DIPEPTIDYLAMINOPEPTIDASE IV IN LYMPHOCYTES OF PATIENTS WITH LYMPHOPROLIFERATIVE DISEASES

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UDC 616.155-392.2-07:[616.155.32-008.931:577.152.342

KEY WORDS: dipeptidylaminopeptidase IV; T and B lymphocytes; lymphoproliferative disease

Dipeptidylaminopeptidase IV (DAP IV), a highly specific serine peptidase, is present in the plasma membrane of many types of cells in man and animals [6, 8, 10, 12]. The enzyme removes dipeptides from the N-end of the polypeptide chain of oligopeptides, by selectively hydrolyzing bonds formed by COOH groups of proline, and also alanine, residues [6, 8, 10, 12]. The physiological functions of DAP IV are not yet quite clear. It has been suggested that the enzyme is involved in the metabolism of biologically active peptides and regulation of the immune response [6]. The attention of enzymologists and clinicians has been drawn to the enzyme because of its narrow substrate specificity, its localization in the cell membrane, and its presence mainly in (helper) T lymphocytes [6, 7, 10, 11]. These data suggested that determination of DAP IV may be used in the differential diagnosis of lymphoproliferative diseases of T-cell phenotype [5, 6]. Meanwhile, there are indications in individual publications that DAP IV is present not only in T cells, but also in B lymphocytes [9, 13]. Considering the contradictory nature of the data, and also the fact that in most cases the enzyme has been determined by a histochemical method, which gives only a qualitative estimate, we set out to determine DAP IV activity in a pathological population of lymphocytes from patients with malignant lymphoproliferative diseases.

EXPERIMENTAL METHOD

Cells from patients with various forms of lymphoproliferative diseases were used. The diagnosis was based on the morphological and immunochemical characteristics of the cells and also on a clinical examination of the patients. The phenotype to which the lymphocytes belonged was determined by the immunofluorescence method, based on expression of strain-specific and differential antigens, identified by monoclonal antibodies: SD2,3 (T cells), SD4 (helper T cells, Th), SD8 (suppressor T cells, TS), SD5 (T1 antigen of B-CLL*-lymphocytes), VEP13 (natural killer cells), Leu7 (Fcγ-receptor on $T8^+$ cells), OKIa1, SD19,20,24 (B cells); and also by the degree of expression of surface immunoglobulins and the number of cells carrying a receptor for sheep's (T cells) and mouse (B cells) erythrocytes [4]. The cells associated with myeloid diseases were phenotyped with the use of commercial OKM1, OKM5, Vim2, VimC6, and My9 monoclonal antibodies.

Altogether 14 patients were studied: with chronic T-cell leukemia — T-CLL (Table 1, Nos. 1 and 2), with Cesari's disease (No. 3), with T_γ lymphocytosis (No. 4), with chronic B-cell leukemia — B-CLL (Nos. 5 and 6), with hair-cell leukemia — HCL (Nos. 7 and 8), with non-Hodgkin's lymphoma — NHL (Nos. 9-12), and with chronic and acute myeloid leukemia — CML and AML respectively (Nos. 13 and 14).

Lymphoid cells were isolated from a concentrate of lymphocytes obtained after sessions of lymphocytophoresis, or from peripheral blood by gradient centrifugation in Verografin—Ficoll [1].

*CLL) chronic lymphatic leukemia.

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0007-4888/89/0007-1005$12.50

TABLE 1. Immunologic Characteristics and Activity of DAP IV in Pathological Cells Associated with Lymphoproliferative and Myeloproliferative Diseases

No.	Immunologic phenotype and nosologic forms	T-cell markers, %*					Protein, mg/10^8 cells	Activity of DAP IV nmoles/min	
		OKT3*	$OKT4^+$	$OKT8^+$	Leu7	Vep13		per milligram protein	per 10^8 cells
1	T-cell forms: CLL	94	78	96	0	1,4	3,0	2,5	7,5
2	The same	94	2	93	67	60	3,1	0,26	0,97
3	Cesari's disease	91	80	9	—	—	3,0	0,25	0,75
4	T γ-lymphocytosis	93	13	92	81	2	3,1	0,53	1,2
		B-cell markers, %							
	B-cell forms:	HLA-DR	B4	Ia	SIg	EM			
5	CLL	75	90	75	0	73	4,7	0,23	1,1
						46	1,2	0,38	0,45
6	The same	83	—	83	79±80μλ**	—	3,0	0,22	0,66
7	HCL	98	96	98			2,5	0,02	0,05
8	The same NHC (prolymphocytic variant)	93	90	93	93μχ	5	2,8	0,16	0,43
10	The same	77	70	77	+++/++ 53±64	26	2,5	0,18	0,45
11	Lymphoblastic variant	74	84	74	+/++	11	2,3	0,2	0,42
12	Follicular variant	95	84	95	93 ++/+++	26	7,0	0,17	1,19
		Makers of myeloid cells, %							
		OKM1	OKM5	Vim2	VimC6	MJ9			
13	CML	87	69	96	13		10,0	0,098	
14	AML					58	5,3	0,053	
	Normal (n = 4)	T-cells, %		B-cells, %					
		70		10			—	0,079	—

Legend. *) Percentage of cells expressing the given marker relative to total number of cells, **) degree of expression of membrane immunoglobulins, n) number of observations.

Contaminating erythrocytes were removed by hemolysis in 0.83% NH_4Cl solution. The cells were washed 3 times with 0.15 M NaCl solution in phosphate buffer (pH 7.2). The number of lymphoid cells in the final suspensions was over 90%; in CLL the cell composition consisted morphologically of lymphocytes, in HCL 94% of the lymphoid cells were identified as "hair" cells, and in NHL, besides small lymphocytes, a high proportion consisted of prolymphocytes and lymphoblasts.

To determine enzyme activity lysates were obtained by destruction of the cells [(20-40) × 10^6 cells/ml], suspended in 0.15 M NaCl (pH 7.2) on a type UZDN-2 ultrasonic disintegrator (3 times, each for 30 sec, frequency 22 Hz, 4°C). After sonication the undissolved material was removed by centrifugation. Activity of DAP IV was determined fluorometrically as hydrolysis of the synthetic substrate 7-amino-4-methylcoumarylamide of glycyl-L-proline (Gly-Pro-MCA), synthesized at the Institute of Biological and Medical Chemistry, Academy of Medical Sciences of the USSR, by V. F. Pozdnev. The sample (500 μl) contained 4 × 10^5 M substrate, 50-100 μl lysate, and 0.1 M phosphate buffer (pH 7.8). Hydrolysis was carried out at 37°C for 30 min; after the reaction stopped (pH 4.0) the methylcoumarylamine (MCA) formed was determined on an "Opton" fluorometer. Protein was determined by Lowry's method.

EXPERIMENTAL RESULTS

Preliminary experiments showed that hydrolysis of Gly-Pro-MCA in lymphocyte lysates was due to the action of DAP IV. This was shown by data on the pH-optimum of the enzyme (pH 7.8) and inhibition of its activity of p-methylsulfonyl fluoride, a serine proteinase inhibitor [10, 14].

The results of determination of DAP IV in lysates of lymphocytes of patients with T- and B-cell forms of leukemia were as follows. Highest activity of the enzyme was found in the peripheral blood cells of a patient with a rare variant of T-CLL (Table 1, No. 1), which expressed antigens Th and Ts simultaneously. This level of activity was about 30 times higher than DAP IV activity in the donors' mononuclear cells. DAP IV activity in pathological lymphoid cells with the phenotype of mature Th cells obtained from a patient with Cesari's syndrome

(No. 3) was approximately 10 times less than in the case examined above. The same level of activity also was found in morphologically mature cells from a patient with a rare variant of T-CLL, which expressed the Ts antigen and antigens of natural killer cells (No. 2). These results indicate that DAP IV is evidently present not only in Th. This conclusion was confirmed by analysis of lymphocytes of a patient with Tγ-lymphocytosis (No. 4), 85% of which were evidently represented by normal Ts. DAP IV activity in the cells of this patient was almost twice as high as in cases of T-CLL and Cesari's disease. Thus no experimental proof was obtained that DAP IV is a marker only of lymphocytes of the Th subpopulation. Since the highest activity of the enzyme was found in immature T cells, corresponding to the early thymic stage of differentiation (No. 1), it can be tentatively suggested that differences in expression of DAP IV are linked with the stage of differentiation and not with belonging to a particular immunologic subpopulation of T cells.

In all cases of B-cell forms of malignant lymphoproliferative diseases DAP IV activity was found to be rather lower than in T cells. The B cells studied were at different stages of differentiation. However, unlike T cells, correlation was not observed between DAP IV activity and the level of their differentiation. In cases with myeloid proliferation, low DAP IV activity also was found (Table 1).

The methods of expression of DAP IV activity shown in Table 2 are mutually complementary. On the other hand, they enable activity of the enzyme to be assessed relative to total cell protein, and on the other hand, its activity can be characterized per cell [2, 3, 5]. Because of differences in the protein content of the cells studied, the ratio between DAP IV activity determined in different patients by the two methods of calculation differed somewhat.

Unfortunately an adequate control was not available, for as the normal we used an unfractionated pool of lymphoid cells. Since 70% of the lymphocytes were T cells, in which DAP IV activity is almost 7 times higher than in monocytes [9], this suggests that the values we obtained for DAP IV activity relate to T lymphocytes (Table 1).

The results indicate that expression of DAP IV is not a characteristic feature of T lymphocytes only, but it is more or less characteristic of other cell populations also. The results indicate also that quantitative determination of DAP IV may prove useful when assessing the level of differentiation of a pathological T-cell population, whereas it has little informative value in the differential diagnosis of tumors of T-cell origin.

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