the immunoreactive material was not associated with the nuclei.

The immunoelectron-microscopic method showed unequivocally that the protein was associated with the plasma membranes of the reactive cells, as demonstrated by the deposition of a dark reaction product (Figs. 3–5). Once again, only a small proportion of the total cells was reactive, with strongly stained plasma membranes clearly evident.

Fig. 4 shows that the reaction product was absent from part of the membranes where the two cells are in close apposition and, although

Fig. 4. Medium-power (magnification × 5500) electron micrograph of one complete cell and part of another (BHK-21/C13/3P) Note the strong reaction associated with the plasma membranes, except at one place where the cells are in close apposition. Immunoperoxidase method.
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Fig. 5. High-power (magnification x 12600) electron micrograph of a part of a cell (BHK-21/C13/3P) showing Tamm–Horsfall-glycoprotein-like immunoreactivity associated with the plasma membrane and microvilli

The two elliptical profiles within the cytoplasm are either vesicles or invaginations of the plasma membrane. Immunoperoxidase method.

relatively uncommon, this was not an isolated example. In all instances it seems probable that the paired cells are the product of a recent cell division. Control preparations were in all cases negative, thus demonstrating the specificity of the immunofluorescence and immunoperoxidase reactions. The cell line BHK-21/ICRF yielded negative results by all the techniques used.

Discussion

The status of cells derived from a variety of organ explants, including kidney, and maintained by subculture over long periods has been a matter of considerable controversy. A decade or so ago it was widely held that in such cultures all the cells, or at least a very high proportion of them, were fibroblasts or endothelial cells or both (Franks & Wilson, 1970; Franks, 1972). However, subsequent evidence suggests that the dominant cell in the dog kidney cell line, MDCK, has epithelial-cell properties. This conclusion was reached from the results of electrophysicochemical (Richardson et al., 1981), biochemical, hormonal (Ishizuka et al., 1978; Horster, 1980; Saier, 1981) and morphological (Rindler et al., 1979) studies. McRoberts et al. (1981) concluded that ‘MDCK cell monolayers are morphologically, functionally and biochemically similar to epithelia from the distal tubule of the kidney’. Although the morphological evidence for the last part of the statement does not seem totally compelling, there seems little doubt that MDCK cells are derived from some region, or regions, of the renal-tubular epithelium.

Although BHK-21/C13 cell lines have not been studied so intensively in this respect as have MDCK cells, there seems to be no reason a priori why they should not also contain cells of epithelial origin. This possibility is strengthened both by the earlier evidence from immunofluorescence studies (Bloomfield et al., 1977) and by the present observations, which demonstrate a protein clearly related to, if not identical with, Tamm–Horsfall glycoprotein as being associated with a proportion

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of BHK-21/3P and BHK-21/2P cells. But other explanations are possible.

The results obtained by immuno-electron-microscopy show that the protein is plasma-membrane-associated just as it is in the cells of the thick ascending limb of the loop of Henle and of the early distal convoluted tubule of the hamster (Sikri et al., 1979), rat (Sikri et al., 1982) and human (Sikri et al., 1981b). The complete lack of reaction in BHK-21/ICRF cells remains unexplained.

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References


