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A monoclonal antibody detecting dipeptidylpeptidase IV in human tissue*

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Summary. Dipeptidylpeptidase IV (DPP IV) occurs among others in exocrine epithelia, hepatocytes, renal tubuli, endothelia, and myofibroblasts of man and laboratory animals. Also Tμ lymphocytes and their varying differentiated neoplastic counterparts reveal this enzyme activity. The present paper describes a new monoclonal antibody recognizing DPP IV.

Additional efforts have been taken to detect the subcellular localization of DPP IV and its isoelectric focusing pattern in different tissue types. The monoclonal antibody anti-DPP IV (clone II-19) shows a reaction pattern indistinguishable from the corresponding enzymehistochemical reaction. These findings were further substantiated by immunoblotting analysis. In line with the results of direct enzyme measurements in different subcellular fractions a considerable portion of the enzyme is localized in the membrane fraction.

Key words: Dipeptidylpeptidase IV – Monoclonal antibody – Enzyme histochemistry

Introduction

The peptidase cleaving glycyl-proline-β-naphthylamide into glycyl-proline and naphthylamine is a well studied enzyme, generally referred to as dipeptidylpeptidase IV (DPP IV) (Hopsu-Havu and Glenner 1966; McDonald et al. 1971). Its occurrence in exocrine glands, hepatocytes, renal tubules, and endothelial cells has been extensively studied, mainly in laboratory animals (Gossrau 1979; Lojda 1979; Fukasawa et al. 1981; Sahara et al. 1981). In

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man DPP IV also covers the surface of fibroblasts (Saison et al. 1983), furthermore, the myofibroblasts occurring in connective tissue and in the stroma of placental villi have been shown to contain a considerable level of enzyme activity, as have the other cell types (Feller et al. 1985).

In the human haematopoietic system DPP IV occurs in T-lymphocytes (Lojda 1977; Lojda 1981) later shown to be T-lymphocytes bearing Fc-receptors for IgM (Tμ lymphocytes) (Feller et al. 1982). In functional terms this subgroup encompasses helper-T-cells (Moretta et al. 1977). In these lymphocytes DPP IV is predominantly located on the cell surface (Mentlein et al. 1984).

In studies using polyclonal antisera, raised against DPP IV it seemed probable that the enzyme activity encountered in different tissue sources was generated by an identical protein or closely related enzyme variant (Fukasawa et al. 1981; Sahara et al. 1981). To extent the technical possibility of studying DPP IV we report here on the production of a monoclonal antibody raised with purified DPP IV obtained from human placenta. The characteristic reaction of this antibody with different cell types was further related to the results of isoelectric focusing and immunoblotting analysis.

Material and methods

Snap frozen tissue samples of different human organs were studied. In addition biopsies of full term human placenta ($n=3$), T-lymphoblastic lymphomas and leukaemias ($n=23$) and cytospin preparations from separated human T-lymphocytes (Böyum 1968; Weiner et al. 1973) were utilized for enzymehistochemical and immunohistochemical techniques. For isoelectric focusing samples of 5×10^7 T-lymphocytes or 15 g of the different tissue types were homogenized, treated with Triton X-100 (0.5%/v/v) for 30 min and centrifuged at 100,000 *g* for 30 min. The cell and partical free supernatant was subjected to direct enzyme measurement (Grabske et al. 1979).

The enzymehistochemical reaction for DPP IV was performed according to Lojda (1977). As substrate glycyl-proline-4-methoxy-β-naphthylamide was used (Bachem, Bubendorf, Switzerland) and as coupler Fast Blue B (Sigma, München, FRG). Sections were incubated for 10–30 min at room temperature.

The immunohistochemical reactions were performed by an immunoperoxidase and immunoalkaline phosphatase method (Stein et al. 1982; Feller and Parwaresch 1983).

Isolelectric focusing followed the technique of Vesterberg (1972) using "Multifor 2117" (LKB, Bromma, Sweden). The pH-gradient in the polyacrylamide gel was set up using a mixture of ampholine pH 2.4–4 (1.6 ml), pH 4–6 (0.4 ml), pH 5–7 (1.6 ml). 40 μl samples with a total DPP IV activity of 15 mU were used. The focusing was run for 4 h at 4° C with an initial voltage of 400 V and a final voltage of 1,300 V.

For the immunoblot a transfer from agarose gel to a nitrocellulose membrane (BA-85; Schleicher and Schüll, Dassel, FGR) was performed. The nitrocellulose prints were stained with Coomassie blue, and enzymehistochemically with glycyl-proline-4-methoxy-β-naphthylamide. Furthermore, one print was incubated with the monoclonal antibody II-19 for 12 h at room temperature at a dilution of 1:100 in PBS. The print is further incubated with alkaline phosphatase conjugated anti-mouse IgG + IgM and development with 5-bromo-4-chloro-3-indolyl phosphate of pH 9.5 for 20 min at 37° C. The negative control was developed in the same manner without incubating with primary monoclonal antibody II-19 (Peltre et al. 1982; Leary et al. 1983).

For the isolation of subcellular fractions lymphocytes were suspended and centrifuged in a mixture of 0.13 M Hepes, 0.9% NaCl, and 0.5 mM $MgCl_2$ and subsequently washed in 0.5 mM sucrose ($400 \times g$, 10 min, 4° C). Cells were suspended in Hepes-Sucrose (1:3/v/v).

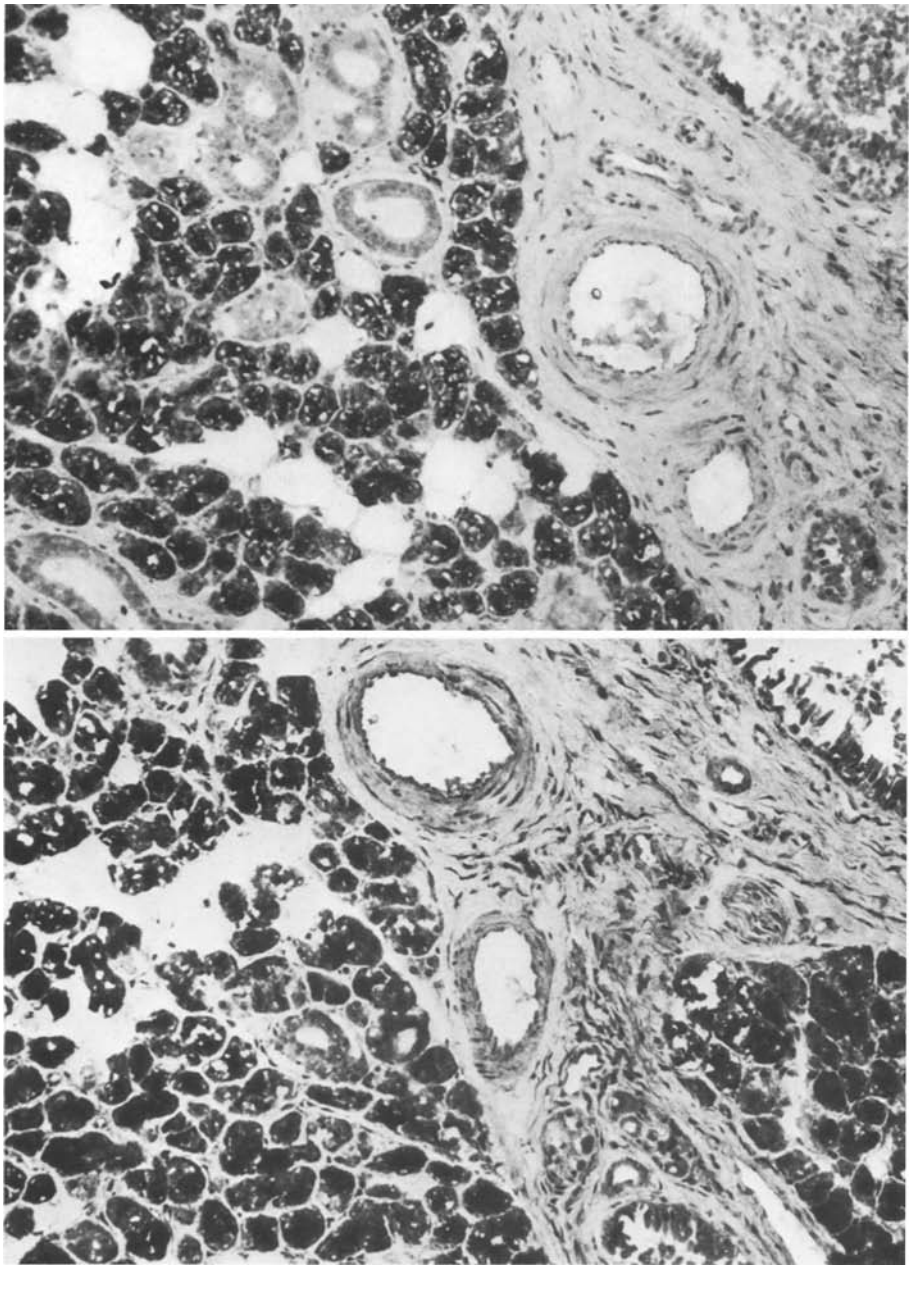


Fig. 1 a, b. Histological distribution pattern of DPP IV in human salivary gland. Enzyme activity is confined to acinar cells and intercalated ducts. **a** Enzymehistochemical reaction. **b** Immunohistochemical reaction with monoclonal anti-DPP IV-antibody (II-19). Nuclear counterstaining, $\times 150$

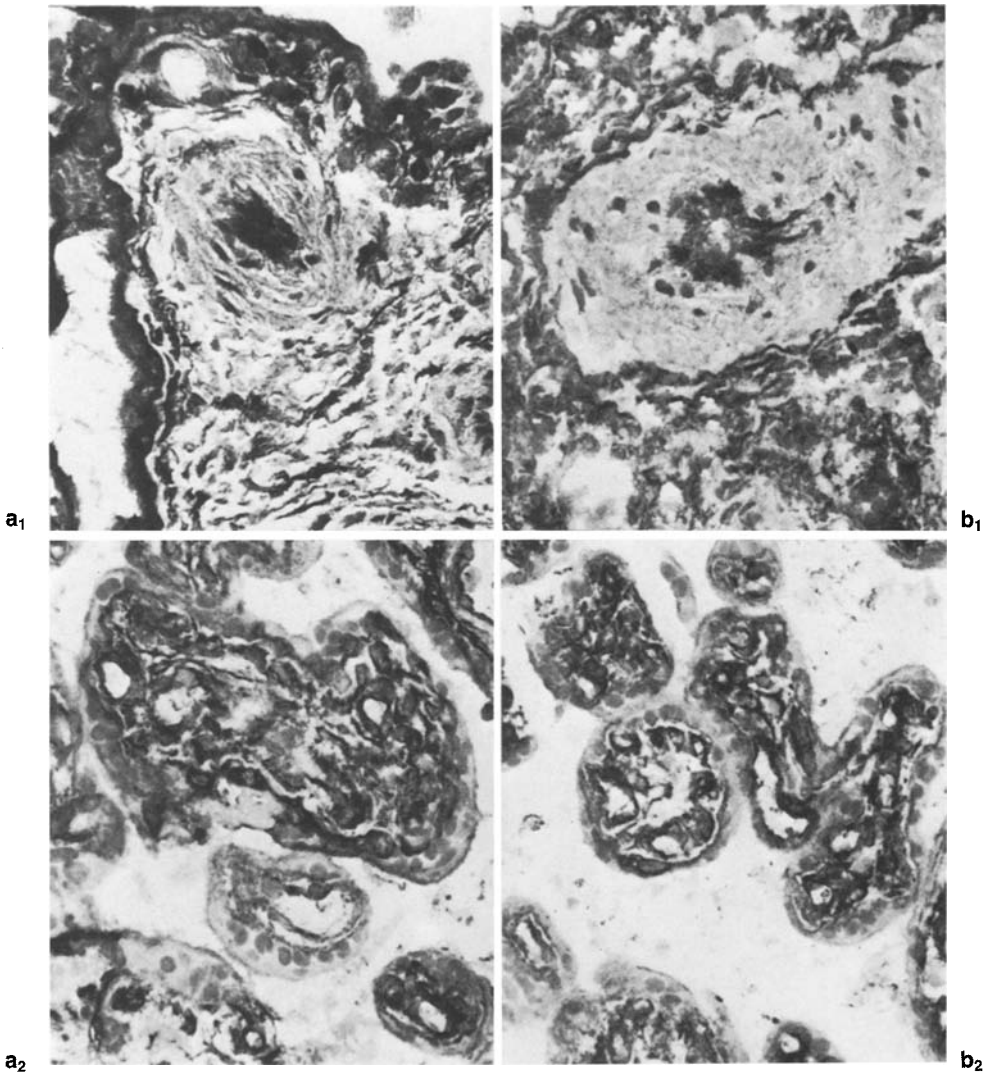


Fig. 2 $\mathbf{a}_{1+2}$, $\mathbf{b}_{1+2}$. Histological distribution pattern of DPP IV in human placenta. Enzyme activity is visiable in endothelia cells of blood vessels ($\mathbf{a}_1/\mathbf{b}_1$) and to myofibroblasts in the viluos stroma ($\mathbf{a}_2/\mathbf{b}_2$). **a** Enzymehistochemical reaction. **b** Immunohistochemical reaction with monoclonal anti-DPP IV-antibody (II-19). Nuclear counterstaining. × 340

Using the method of Ferber et al. (1972) cells were disrupted in a nitrogen cavitation bomb by incubation at 50 atm for 30 min. The cell suspension was homogenized and differential centrifugation was performed (nuclei: $450 \times g$; lysosomes and mitochondria: $20{,}000 \times g$; small membrane fragments: $105{,}000 \times g$). From different subcellular fractions DPP IV was extracted with Trition X-100 as described above.

Monoclonal antibodies were generated according to Köhler and Milstein (1975). Balb/c mice were immunized with 0.5 mg of purified DPP IV from human placenta (Püschel et al. 1982) three times in intervalls of two weeks. Two weeks after the last dose, the animals were boostered with 0.25 mg (4th day) and 0.175 mg (3rd, 2nd, and 1st day before fusion). Culture supernatants were tested on cryostat sections of normal human tonsils and of human placenta using the immunoperoxidase method (Stein et al. 1982).

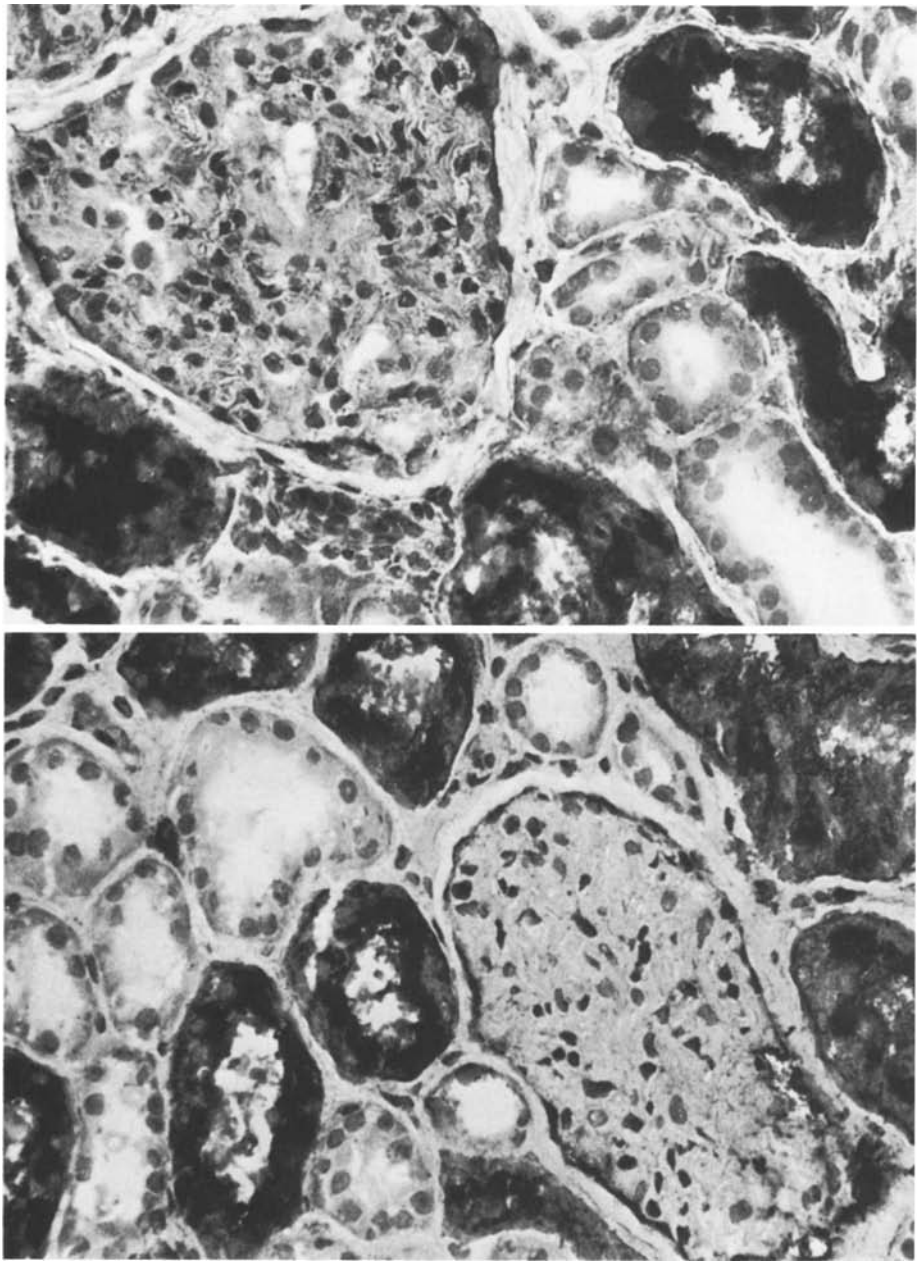


Fig. 3a, b. Histological distribution pattern of DPP IV in human kidney. Enzyme activity is confined to epithelia cells of proximal convoluted tubules and endothelia of glomerula whereas collecting tubules are DPP IV negative. **a** Enzymehistochemical reaction. **b** Immunohistochemical reaction with monoclonal anti-DPP IV-antibody (II-19). Nuclear counterstaining, × 340

Table 1. Reaction pattern of four monoclonal antibodies raised against purified placental DPP IV compared to enzymhistochemical staining pattern of DPP IV

	II-19	V-22	V-8	III-19	DPP IV
Myofibroblasts of placenta	++	+	(+)	−	++
Myofibroblasts of palmar fibromatosis	++	+	(+)	−	++
Endothelial cells	+	+	(+)	−	+
Epithelial cells	−	−	+	−	−
T-lymphocytes	+	−	−	−	+
Other leucocytes	−	−	−	+	−

Results

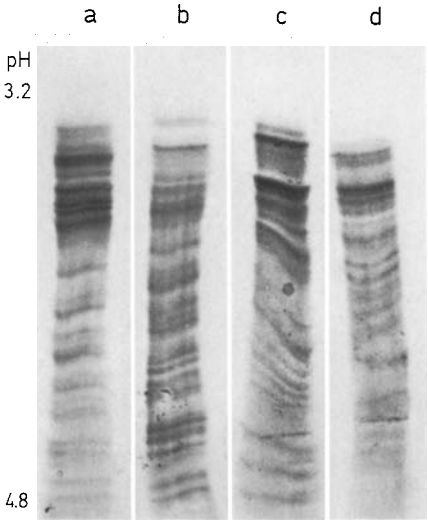
In enzyme histochemistry full term placenta showed a strong diffuse positivity in stromal cells of the villi recognized as myofibroblasts. In human tonsils enzyme activity was confined to lymphocytes, lying in the interfollicular areas and to lymphocytes within the epithelial covering of the tonsillar tissue. In the germinal center of secondary follicles only single cells were positive. Blood T-lymphocytes showed a positive reaction in $59\% \pm 8\%$. In human liver the enzyme activity was confined to the areas around the bile canaliculi (Fig. 1a). In addition moderate activity was detectable in salivery glands, pancreas and renal tubules (Fig. 2a) and sinus lining cells of the spleen. In all tissue sites vascular and lymphatic endothelium showed a positive enzyme reaction (Fig. 3a). 7 out of 23 T-lymphoblastic lymphomas and leukaemias (Feller et al. 1984) showed a positive reaction to DPP IV in a focally accentuated pattern.

In the preparation of the monoclonal antibody fusion resulted in 152 growing clones. Four supernatants with reaction patterns that on the first glance were indistinguishable from or closely related to that of DPP IV were selected for production. These showed a positivity in myofibroblasts of human placenta and in lymphocytes in the interfollicular areas of human tonsils.

Supernatant of the clone II-19 showed a reaction pattern identical to that of enzyme histochemical staining for DPP IV (Table 1). A similar pattern was shown by a second clone (V-22). In contrast to the first, this monoclonal revealed a weaker staining with myofibroblasts and almost no reactivity with T-lymphocytes. The third clone (V-8) exhibited additional strong staining of epithelial coverings. The fourth clone (III-19) revealed a highly restricted occurrence, confined to neutrophils.

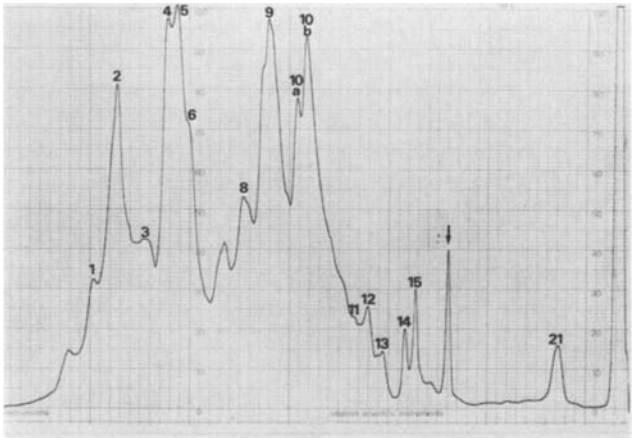
In all organs described for the enzym-histochemical reaction we found an identical reaction pattern using the monoclonal anti-DPP IV antibody (clone II-19) (Fig. 1b, 2b, 3b). The number of positive blood T-lymphocytes amounted to $57\% \pm 6\%$. Seven cases of T-lymphoblastic lymphomas and laeukemias reacted positively with the monoclonal anti-DPP IV-antibody. These were those cases which had been described to be positive also on enzymehistochemical examination.

Isoelectric focusing pattern of DPP IV from different tissue types including T-lymphocytes is shown in Fig. 4. Human full term placenta, tonsillar

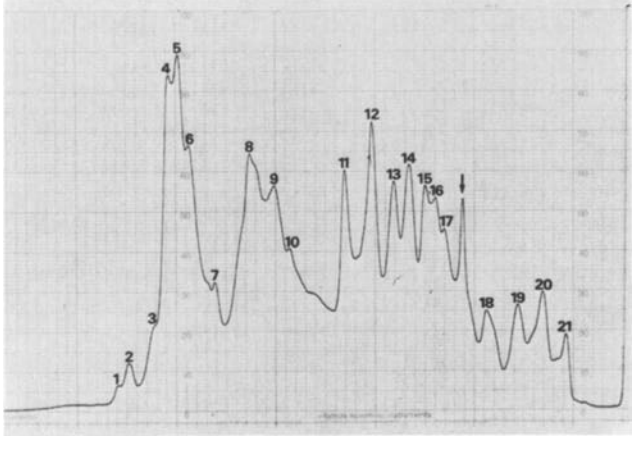


a

Fig. 4. a Isoelectric focusing pattern of DPP IV of (a) placenta, (b) palmar fibromatosis (Dupuytren's contracture), (c) kidney, (d) liver. In each colum a total of 15 mU enzyme activity was run. **b** Densitograms of isoelectric focusing of DPP IV from T-lymphocytes ($\mathbf{b}_1$) and human placenta ($\mathbf{b}_2$). Note the lack of bands 16–20 in T-lymphocytes. ---> : origin



$\mathbf{b}_1$



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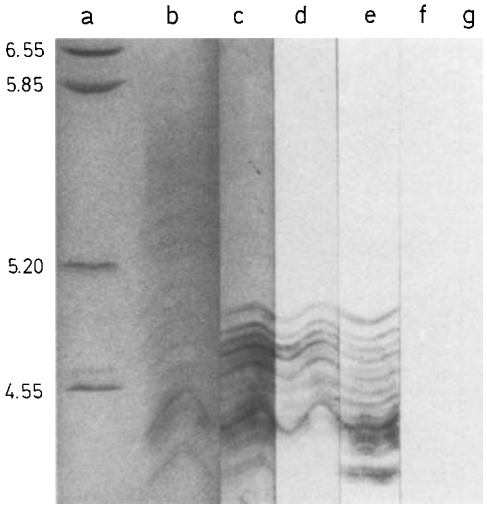

Fig. 5. Isoelectric focusing of partially purified DPP IV from human placenta. (a) Marker proteins (Pharmacia, broad pI Kit, pH 3–10) (Coomassie blue), (b) purified (Coomassie blue) DPP IV, (c–g) DPP IV transferred to nitrocellulose membrane with the blotting technique; (c, d) enzymehistochemical stain (c: 12 h, (d): 1 h developed for DPP IV); (e) incubation with monoclonal antibody II-19 and immunohistochemical development; (f–g) controls without the monoclonal antibody II-19 and immunohistochemical development

tissue, and blood T-lymphocytes as well as T-lymphoblastic lymphomas and leukaemias showed at a minimum of 10 isoenzyme bands with identical loci within a pH range from 3.2–4.7 (Fig. 4a, b). Beyond that human placenta showed 9 additional bands within a pH range from 4.7 to 4.85.

In Fig. 5, the specificity of the monoclonal antibody II-19 was demonstrated with the blotting technique following isoelectric focusing of partially purified DPP IV (Fig. 5b). All bands of the purified DPP IV (Fig. 5b) found within a pH range 3.8–4.9 were also visualized by the enzymehistochemical staining (Fig. 5c, d). An identical banding pattern could be demonstrated after incubating the nitrocellulose membrane with monoclonal II-19 and immunohistochemical staining of the print (Fig. 5e). The control runs showed no single band.

To localize the subcellular distribution of DPP IV in human lymphocytes several subcellular fractions were separated by differential centrifugation and subjected to direct enzyme assay. The heaviest fraction containing nuclei and large debris had about 25% of the net DPP IV activity. About 50% of the enzyme was found in the $20{,}000 \times g$ fraction. The remaining 25% were localized in the microsomes. The particle-free supernatant fluid had no DPP IV activity.

Discussion

The detection and separation of various T-cell subpopulations with different expression of Fc-receptors allowed the recognition of functional subpopulations (Moretta et al. 1977; Heijnen et al. 1979). Similar observations were made for T-cells which were described by the detection of the antigens T4 (helper/inducer T-cells) and T8 (suppressor/cytotoxic T-cells) using monoclonal antibodies (Reinherz et al. 1979, 1980). Separating T-cells according to their Fc-receptors or to the T4 or T8 antigen made it possible

to relate enzymehistochemical reaction patterns of T-lymphocytes to well defined subpopulations. The so called dot-like reaction pattern for nonspecific esterase was recognized in T-cells, bearing Fc-receptors for IgM (Grossi et al. 1978). However, it is a major disadvantage of most enzymes, that they are widely distributed in the haematopoietic system. Apart from its occurence in T-lymphocytes nonspecific esterase is detectable in B-lymphocytes and erythropoietic cells as well as in monocytes and macrophages (Radzun et al. 1983). The detection of DPP IV in T-lymphocytes was a promising development, because of its high specificity in the haematopoietic system (Lojda 1981). Beyond that, DPP IV ws detectable in 89% ± 5% of the T-cells bearing Fc-receptors for IgM (Feller et al. 1982). Neither enzymehistochemically nor fluorophotometrically DPP IV was detected in B-lymphocytes.

Because of a partially identical isoelectric focusing pattern of DPP IV from different organs including blood T-lymphocytes, it was to be expected that placental DPP IV would be similar to DPP IV from T-lymphocytes. Isolated DPP IV from placenta was used for immunizing mice and raising monoclonal antibodies. Using enzyme-histochemistry and the monoclonal anti-DPP IV antibody (clone II-19) for immunohistochemistry the number of positive T-lymphocytes as well as the reaction pattern in different human organs were identical for both techniques. Apart from this, 7 out of 23 T-lymphoblastic lymphomas and laeukemias showed a clear cut reactivity both enzyme-histochemically and immunohistochemically. These investigations already show that the monoclonal antibody detects DPP IV and the results of immunoblotting further substantiated these findings. Identical banding patterns were visualized by enzyme-histochemistry and immunohistochemistry.

If we consider the results of direct enzyme measurement in different subcellular fractions, the enzyme-histochemical findings appeared contradictory. With the former, 25% of the net cellular activity was localized in the microsomal fraction which contains fragments of the cell membrane, whereas the latter revealed no morphological correlate for this finding. However, it is generally accepted that enzyme-cytochemical techniques at the light microscopical level are not appropriate for the detection of membrane bound activity. In contrast there is general agreement that monoclonal antibodies allow the detection of surface membrane bound antigens. It was thus reasonable to assume that the monoclonal antibody recognizing DPP IV would contribute the visualization of the membrane bound portion. Correspondingly, the immunocytochemical investigations showed that the DPP IV antigen was also recognizable on the surface membrane of T-lymphocytes (Mentlein et al. 1984). Similar results were obtained by fluorescence activated cell sorter analysis using II-19 (Scholz et al. 1985). Antigen distribution on the cell surface differed from cell to cell, from a strong ring shaped positivity to a weak surface staining, just detectable at the immunocytochemical level. The binding of the monoclonal antibody to the cell surface enables a separation of DPP IV positive and negative T-cell subpopulations. Investigations of Scholz et al. (1985) showed that DPP IV

positive T-lymphocytes contained 71% ± 10% T4 positive and 22 ± 3% T8 positive cells. Furthermore, there is an association in PHA stimulated T-cells between DPP IV expression and interleukin 2 (IL2) production. Purified DPP IV fraction showed a higher IL 2 production than the purified T4 fraction.

Taken together, the monoclonal anti-DPP IV-antibody (II-19) enables an immunological diagnosis of T-cell neoplasia to be made because of its high restriction and specifity within the haematopoietic system. Furthermore, the detection of DPP IV in a defined T-cell population allows the description of that T-cell population which shows highest IL2 production.

Finally reference should be made to the close relationship between DPP IV from different tissue sources including T-lymphocytes and myofibroblasts. Not only was a more or less similar (if not identical) IEF pattern found between the samples of different origins but great similarity was also disclosed in the antigenic behaviour of DPP IV protein localized in different cells.

To what extent the different intensity of antigen expression of DPP IV on the cell surface of Tμ lymphocytes is a mirror of a defined functional status is a question which will be followed in further investigations.

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