A Fischer rat substrain deficient in dipeptidyl peptidase IV activity makes normal steady-state RNA levels and an altered protein

Use as a liver-cell transplantation model

Nancy L. THOMPSON,*§ Douglas C. HIXSON,* Helen CALLANAN,* Marilyn PANZICA,* Donna FLANAGAN,* Ronald A. FARIS,* Wanjin HONG,†‖ Sabine HARTEL-SCHENK‡¶ and Darrell DOYLE†

*Department of Medical Oncology, Rhode Island Hospital/Brown University, Providence, RI 02903, U.S.A., †Department of Biological Sciences, State University of New York at Buffalo, Buffalo, NY 14260, U.S.A., and ‡Department of Molecular Biology and Biochemistry, Freie Universität Berlin, D-1000 Berlin 33, Germany

Dipeptidyl peptidase IV (DPPIV) is a serine exoproteinase expressed at high levels in epithelial cells of kidney, liver and small intestine. Recently Watanabe, Kohima & Fujimoto [(1987) Experientia **43**, 400–401] and Gossrau *et al.* [(1990) Histochem. J. **22**, 172–173] reported that Fischer 344 rats are deficient in this enzyme. We have examined DPPIV expression in Fischer 344 rats available from U.S. and German suppliers and find that livers of the U.S. Fischer rats, in contrast with their German counterparts, express active DPPIV (D+). Northern analysis of liver RNA showed comparable levels of 3.4 kb and 5.6 kb DPPIV transcripts in both D+ rats from the U.S. and German (D−) rats. Monoclonal antibody (MAb) 236.3 to DPPIV immunoprecipitated a 150 kDa enzymically active (105 kDa, denatured) protein from surface-labelled D+ hepatocytes and reacted with canalicular and sinusoidal membranes (as shown by immunofluorescence microscopy). MAb 236.3 failed to immunoprecipitate a labelled peptide from D− cell extract or to stain D− liver sections. Polyclonal antibody (PAb) specific for DPPIV immunoprecipitated an enzymically active peptide from D+ hepatocyte extracts and a smaller, inactive peptide from D− hepatocyte extracts. Peptide maps of DPPIV immunoprecipitated from D+ extracts with MAb 236.3 and PAb were identical, but differed from that of the D− hepatocyte component recognized by PAb. The molecular basis of the DPPIV deficiency in the D− rats thus appears to be the translation of an enzymically inactive protein missing the epitope recognized by MAb 236.3. We have exploited these D− rats as hosts for syngeneic transplantation of liver cells from D+ Fischer rats. DPPIV expression is stable in the transplanted cells and allows them to be readily distinguished from the surrounding D− tissue.

INTRODUCTION

Dipeptidyl peptidase IV (DPPIV) (EC 3.4.14.5) is a serine exoproteinase which cleaves dipeptides *in vitro* from the *N*-terminus of oligopeptides with a penultimate proline or alanine residue (Hopsu-Havu & Glenner, 1966). Physiologically reactive substrates *in vivo* are not known, but potential substrates identified by sequence include substance P, kentsin and interleukin 2 (Hartel *et al.*, 1988*a*). The localization of DPPIV in the extracellular matrix of liver as well as on the hepatocyte cell surface (Walborg *et al.*, 1985), and its reported binding to collagen (Bauvois, 1988; Hanski *et al.*, 1988) and fibronectin (Piazza *et al.*, 1989), have suggested that one of its functions *in vivo* may be in cell–matrix interactions. However, high tissue concentrations of DPPIV are found in kidney and small-intestine brush-border membranes, hepatocyte bile-canalicular membranes and on other cell types (Hopsu-Havu & Ekfors, 1969; Gossrau, 1981) that are not in contact with extracellular matrix, suggesting that additional functions exist for this enzyme.

Watanabe *et al.* (1987) reported that the activity of membrane-bound DPPIV was markedly reduced in kidney membranes from Fischer 344 rats compared with Wistar strain rats, whereas the other peptidase activities examined were equivalent between the strains. Tiruppathi *et al.* (1990*a*,*b*) recently showed that a deficiency of renal DPPIV is characteristic of Fischer rats from Japan, but not those obtained from three different U.S. suppliers. Moreover, Gossrau *et al.* (1990) demonstrated that tissues from Fischer 344 rats obtained in Germany also lack histochemically detectable DPPIV and fail to react in immunohistochemical assays with either polyclonal antisera or monoclonal antibodies to DPPIV. We have examined the expression of DPPIV in livers of Fischer 344 rats obtained from both U.S. and German suppliers to determine the molecular basis for the enzyme deficiency and have transplanted cells from the DPPIV-positive (D+) to DPPIV-negative (D−) rats to determine the stability of the phenotype and its utility as a syngeneic transplantation model.

EXPERIMENTAL

Animals and procurement of cells and tissues

Fischer 344 adult male rats were obtained in the U.S.A. from Charles River, Wilmington, DE, U.S.A. German Fischer 344 adult male and female rats were obtained from Charles River Wiga, München, Germany. All animals were maintained on normal rat chow *ad libitum* under standard conditions. All

Abbreviations used: DPPIV, dipeptidyl peptidase IV; MAb, monoclonal antibody; PAb, polyclonal antibody; D+, Fischer 344 rats from U.S. suppliers with DPPIV enzymic activity; D−, Fischer 344 rats from German supplier deficient in DPPIV activity; AFC, amino-4-trifluoromethylcoumarin; MNA, 4-methoxy-β-naphthylamide; PBS, phosphate-buffered saline; poly(A)$^+$, polyadenylated; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RIP, radioimmunoprecipitation.

§ To whom correspondence should be addressed.
‖ Present address: Institute of Molecular and Cell Biology, National University of Singapore, Singapore 0511.
¶ Present address: Johns Hopkins University School of Medicine, Baltimore, MD 21205, U.S.A.

procedures involving animals were carried out in accordance with the policies and guidelines for proper care and humane treatment of research animals of the Rhode Island Hospital/Brown University Animal Care and Use Committee. In experiments comparing cells and tissues from the U.S. and German rats, care was taken to use male animals of approximately the same age. Hepatocytes were obtained by a modification (Hixson *et al.*, 1983) of the collagenase perfusion technique of Bonney *et al.* (1974). Viability of the isolated hepatocytes was greater than 80% by Trypan Blue dye exclusion. Tissues for RNA extraction and frozen sections were flash-frozen in liquid N_2 immediately after dissection and stored at −70 °C.

RNA extraction and Northern hybridization

Total RNA was prepared as described by Chirgwin *et al.* (1979). Polyadenylated [Poly(A)$^+$] RNA was prepared from total RNA by oligo(dT)–cellulose chromatography (Davis *et al.*, 1986). Aliquots [10 μg of total or 4 μg of poly(A)$^+$] of RNA were separated by agarose/formaldehyde-gel electrophoresis, transferred to Nytran (Schleicher and Schuell, Keene, NH, U.S.A.) filters and hybridized as described previously (Thompson *et al.*, 1989). The 2.0 kb endonuclease-*Eco*RI fragment of the λc2 cDNA clone of rat DPPIV (Hong & Doyle, 1987) and the 1.25 kb *Pst*I fragment of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Fort *et al.*, 1985) were labelled for use as probes with [^{32}P]dCTP (New England Nuclear, Boston, MA, U.S.A.; 3000 Ci/mmol) using a random primer labelling kit (Boehringer Mannheim Biochemicals, Indianapolis, IN, US.A.).

Antibodies and immunochemical protocols

Monoclonal antibody (MAb) 236.3 specific for rat DPPIV has been described (Hixson *et al.*, 1984; Walborg *et al.*, 1985). The IgG fraction of a rabbit antibody raised against purified rat liver DPPIV (Hartel *et al.*, 1987) was used for immunoprecipitation analysis.

Surface labelling of hepatocytes with ^{125}I (as Na^{125}I; Amersham, Arlington Heights, IL, U.S.A.; 500 mCi/ml) was performed by the lactoperoxidase/glucose oxidase procedure of Keski-Oja *et al.* (1977). Procedures for the immunoprecipitation of radiolabelled antigens using *Staphylococcus aureus* (IgGsorb; Enzyme Center, Boston, MA, U.S.A.) have been described (Hixson *et al.*, 1983). One-dimensional SDS/PAGE was performed as described by Laemmli (1970). One-dimensional peptide mapping was performed by digesting gel slices containing labelled bands with *S. aureus* V8 proteinase (Miles Scientific, Naperville, IL, U.S.A.) (Cleveland, 1983) as described previously (Hixson *et al.*, 1985). Apparent molecular masses were calculated from prestained protein standards (myosin, 200 kDa; β-galactosidase, 116 kDa; phosphorylase *b*, 97.4 kDa; BSA, 66.2 kDa; ovalbumin, 42.7 kDa; Diversified Biotech, Hyde Park, MA, U.S.A.) run concurrently with radioactively labelled samples.

Comparison of the reactivity of two different antisera was determined by immunodepletion analysis (Hixson *et al.*, 1985). Briefly, aliquots of ^{125}I-labelled extracts were sequentially immunoprecipitated with the first antibody until SDS/PAGE analysis indicated that all reactive components had been removed. The immunodepleted extracts were then immunoprecipitated with the second antibody and the immunoprecipitates analysed by SDS/PAGE. If the second antibody showed no reactivity with the depleted extract, this was interpreted as evidence that the first antibody recognized all components reactive with the second.

Immunoadsorption experiments were performed by incubating 5×10^6 hepatocytes in 0.5 ml of anti-DPPIV antisera for 2 h at 4 °C. Hepatocytes with adsorbed antibody were then recovered by centrifugation at 100 *g*, washed three times in phosphate-buffered saline (PBS; 140 mM-NaCl/4 mM-KCl/2 mM-KH_2PO_4/20 mM-Na_2HPO_4, pH 7.4) and finally resuspended in 0.5 ml of 0.1 M-citrate buffer, pH 2.2, for 10 min to elute adsorbed antibody. After neutralization to pH 7.4 with 1 M-Tris, the eluted antibodies were used for immunoprecipitation analysis.

Immunofluorescence was performed on frozen sections of rat liver, 4–6 μm thick, after fixation in cold (0 °C) acetone for 10 min. Incubation with MAb 236.3 to DPPIV was followed by fluorescein-conjugated affinity-purified goat anti-mouse IgG as previously described (Hixson *et al.*, 1983). Sections were examined on a Nikon Microphot-FX fluorescence microscope equipped with an epifluorescence illuminator.

Assay of DPPIV activity

DPPIV was identified after SDS/PAGE in unfixed gels by its enzymic activity using an overlay technique (Smith, 1984) in which cellulose acetate strips impregnated with the fluorogenic substrate Ala-Pro-amino-4-trifluoromethylcoumarin (Ala-Pro-AFC) (Enzyme Systems Products, Livermore, CA, U.S.A.) were placed over the gels, incubated 30 min at 37 °C, and then observed and photographed under u.v. light (Piazza *et al.*, 1989). DPPIV activity in acetone-fixed frozen liver sections was assayed by histochemical staining (Lojda *et al.*, 1979), using Gly-Pro-methoxy-β-naphthylamide (Gly-Pro-MNA) (Sigma, St. Louis, MO, U.S.A.) as substrate.

Transplantation studies

Freshly isolated U.S.-Fischer-rat hepatocytes were transplanted into either the liver or pancreas of German Fischer rats. Briefly, an abdominal incision was made under metophane anaesthesia to expose the liver or splenic portion of the pancreas and 5×10^6 donor hepatocytes (in 0.5 ml of Hanks balanced salt solution) were slowly injected beneath the capsule of each organ with a 1 ml syringe and a 22-gauge needle. Leakage of cells was minimized by applying a Gelfoam sponge (Upjohn, Kalamazoo, MI, U.S.A.) to the site of injection. After transplantation of cells, the incision was closed with silk suture and stainless-steel clips and the animals were monitored carefully during recovery. Recipient rats were killed by CO_2 asphyxiation at 7 and 17 days after surgery, and tissues were excised and rapidly frozen in hexane chilled by a solid-CO_2/acetone bath. Transplanted donor cells were detected in frozen tissue sections by indirect immunofluorescence or immunohistochemical assays for DPPIV.

RESULTS

Localization of DPPIV in Fischer-rat liver

DPPIV enzyme histochemistry on acetone-fixed frozen sections of liver from U.S.Fischer rats revealed dark-red reaction product abundant along the bile-canalicular membranes of adjacent hepatocytes, as well as weaker staining of the sinusoidal membranes and bile ducts (Fig. 1*a*). Indirect immunofluorescence microscopy with MAb 236.3 on duplicate sections of U.S.-Fischer-rat liver gave a staining pattern identical with the histochemical result (Fig. 1*c*). By contrast, no histochemical reaction product was detected in sections of liver from German Fischer rats (Fig. 1*b*), confirming the work of Gossrau *et al.* (1990). In addition, MAb 236.3 failed to react in indirect immunofluorescence microscopy with this tissue (Fig. 1*d*). Sections of German-Fischer-rat liver stained without fixation also gave a negative result (not shown), indicating that sensitivity of the protein to fixation was not responsible for the absence of staining and suggesting that either the D− German Fischer rat does not have the enzyme or it produces an inactive form of DPPIV that is not recognized by MAb 236.3.

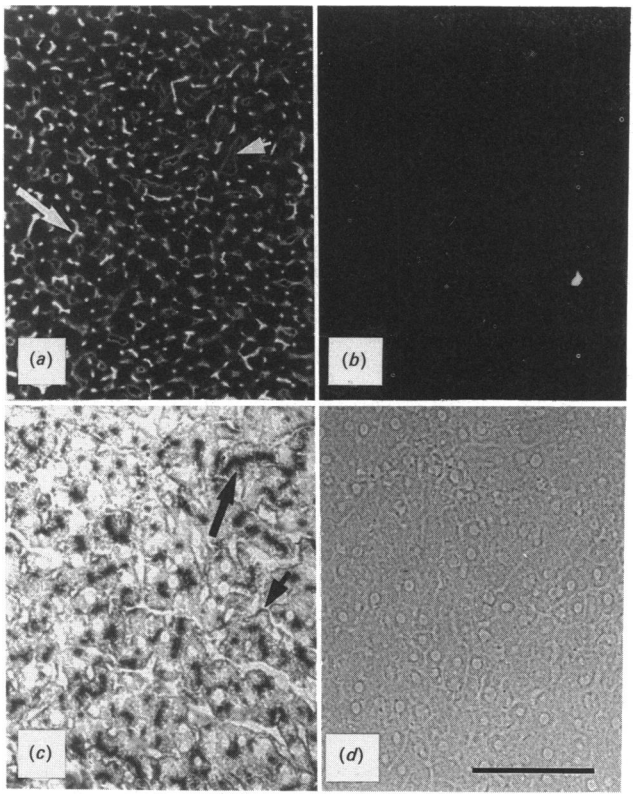

Fig. 1. Antibody and histochemical localization of DPPIV in livers of U.S. and German Fischer rats

(*a*) and (*b*) Indirect immunofluorescence microscopy of acetone-fixed frozen liver sections with MAb 236.3 localizes DPPIV to the hepatocyte bile-canalicular membrane (long arrow) with lighter sinusoidal membrane domain staining (short arrow) in the U.S. Fischer rat (*a*). No antibody reactivity is seen in the German Fischer rats (*b*). Magnification ×150. (*c*) and (*d*) DPPIV histochemical staining of acetone-fixed frozen liver sections shows abundant dipeptidase reaction product localized in the bile-canalicular region (long arrow) and slight reactivity along the sinusoids (short arrow) of the U.S. Fischer rats (*c*). No enzyme activity is seen in the German Fischer rats (*d*). Magnification ×160. The bar represents 100 μm.

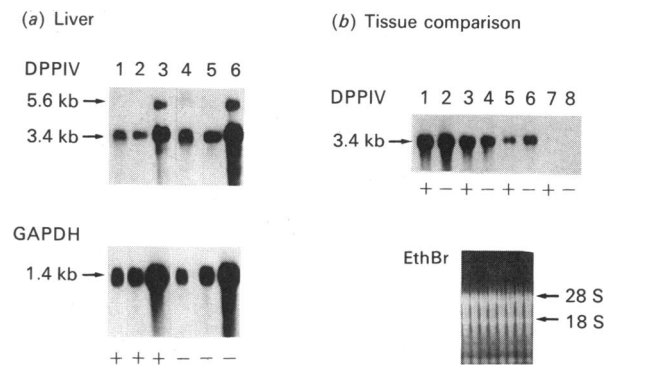

Fig. 2. Northern-blot analysis of DPPIV RNA expression in tissues from U.S. and German Fischer rats

A portion (10 μg) of total RNA [lanes 1, 2, 4 and 5 (*a*) and lanes 1–8 (*b*)] or 4 μg of polyadenylated RNA (pA+, lanes 3 and 6, *a*) was electrophoresed on agarose/formaldehyde gels containing ethidium bromide (EthBr), blotted to Nytran, and hybridized to ^{32}P-labelled cDNA probe for DPPIV. The blot shown in (*a*) (top) was stripped of DPPIV probe and rehybridized to GAPDH probe (bottom left). The blot shown in (*b*) was photographed under u.v. light (bottom right) before hybridization with DPPIV probe in order to reveal the RNA. The positions of the 28 S and 18 S ribosomal RNAs are indicated. RNAs extracted from tissues of U.S. Fischer rats are indicated by '+', whereas those from German Fischer rats are indicated by '−'. Tissue sources are as follows: (*a*) lanes 1–6, liver; (*b*) lanes 1 and 2, kidney; lanes 3 and 4, small intestine; lanes 5 and 6, liver; lanes 7 and 8 heart. (*a*) Samples 1 and 2 are duplicate RNA preparations from different D+ animals, whereas samples 4 and 5 are duplicate preparations from different D− animals.

DPPIV RNA expression

Northern-blot hybridization of total RNA isolated from livers of D+ and D− Fischer rats showed that the 3.4 kb DPPIV mRNA was expressed by both and that steady-state levels were approximately equal between them (Fig. 2*a*). Isolation of poly(A)$^+$ RNA from these livers demonstrated that the 3.4 kb species as well as a novel 5.6 kb RNA species were polyadenylated in both strains (Fig. 2*a*), suggesting a non-transcriptional mechanism for the loss of DPPIV activity in the D− strain. Hybridization of the same blots to probe for the glycolytic enzyme GAPDH was used to standardize DPPIV expression by densitometry ratios of the resulting autoradiographs, since the former 'housekeeping' gene should be expressed at equivalent levels in both strains. When this calculation was made, steady-state levels of DPPIV mRNA in the D− livers were found to be 1.1–1.5 times those of D+ livers. In addition to liver, DPPIV RNA levels were compared in several other tissues from these rats (Fig. 2*b*). Confirming previous observations in Sprague–Dawley-rat tissues (Hong *et al.*, 1989*a*, *b*), the highest levels of the 3.4 kb transcript were found in kidney, followed by small intestine and liver, with only barely detectable levels found in heart (Fig. 2*b*). The 5.6 kb RNA was also apparent in long autoradiographic exposures of total RNA, particularly from kidney (result not shown).

Radioimmunoprecipitation of DPPIV from surface-labelled hepatocytes and *S. aureus* V8-proteinase peptide maps

To examine further the expression of DPPIV on D+ and D− hepatocytes, isolated viable cells from both substrains were surface labelled with ^{125}I and used to prepare detergent extracts for immunoprecipitation analysis with monoclonal and polyclonal (PAb) anti-DPPIV antibodies. As shown in Fig. 3*a*, both MAb 236.3 and PAb immunoprecipitated a 105 kDa band from extracts of ^{125}I-labelled D+ hepatocytes (Fig. 3*a*, lanes 1 and 2), but PAb showed no reactivity when extracts were first immunodepleted with MAb 236.3 (lane 3), thus confirming the identity of the component recognized by PAb as DPPIV. If immunoprecipitates from D+ hepatocyte extracts were separated by PAGE without boiling, primarily the 150 kDa monomeric from of DPPIV was seen (lane 4). As expected from its lack of reactivity on frozen sections of D− liver, MAb 236.3 failed to immunoprecipitate any labelled component from extracts of D− hepatocytes (lane 5).

To determine if D− rats expressed an enzymically inactive form of DPPIV, a two-step analysis was used. The first step involved determining whether D− hepatocytes were able to bind specifically anti-DPPIV antibodies from anti-DPPIV antiserum which could immunoprecipitate enzymically active DPPIV from detergent extracts of surface-labelled D+ hepatocytes. As shown in Fig. 3(*b*), antibodies eluted from the surface of both D+ (lanes 1 and 5) and D− (lanes 2 and 6) hepatocytes were capable of immunoprecipitating a component identical in size with DPPIV immunoprecipitated with MAb 236.3 (lanes 3 and 4). When the immunoprecipitates were boiled before electrophoresis, the characteristic 105 kDa denatured band was seen (Fig. 3*b*, lanes 1–3), whereas primarily the 150 kDa monomer was seen in

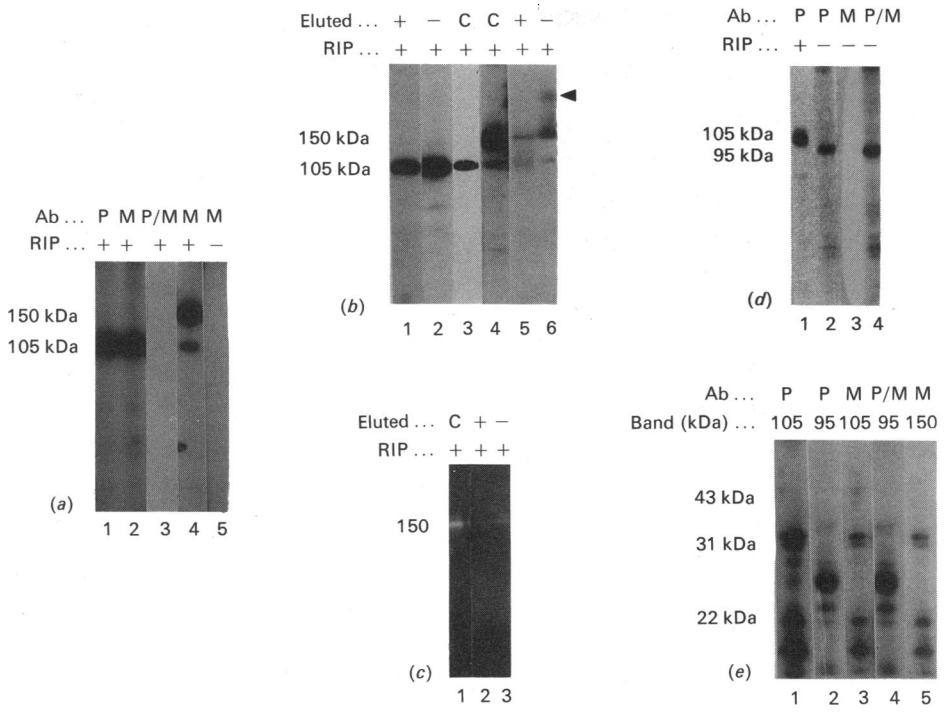

Fig. 3. Radioimmunoprecipitation (RIP) analysis of DPPIV

(*a*) Immunoprecipitation of DPPIV by monoclonal and polyclonal antibodies from extracts of surface-labelled hepatocytes. Hepatocytes were isolated by collagenase perfusion, surface-labelled with ^{125}I and used to prepare detergent extracts for RIP analysis with either polyclonal (P) or monoclonal (M) antibody specific for DPPIV. Both antibodies immunoprecipitate a 105 kDa band from surface-labelled U.S.-Fischer (+)-rat hepatocytes when samples are boiled before electrophoresis (lanes 1 and 2). No DPPIV could be immunoprecipitated with PAb from D+ extracts immunodepleted by MAb (P/M) (lane 3). Unboiled samples show primarily the 150 kDa monomeric form of DPPIV (lane 4). MAb fails to immunoprecipitate a band from extracts of German-Fischer (−)-rat hepatocytes (lane 5). (*b*) RIP of DPPIV from extracts of U.S.-Fischer-rat hepatocytes by PAbs bound by, and eluted from, the surface of U.S.- and German-Fischer-rat hepatocytes. Hepatocytes (5×10^6) were incubated with 0.5 ml of PAb for 2 h at 4 °C, washed three times in PBS, and bound antibody was eluted in 0.1 M-citrate, pH 2.2. Eluted antibody was used for immunoprecipitation of DPPIV from extracts of U.S.-Fischer-rat hepatocytes. Lanes 1 and 5, RIP with antibody eluted from U.S.-(+)-Fischer-rat hepatocytes; lanes 2 and 6, RIP with antibody eluted from German-(−)-Fischer-rat hepatocytes. Lanes 3 and 4, control (C) RIPs with MAb 236.3. Samples in lanes 1–3 were boiled, where those in lanes 4–6 were unboiled. The arrow denotes the position of approx. 300 kDa DPPIV dimer visible in lane 6. (*c*) Dipeptidase activity of immunoprecipitates by substrate/gel-overlay technique. Lanes 4–6 (unboiled RIPs) of the gel shown autoradiographed in (*b*) were overlaid with a sheet of cellulose acetate impregnated with the DPPIV fluorogenic substrate Ala-Pro-AFC. Enzyme activity is shown by the white bands visible on the cellulose acetate under u.v. light corresponding to the 150 kDa components immunoprecipitated from hepatocyte extracts. Lane 1, control RIP with MAb 236.3; lane 2, RIP with PAb eluted from U.S.-(+)-Fischer-rat hepatocytes; lane 3, RIP and PAb eluted from German-Fischer-(−)-rat hepatocytes. (*d*) RIP of a 95 kDa band from extracts of German-(−)-Fischer-rat hepatocytes by PAb. In contrast with the 105 kDa band immunoprecipitated from extracts of U.S.-(+)-Fischer-rat hepatocytes (lane 1), PAb immunoprecipitated a 95 kDa band from extract of German-(−)-Fischer-rat hepatocytes (lane 2). MAb failed to immunoprecipitate a labelled band from D− extracts (lane 3), and D− extracts immunodepleted with MAb still contained the 95 kDa component (lane 4). (*e*) V8-proteinase maps of immunoprecipitated DPPIV and 95 kDa component. Bands immunoprecipitated by DPPIV antibody in (*b*) and (*d*) were sliced from dried gels, digested with V8 proteinase, and separated by electrophoresis on 12.5% gels, which were then dried and autoradiographed to produce the peptide maps shown. Lanes 1, 2 and 4 are peptide maps of the labelled bands immunoprecipitated with PAb in lanes 1, 2 and 4 respectively of (*d*). Lanes 3 and 5 correspond to V8 digestions of the 105 kDa and 150 kDa bands of DPPIV immunoprecipitated with MAb 236.3 in (*b*), lanes 3 and 4 respectively. The position of molecular-mass markers are shown on the left.

unboiled immunoprecipitates (Fig. 3*b*, lanes 4–6), with some higher-molecular-mass material, probably representing dimer, observed as well (lane 6, arrowhead). The identity of these 150 kDa bands as DPPIV was confirmed by substrate overlay of the gel containing the unboiled samples (Fig. 3*c*). DPPIV activity was associated with the 150 kDa bands immunoprecipitated by the bound and eluted PAb (Fig. 3*c*, lanes 2 and 3) as well as the MAb 236.3 control (Fig. 3*c*, lane 1). Having thus established the presence of a cross-reacting component on D− hepatocytes, direct immunoprecipitation analysis of ^{125}I-labelled D− hepatocyte extracts with PAb was done (Fig. 3*d*). In contrast to the 105 kDa band immunoprecipitated from D+ hepatocyte extracts (Fig. 3*d*, lane 1), PAb immunoprecipitated a 95 kDa component from D− hepatocytes (Fig. 3*d*, lane 2). This component was not removed by prior depletion with MAb 236.3 (Fig. 3*d*, lanes 3 and 4), indicating that the reactive epitope was either not present or not exposed on the 95 kDa protein.

To explore further the relationship of the 95 kDa band to the 105 and 150 kDa forms of DPPIV, *S. aureus*-V8-proteinase peptide maps were generated from each labelled band and compared (Fig. 3*e*). DPPIV immunoprecipitated from U.S. Fischer rats by both the polyclonal (Fig. 3*e*, lane 1) and monoclonal (Fig. 3*e*, lanes 3 and 5) antibodies gave identical peptide maps no matter whether boiled (Fig. 3*e*, lanes 1 and 3) or unboiled (Fig. 3*e*, lane 5) immunoprecipitates were used. However, the V8 peptide map obtained for the 95 kDa component from the D− hepatocytes (Fig. 3*e*, lanes 2 and 4) differed in that it lacked a major labelled peptide of approx. 17 kDa, did not display labelled peptides larger than approx. 32 kDa, and contained a doublet of labelled bands at about 32 kDa, somewhat

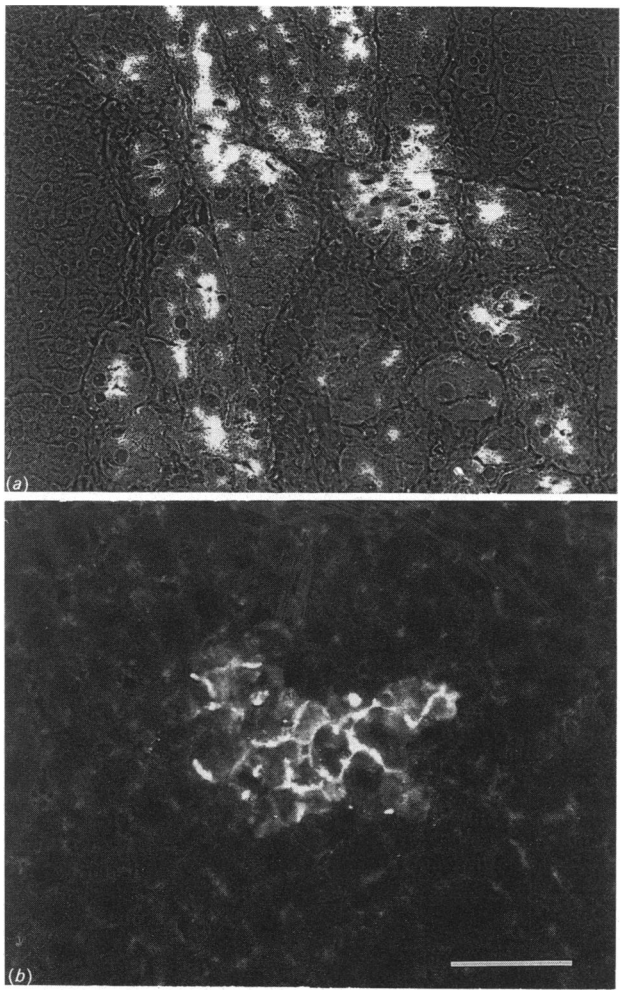

Fig. 4. Transplantation system

(*a*) Colonies of U.S.-Fischer-rat hepatocytes detected by DPPIV histochemical staining 17 days after transplantation to the pancreas of a German Fischer rat. Histochemical staining of acetone-fixed frozen sections shows that the transplanted U.S.-Fischer-rat hepatocytes visible as colonies in the pancreas are positive for DPPIV activity (white histochemical reaction product), whereas the surrounding pancreatic tissue of the German Fischer rat is negative. Phase contrast; magnification × 185. (*b*) Immunofluorescent localization of U.S.-Fischer-rat hepatocytes 7 days after transplantation to the liver of a German Fischer rat. Indirect immunofluorescence on acetone-fixed frozen sections with MAb 236.3 reveals a colony of positive-staining cells with the characteristic canalicular DPPIV localization visible against the surrounding negative tissue. Magnification × 320. The bar represents 50 μm.

larger than a similar doublet seen in V8 maps of DPPIV from D+ hepatocytes.

Transplantation system

The availability of (1) syngeneic rat substrains with and without active DPPIV and (2) histochemical and immunocytochemical assays to detect DPPIV suggested that these rats could be employed as a transplantation system to examine the stability of DPPIV expression as well as the fate of the small number of D+ normal or preneoplastic cells transplanted to D− hosts. To investigate this possibility, hepatocytes isolated from D+ rats were transplanted beneath the capsule of liver and pancreas of D− rats. Colonies of DPPIV-histochemically-positive hepatocytes were found in the vicinity of the injection site in the pancreas of a German Fischer rat 17 days after transplantation (Fig. 4*a*), indicating that DPPIV expression was stable in the transplanted cells. Similarly, the D− phenotype of the host appeared to be stable, since there was no indication of enzymically active DPPIV in the surrounding tissue. No infiltration of lymphoid cells was seen in the area, suggesting that an immune response was not raised against the transplanted cells. Hepatocytes from the D+ rat could also be detected after transplantation of the liver of D− hosts by immunofluorescent staining with MAb 236.3 (Fig. 4*b*). Colonies of cells positive for this antibody and with characteristic canalicular staining were clearly distinguishable in livers of host animals 1 week after transplantation.

DISCUSSION

We have investigated the molecular basis for the lack of DPPIV activity in livers of German Fischer rats. As reported recently (Tiruppathi *et al.*, 1990*a*, *b*), renal membranes of Fischer 344 rats from Japanese suppliers are deficient in DPPIV activity, whereas renal membranes from Fischer rats available from U.S. suppliers possess normal levels of this enzyme. Similarly, Gossrau *et al.* (1990) found that kidney, as well as other tissues of Fischer rats from Germany, also lack this enzyme, as demonstrated by histochemical and antibody reactivity, whereas levels of other membrane-bound enzymes examined were normal. We have confirmed and extended these findings to show that, despite the lack of functional enzyme, German Fischer rats have comparable steady-state levels of the 3.4 kb DPPIV transcript found in U.S. Fischer rats, as well as a previously unreported 5.6 kb RNA species hybridizing with DPPIV cDNA. The presence of the 5.6 kb transcript for DPPIV was not reported by Hong *et al.* (1989*a*,*b*), who originally cloned DPPIV as gp110 from a rat kidney expression library, most likely because the RNA preparations used for Northern hybridizations in those studies were total RNA and, in our experience, the larger transcript is much less abundant in total RNA as against poly(A)$^+$-selected RNA. The relationship between the two transcripts is not presently known. DPPIV has been reported to be a single-copy gene (Hong *et al.*, 1989*a*). Independent cDNA clones have been obtained from a rat liver library by Ogata *et al.* (1989), which vary from the kidney clones obtained by Hong *et al.* (1989*a*,*b*) in five amino acid residues in the coding region and which, owing to a frameshift caused by one of these residues, limits the open reading frame from 792 residues in gp110 to 767 in DPPIV. In addition, Ogata *et al.* (1989) reported the presence of more than one poly(A) signal, which may imply polymorphism in the 3′ non-coding region of the mRNA. Alternatively, the mRNAs may initiate at distinct promoters on a single gene, as was recently found for another rat plasma-membrane ectoenzyme, γ-glutamyl transpeptidase (Chobert *et al.*, 1990).

Lack of active DPPIV in hepatocyte membranes was demonstrated by histochemical assay in frozen sections of German-Fischer-rat liver using specific substrate as well as by immunoprecipitation with antibodies to DPPIV and substrate-overlay assay of the undenatured immunoprecipitated material separated by SDS/PAGE. We found that surface-labelled hepatic membranes from German Fischer rats lack the 105 kDa band, corresponding to DPPIV monomer, that is found in membranes of U.S. Fischer rats. Tirrupathi *et al.* (1990*a*) reported similar findings for renal membrane proteins of Japanese Fischer rats separated by SDS/PAGE. All other membrane proteins compared between hepatocytes of the German and U.S. Fischer rats were present in equal abundance, indicating that the DPPIV deficiency is not due to a generalized aberrant processing and transport of cell-surface glycoproteins. Deficiencies of another proteinase associated with kidney brush-border membranes, merpin, as well as the production of an immunologically related

altered protein, have been previously demonstrated in some inbred mouse strains (McKay *et al.*, 1985).

The 95 kDa band immunoprecipitable from extracts of surface-labelled D− hepatocytes by polyclonal antibody may represent an inactive truncated form of the enzyme. This band is not seen on autoradiographs of immunoprecipitates from D+ extracts and thus cannot be attributed to another cross-reactive surface molecule. A DPPIV-immunoreactive species on the surface of D− hepatocytes is also indicated by the ability of these cells to bind antibodies which can then specifically immunoprecipitate enzymically active DPPIV from D+ hepatocyte extracts. The nature of this molecule is presently not clear. V8 peptide maps of the 95 kDa band show that it clearly differs from DPPIV immunoprecipitated from D+ hepatocytes. MAb 236.3 is not directed against the active site of the enzyme, since it does not decrease DPPIV activity in a quantitative assay (G. Piazza & D. Hixson, unpublished work), suggesting that loss of this epitope alone is not responsible for lack of enzymic activity.

Although two forms of DPPIV have been purified from rat liver plasma membranes, a detergent-soluble membrane form and a soluble form released by papain which is approx. 4 kDa smaller, these forms are identical, except that the *N*-terminal 35 amino acids, including the signal peptide which anchors the enzyme in the plasma membrane, are missing from the smaller, soluble, form (Ogata *et al.*, 1989). A smaller, soluble, form has also been observed to account for much of the DPPIV activity in rat hepatoma cells (Hartel *et al.*, 1988*b*), but is not likely to correspond to the 95 kDa band we observe in D− extracts.

The availability of rats deficient in DPPIV has permitted functional studies of the role of this enzyme in various physiological and pathological processes, such as peptide hydrolysis and transport in renal brush-border membranes (Tiruppathi *et al.*, 1990*a*,*b*) and in passive Heymann nephritis (Natori *et al.*, 1989). We have chosen to exploit the D− rats as a syngeneic host for the transplantation of small numbers of hepatic cells from D+ animals in order to study their colonization and differentiation potential. Such a system obviates the need to manipulate the transplanted cells by introducing markers like recombinant DNA expression vectors or to use anti-allogeneic sera and F_1 animals in order to identify the transplanted cells in the same kind of tissue. It also overcomes the problem of immunological rejection or the use of nude mice. Since DPPIV is expressed normally in a variety of cell types, including hepatocytes and ductular cells of liver, and its expression begins at a defined stage of development (Hong *et al.*, 1989*a*, *b*), this marker will allow identification of transplanted fetal as well as adult liver cells. We have determined that the expression of DPPIV is stable in transplanted hepatocytes and that they may be identified both by enzymic activity and by immunofluorescence. Further studies will need to examine the developmental potential of cells isolated from carcinogen-treated animals as well as putative stem cells in this system.

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