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Changes of Dipeptidylpeptidase IV as a Membrane Marker of Lymphocytes in Acute and Chronic Liver Diseases – Biochemical and Cytochemical Investigations

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Summary

Lymphocytic dipeptidylpeptidase IV (DPP-IV, E.C. 3.4.14.5) is described as a marker enzyme of immunostimulant T-lymphocytes as well as functional characteristic of interleukin-2-producing cells. Cytochemical staining of DPP-IV positive lymphocytes and measurements of DPP-IV activity in mononuclear cells and in sera of patients suffering from different kinds of liver diseases were performed to evaluate the average activities in positive cells. The results demonstrated that this serine exopeptidase exhibits extremely low activity in autoimmune chronic hepatopathies. On the contrary, hepatitis-B-associated liver diseases were connected with markedly increased values. Furthermore, significant differences of DPP-IV activity were found in different kinds of acute and chronic liver diseases. These findings are discussed in connection with the participation of dipeptidylpeptidase IV in impaired immunoregulation of the altered liver.

Key words:
Dipeptidylpeptidase IV – Liver disease – Lymphocytes – Immunoregulation

Introduction

Chronic diseases of the liver have been described as being closely connected with immunological disorders, often only as epiphenomena, but in other cases also as being of aetiological importance (Berg 1979, Eddleston *et al.* 1974, Hütteroth *et al.* 1979, Mackay 1982, Meyer zum Büschenfelde and Manns 1984, Poralla 1989). However, little is known about immunomodulating mechanisms at the enzymatic level.

Dipeptidylpeptidase IV is a well-identified serine proteinase (Fukasawa *et al.* 1978, Ogata *et al.* 1989) that was first described in the rat kidney (Hopsu-Havu and Glenner 1966) by its ability to cleave glycyl-proline from the N-terminus of peptides (Heymann and Mentlein 1978, Hopsu-Havu and Ekfors 1969). The enzyme exists as a sialoglycoprotein (Reutter *et al.* 1989) in a number of mammalian cells and tissues as a plasma membrane ectoenzyme (Gossrau 1979, Hopsu-Havu and Ekfors 1969, Kenny 1977). The molecular weight of one subunit of the dimere is about 105 kDa

and different molecular forms have been discussed (Křepela *et al.* 1983). The exact biological function, however, cannot yet be clearly described. Fibrine (Küllertz *et al.* 1981), substance P (Heymann and Mentlein 1978) and collagen (Hopsu-Havu and Ekfors 1969) could be the possible substrates for this dipeptidase with high specificity for X-proline peptides (Kato *et al.* 1978). A central role in the fibronection-mediated interaction of hepatocytes with the extracellular matrix is discussed by Piazza *et al.* (1989). In lymphoid organs the hypothesis is suggested that DPP-IV in particular corresponds to the T-helper-subpopulation (Feller *et al.* 1982, Crockard *et al.* 1984, Schön *et al.* 1987). Moreover, Scholz *et al.* (1985) found that the expression of DP-IV is indeed associated with the capacity of T-cells to produce interleukin-2 (IL-2).

It seemed to be of special interest to investigate the activity of lymphocytic DPP-IV in chronic liver diseases to show its possible correlations to the immunopathogenesis of these diseases.

Material and Methods

The experimental group included 125 patients suffering from different kinds of acute and chronic liver diseases while the control group consisted of 31 healthy blood donors. The first group consisted of 14 patients with acute virus hepatitis (AVH), 6 cases of chronic persistent hepatitis (CPH) and 24 patients with chronic active hepatitis (CAH) of different origin. There were also 26 patients with alcoholic liver cirrhosis (AC), 22 forms of non-alcoholic liver cirrhosis (NAC), 7 examples of primary biliary cirrhosis (PBC), 7 cases with secondary biliary cirrhosis (SBC) and 19 patients with fatty liver disease and hepatosis (HFL). The diagnosis was confirmed by liver biopsies, investigation of hepatitis-B-virus markers and autoantibodies as well as by conventional clinical chemical tests.

Mononuclear cells (MNC) were isolated from heparinized peripheral venous blood by density gradient centrifugation according to Boyeum (1968) with minor modifications. The resulting suspension consisted of 91 ± 4 % lymphocytes, 95 % of cells were viable in Trypan blue staining. Using a chromogenic substrate (glycylprolyl-4-methoxy-beta-naphtylamide) we determined the percentage of DPP-IV-positive lymphocytes by enzyme cytochemical staining by the method of Lojda (1977). Assays of the activity of dipeptidylpeptidase IV in homogenates of mononuclear cells and in the serum were performed according to Schön *et al.* (1984) with glycyl-prolyl-para-nitroanilide as the chromogenic substrate (final substrate concentration 2 mmol/l; incubation at 37 °C for one hour after homogenization; Eppendorff photometer at 405 nm). Proteins were measured according to Lowry *et al.* (1951) and activity in the serum according to Hütteroth *et al.* (1979). Comparing both results we calculated *average DPP-IV activity in DPP-IV positive cells* (AAPC) as one aspect of mean DPP-IV activity in single reactive T-lymphocyte (Stuhec and Dietrich 1988).

Statistics were carried out by a modified Welch t-test (Sachs 1984).

Results

Determination of DPP-IV parameters in patients with acute and chronic hepatobiliary diseases (Tab. 1) revealed significant differences in cytochemical and biochemical values among various kinds of liver diseases but there was no correlation between DPP-IV activities in the serum and lymphocytes (correlation matrix not shown). This supports the hypothesis that the rise of DPP-IV levels in the sera of patients with liver diseases was due to the membrane ablation of the liver enzyme itself and not due to changes of the lymphocytic enzyme. However, the enzyme activity of lymphocytic DPP-IV is substantially decreased in cases of alcoholic liver cirrhosis so that it can be considered as a sign of impaired immunoregulation in these cases. Serum DPP-IV activities were increased in nearly

all types of hepatobiliary diseases. Especially high levels in primary biliary cirrhosis seems to be of diagnostic interest.

Table 1

Dipeptidylpeptidase IV (DPP-IV) in mononuclear cells (MNC) of peripheral blood and serum in hepatobiliary diseases

AVH – acute virus hepatitis, CPH – chronic persistent hepatitis, CAH – chronic active hepatitis, AC – alcoholic liver cirrhosis, NAC – liver cirrhosis of non alcoholic origin, PBC – primary biliary cirrhosis, SBC – secondary biliary cirrhosis, HFL – hepatosis and fatty liver

	DPP-IV positive MNC	DPP-IV activity (homogenized MNC)	Average activity in positive cells (AAPC)		Serum DPP-IV activity
	(%)	nkat/ Gpt.	nkat/ g protein	nkat/ Gpt. positive cells	nkat/l
Controls (n=31)	21.80 ±5.36	11.44 ±2.81	9.14 ±2.51	55.40 ±25.66	410 ±90
AVH (n=14)	15.77** ±4.37	11.52 ±5.75	7.31* ±2.47	83.06 ±44.87	903** ±406
CPH (n=6)	15.75 ±4.35	13.53 ±4.54	9.74 ±5.94	76.39 ±51.45	624* ±202
CAH (n=24)	18.94 ±9.70	8.93 ±5.84	8.05 ±3.80	55.98 ±35.17	626* ±382
AC (n=26)	20.81 ±7.51	7.65** ±4.29	5.71** ±3.23	46.14 ±29.34	588 ±482
NAC (n=22)	18.08 ±11.65	9.18 ±5.67	6.39* ±4.30	76.49 ±60.26	601 ±334
PBC (n=7)	16.83 ±9.17	16.60 ±15.53	7.08 ±3.42	109.51 ±89.72	2334** ±563
SBC (n=7)	31.33 ±10.63	10.50 ±7.34	7.63 ±4.06	43.13 ±50.25	508 ±395
HFL (n=19)	15.84* ±8.57	8.44* ±6.09	6.31* ±2.74	63.70 ±43.41	609* ±404

*Data are means ± S.D.; significantly different from controls: * $p<0.05$, ** $p<0.01$*

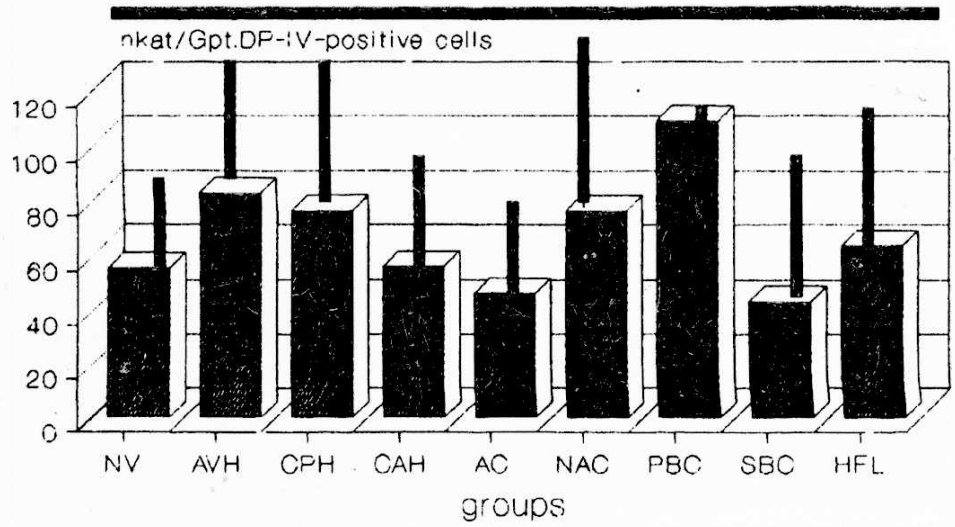

Fig 1.
Average DPP-IV activities in DPP-IV positive mononuclear cells from peripheral venous blood of patients with acute and chronic liver diseases.

The main findings of our investigation concern new aspects of lymphocytic DPP-IV activity (Fig. 1). When calculating the average activities in positive cells (AAPC values), increased activities were encountered in acute viral hepatitis, in chronic persistent hepatitis and they were remarkably high in primary biliary cirrhosis. Lower AAPC levels were found in alcoholic and secondary biliary cirrhosis, but we did not find any significant alterations in patients with chronic active hepatitis. This observation is clearly due to the aetiological heterogeneity of the groups.

Half of the patients with chronic active hepatitis were treated with prednisolone, but lymphocytic DPP-IV values were similar in the two groups. DPP-IV activity in the sera of the group with prednisolone therapy decreased down to the normal range (Tab. 2). This can be considered as an aspect of glucocorticoid-induced membrane stabilization of the liver cell.

In order to assess the influence of viral or autoimmune genesis of liver disease on lymphocytic DPP-IV activity we compared the results (referring to histological classification) in all patients with HBs-Ag positive chronic hepatitis and with autoimmune damage. Such an aetiopathogenic comparison disclosed highly increased AAPC levels in HBs-Ag associated liver disease, whereas AAPC values in autoimmune cases were extremely low (Tab. 3).

Table 2

Comparison of prednisolone-treated patients with chronic-active hepatitis and patients treated without this medication

	DPP-IV positive MNC	DPP-IV activity (homogenized MNC)	Average activity in positive cells (AAPC)		Serum DPP-IV activity
	(%)	nkat/ Gpt.	nkat/ g protein	nkat/ Gpt. positive cells	nkat/l
Prednisolone (n=12)	17.78 ±9.47	8.62 ±4.98	7.89 ±3.16	60.87 ±40.10	459 ±117
Without (n=12)	19.24 ±10.46	9.11 ±6.81	8.23 ±4.29	51.63 ±31.97	824* ±489

*Data are means ± S.D.; significantly different from controls: * $p < 0.05$*

Table 3

Comparison of all hepatitis B surface antigen (HBs-Ag) positive cases of acute and chronic hepatitis and patients with liver diseases of autoimmune origin (AAK) (autoantibodies positive) except of primary billiary cirrhosis

	DPP-IV positive MNC	DPP-IV activity (homogenized MNC)	Average activity in positive cells (AAPC)		Serum DPP-IV activity
	(%)	nkat/ Gpt.	nkat/ g protein	nkat/ Gpt. positive cells	nkat/l
HBs-Ag-positive (n=22)	16.25 ±8.44	11.40 ±5.64	9.17 ±4.57	78.38 ±43.76	777 ±315
AAK-positive (n=8)	26.20* ±6.53	6.35* ±3.42	5.03** ±2.18	26.77** ±14.52	648 ±628

*Data are means ± S.D.; significantly different from controls: * $p < 0.05$, ** $p < 0.01$*

Discussion

Two hypotheses should be discussed in connection with our results. At first, based on the substrate specificity of DPP-IV and N-terminal sequence of IL-2, a limitation of IL-2 effects *via* molecular degradation as well as protective role of lymphocytic DPP-IV against proliferating IL-2 effects are possible. The remarkable increase of AAPC in virus-associated liver disease could limit antiviral mechanisms by suppressing IL-2 in the initial stage. Unhindered virus replication and perpetuation of infection follow this immune hyporeactivity. Alternatively, in autoimmune cases, limitation of the interleukin-2 influence is ineffective because of the extremely low DPP-IV activity. T-cell and following B-cell lines proliferation becomes highly stimulated with resulting autoaggression. Thus, low AAPC would be an aspect of described immunological hyporeactivity in autoimmune hepatitis (Fig. 2).

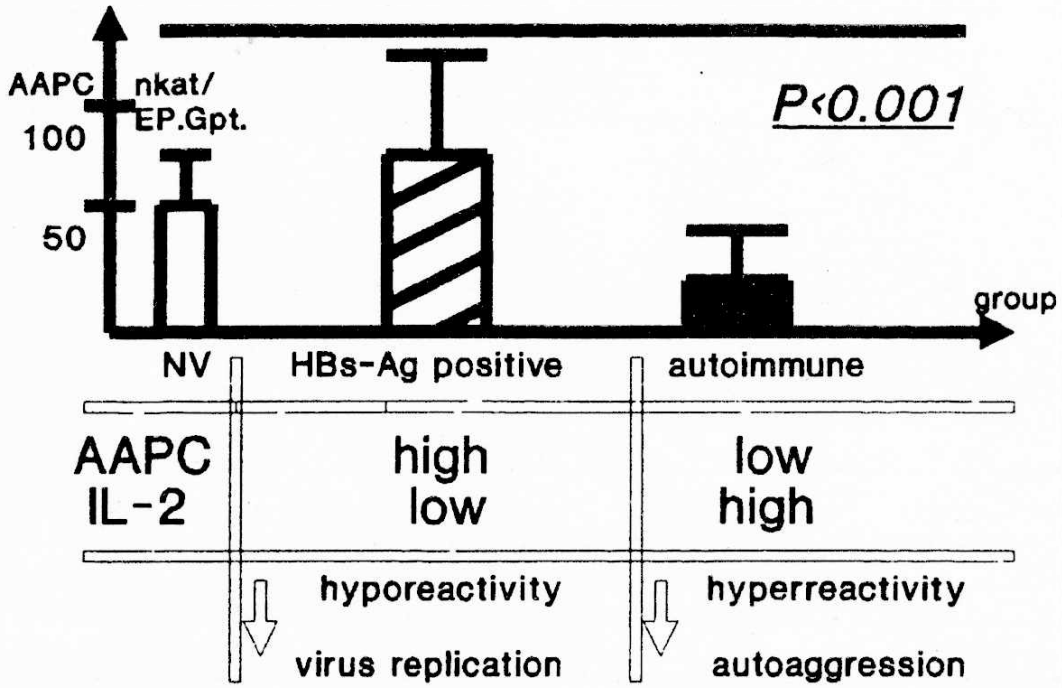

Fig 2.
Hypothetical limitations of interleukin-2 (IL-2) by DPP-IV expression.

Nevertheless, the idea of DPP-IV and IL-2 in lymphocytes as antagonists in the regulation of immune reactivity is only one possibility. A clear-cut connection between IL-2 production and DPP-IV expression is, however, taken for granted. Possibly, DPP-IV forms IL-2 from precursor molecules on the membrane site. This suggests a parallelism of both phenomena. Increased AAPC values and simultaneously raised IL-2 levels in acute viral hepatitis could be interpreted as signs of a sufficiently potent immunodefense against infection agents. In contrast, non-stimulated lymphocytic DPP-IV activities in chronic active hepatitis of viral origin may be connected with a certain immune insufficiency (Fig. 3).

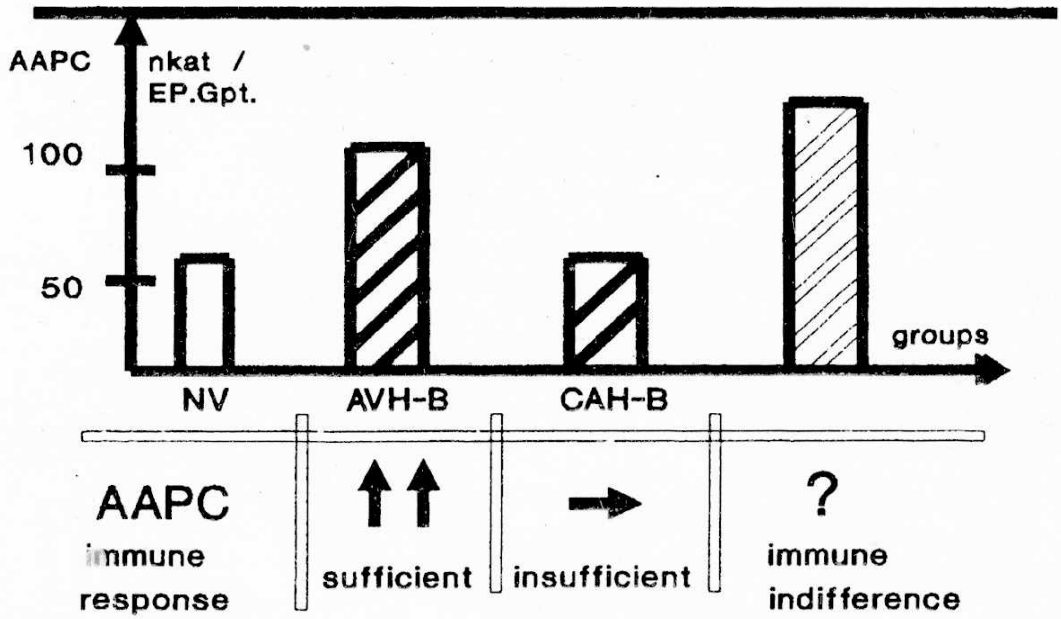

Fig 3.
Model of the parallelism of DPP-IV expression and interleukin-2 (IL-2) production.

Further explanations are difficult, because the connections between DPP-IV and multiple forms of lymphokines are not yet well-understood. Summarizing these recent results with clinical implications we conclude that lymphocytic DPP-IV is related to the development of chronic liver disease, since it is involved in impaired immunoregulatory mechanism.

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