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Dipeptidyl-peptidase IV-β, a novel form of cell-surface-expressed protein with dipeptidyl-peptidase IV activity

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The T-cell activation antigen CD26, is a type II membrane glycoprotein with intrinsic dipeptidyl-peptidase IV (DPP IV) activity, characterized by its capacity to cleave off N-terminal dipeptides containing proline as the penultimate residue. Independent of its catalytic activity, CD26 has also been characterized as adenosine deaminase binding protein. By using CD26 negative human C8166 cells, here we describe the existence of another cell-surface protein which manifests CD26-like DPP IV activity. For convenience, this protein will be referred to as DPP IV-β. Consistent with the cell-surface expression of DPP IV-β, intact C8166 cells manifested a high level of DPP IV, whereas, they manifested poor activity against substrates of DPP II known to have an intracellular localization. A partially purified preparation of CD26 from human MOLT4 cells, and the DPP IV-β expressed on intact cells were found to possess similar catalytic activity and pH optimum. In addition, cell-surface CD26 and DPP IV-β on intact MOLT4 and C8166 cells, respectively, resisted digestion by proteolytic enzymes such as trypsin and proteinase K. However, adenosine deaminase activity was not detectable on the surface of C8166 cells in contrast to CD26 positive MOLT4 cells. In accord with this, ^{125}I-labeled adenosine deaminase which binds CD26 was found not to bind DPP IV-β. Gel-filtration experiments using 0.5% Triton X-100 extracts from C8166 and MOLT4 cells, revealed that the apparent molecular mass of DPP IV-β is 82 kDa, whereas that of CD26 is 110 kDa as expected. Taken together, our results suggest that DPP IV-β is a CD26-like protein which could be characterized by distinct properties.

Keywords: CD26; dipeptidyl peptidase IV; adenosine deaminase; C8166 cells; human immunodeficiency virus.

The family of dipeptidyl-aminopeptidase (DPP) contains several peptidases characterized by their capacity to catalyze the cleavage of dipeptides from the N-terminal end of polypeptide substrates with different specificities (McDonald and Schwabe, 1980). In this family, dipeptidyl-peptidase IV (DPP IV) also known as CD26, cleaves dipeptide motifs from the N-terminus of polypeptides provided that the penultimate residue is proline; alanine could replace proline but at a much lower efficiency (Heins et al., 1988; Rahfeld et al., 1991).

DPP IV/CD26 is a cell-surface-expressed serine-exopeptidase with a wide tissue expression, particularly on epithelial cells of the intestine, prostate gland and kidney proximal tubules (for a recent review see Fleischer, 1994). The expression of CD26 on the peripheral blood T lymphocytes is highly enhanced upon T-cell activation, whereas its expression *in vivo* is a marker of the T cell subset, memory T cells which respond to recall antigens (Morimoto et al., 1989; Hegen et al., 1990; Schön and Ansorge, 1990). In T cells, CD26 is physically associated with the membrane-linked tyrosine phosphatase CD45, and modulation of CD26 with anti-CD26 mAb leads to a comitogenic effect on T cell activation, a process which is associated with an enhanced tyrosine phosphorylation of the ζ chain of the T cell receptor (TCR)/CD3 complex along with an increased CD4-associated p56^{lck} tyrosine kinase activity (Dang et al., 1990a; Torimoto et al., 1991; Mittrücher et al., 1995). These observations and the finding that the cytoplasmic tail of CD26 is composed of only six amino acids, favor the hypothesis that signalisation via CD26 is dependent on its interaction with other membrane-expressed antigens, such as CD45. Thus, the CD26/CD45 complex through tyrosine kinase-dependent transduction pathways (Rudd et al., 1994), may play a regulatory role in the T lymphocyte activation process. In addition to this, CD26 has been shown to bind adenosine deaminase, and consequently be responsible for the presence of adenosine deaminase on the surface of cells (Kameoka et al., 1993; De Meester et al., 1994). Recently, CD26 has been reported to serve as a cofactor of the CD4 antigen for human immunodeficiency virus (HIV) entry into permissive cells (Callebaut et al., 1993; Oravecz et al., 1995; Callebaut and Hovanessian, 1996).

Several groups have cloned cDNAs encoding human, mouse, rat and yeast DPP IV (Darmoul et al., 1992; Tanaka et al., 1992; Marguet et al., 1992; Ogata et al., 1989; Roberts et al., 1992). The deduced amino acid sequence of DPP IV/CD26 in these different species, revealed the presence of a conserved sequence

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Abbreviations. DPP IV, dipeptidyl peptidase IV; NH-Np, *p*-nitroanilide; $PhMeSO_2F$, *phenylmethylsulfonyl* fluoride; iPr_2P-F, diisopropylfluorophosphate; Pefabloc-SC, 4-(2-aminoethyl)-benzenesulfonyl fluoride; $Z(NO_2)$, 4-nitrobenzyloxycarbonyl; HIV, human immunodeficiency virus; FITC, fluorescein isothiocyanate; PE, phycoerythrin; FAPα, fibroblast-activation protein α.

Enzyme. Dipeptidyl-peptidase IV (EC 3.4.14.5).

Gly-Trp-Ser-Tyr-Gly towards the C-terminus which corresponded to the consensus sequence Gly-Xaa-Ser-Xaa-Gly representing the active site of serine proteases (Ogata et al., 1992). The DPP IV activity of CD26 might be required for the regulation of some hormones, and might be essential for the intestinal uptake of certain peptides (Yaron and Naider, 1993). The promoter of the human CD26 gene contains the sequence characteristic of a housekeeping gene but has the capacity to regulate the expression of CD26 mRNA in a tissue-specific fashion (Bohm et al., 1995). Until recently, T lymphocytes were considered to express only a single form of DPP IV/CD26. However, Duke-Cohan et al. (1996) have reported a secreted form of DPP IV of 175 kDa from activated T cells. Here, by using a CD26-negative human T-lymphobastoid cell line, we report the existence of a CD26-like cell-surface 82-kDa protein. The properties of this CD26-like DPP IV activity, which we refer to as DPP IV-β, are described here.

MATERIALS AND METHODS

Materials. The mAb B-F5 (IgG1) specific for human CD4 was purchased from Immuno Quality Product. The following mAbs specific for human CD26 were used: mAb BA5 (IgG2a) from Immunotech S.A., mAb Ta1 (IgG1) from Coulter, mAb 4H12 (IgG1) from Endogen Inc., Mab 1F7 (IgG1) from Dr C. Morimoto (Dana-Farber Cancer Institute, Boston; Dang et al., 1990b), and mAb TA5.9 (IgG1; clone CC1-4C8) against the adenosine-deaminase-binding site in CD26 from Dr E. Bosmans (Eurogenetics, Tessenderlo; De Meester et al., 1994). Peptidase substrates Gly-Pro-, Ala-Ala-, Gly-Arg- and Arg-NH-Np (-NH-Np, *p*-nitroanilide) were purchased from Sigma, whereas Ala-Pro-, Lys-Ala-, Ala-, Pro- and succinyl-Ala-Pro-NH-Np were obtained from A.G.M.V. Halle, Germany. 4-(2-Aminoethyl)-benzenesulfonyl fluoride (Pefabloc-SC) was from Interchim and aprotinin was from Sigma. Protein-A−Sepharose was purchased from P.L. Biochemicals. MAb 71/10 specific for the dsRNA-activated protein kinase (Laurent et al., 1985) was used as a control antibody, and it was prepared in the laboratory.

Buffers. Buffer E contains 20 mM Tris/HCl, pH 7.6, 150 mM NaCl, 5 mM $MgCl_2$, 0.2 mM phenylmethylsulfonyl fluoride ($PhMeSO_2F$), 5 mM 2-mercaptoethanol, aprotinin (100 U/ml) and 0.5% Triton X-100.

Buffer I contains 20 mM Tris/HCl, pH 7.6, 400 mM NaCl, 50 mM KCl, 1 mM EDTA, 0.2 mM $PhMeSO_2F$, aprotinin (100 U/ml), 5 mM 2-mercaptoethanol, 1% Triton X-100 and 20% glycerol.

Buffer PB contains 100 mM Hepes, pH 7.6, 120 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 8 mM glucose and 1% BSA.

FACS buffer contains 1% BSA, 0.01% sodium azide in NaCl/P_i (137 mM NaCl, 8.5 mM $Na_2HPO_4 \cdot H_2O$, 1.5 mM KH_2PO_4 and 3 mM KCl, pH 7.4).

Buffer ADA (for adenosine deaminase) contains 10 mM Hepes, pH 7.4, 138 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 8 mM glucose, 50 μM adenosine and 1 μM nitrobenzylthioinosine (an inhibitor of adenosine uptake).

Cells and preparation of extracts. C8166 cells (from Dr G. Farrar) and the clone M8166 from these cells (from Dr P. Clapham) were generously provided by the Medical Research Council AIDS Directed Programme Reagent Project, UK. The parental Jurkat cells (clone P32) and Jurkat cells transfected in order to express enhanced levels of CD26 (clone J37) were provided by Dr C. Morimoto, Dana-Farber Cancer Institute, Boston, MA, USA (Tanaka et al., 1993). MOLT4 cells are lymphoblastoid $CD4^+$ T cells permissive to HIV infection and which are routinely used in our laboratory. The murine hybridoma T-cell line T54S1 was a gift of U. Blank (Institut Pasteur, Paris). All cells were cultured in suspension medium RPMI-1640 (Bio-Whittaker, Verviers, Belgium) containing heat-inactivated (56°C, 30 min) 10% (by vol.) fetal calf serum. For the preparation of cell extracts, cells were first washed extensively in NaCl/P_i before lysis in buffer E (75 μl/10^7 cells) and the nuclei were pelleted by centrifugation (1000 *g* for 8 min). The supernatant was diluted with one volume of buffer I and centrifuged at 12000 *g* for 10 min. The supernatants (cell extracts) were stored at −80°C.

Purification of CD26. Extracts from MOLT4 cells (material from 10^8 cells) were incubated with mAb 1F7 (2 μl of the ascitic fluid) at 4°C for 45 min before addition of protein-A−Sepharose (400 μl in buffer I) and further incubated at 4°C for 16 h. This immune-complex (mAb 1F7-CD26) preparation bound to protein-A−Sepharose was washed batchwise with 4×4 ml of buffer I followed by 4 ml of buffer PB. Under these experimental conditions CD26 is isolated free of adenosine deaminase probably due to the elevated concentration of NaCl (400 mM) in buffer I which was used for the preparation of the immunoprecipitated CD26. Furthermore, CD26 in its bound state to mAb 1F7 is catalytically active, i.e., capable of cleaving Gly-Pro-NH-Np and Arg-Pro-NH-Np but not Pro-, Arg- and Gly-Arg-NH-Np (Callebaut et al., 1993).

Assay for DPP IV activity. The DPP IV activity was assayed by the cleavage of Gly-Pro-, Arg-Pro-, or Ala-Pro-NH-Np at pH 7.6 (Bauvois et al., 1992) and inhibition of this cleavage by specific inhibitors of DPP IV activity (Schön et al., 1991; Born et al., 1994; Brandt et al., 1995). In a typical assay, 2×10^6 cells in 0.5 ml buffer PB were incubated with 0.5 mM dipeptide-NH-Np at 37°C for 90 min. The reactions were stopped by the addition of 1 M sodium acetate, pH 4.5 (1 ml). After centrifugation at 12000 *g*, the production of NH-Np in the supernatant was assessed by measurement of the absorbance at 405 nm.

Peptidase activity was also assayed using 25 μl of cell extracts after dilution in buffer PB (total reaction volume was 500 μl). Similarly, aliquots of the immunoprecipitated CD26 preparation (50 μl of protein-A−Sepharose bound mAb-CD26 immune complex) were assayed for peptidase activity by suspension in 500 μl of buffer PB containing 0.5 mM of the substrate.

Fluorescence-activated-cell-sorting (FACS) analysis. The cell-surface expression of CD26 and CD4 antigens were monitored by FACS analysis using phycoerythrin (PE)-labeled mAbs BA5, Ta1 (for CD26) and fluorescein isothiocyanate (FITC)-labeled B-F5 (for CD4). PE-labeled mAb B4 specific for CD19 (Coulter) was used as a control for PE-labeled mAbs, whereas, FITC-labeled mouse isotype control antibody MCG1 (IgG1) was used as a control for FITC-labeled mAbs. The surface staining with the other mAbs specific for CD26 was performed by indirect immunofluorescence using FITC-labeled hamster anti-mouse IgG antibodies (BIOSYS, France). Incubations were carried out in FACS buffer at 4°C for 30 min. After each incubation cells were washed twice in FACS buffer, fixed with 1% formaldehyde in FACS buffer and applied to a FACS scan flow cytometer (Beckton Dickinson). For each sample, 10000 cells were analysed with Lysis II Software (Beckton Dickinson).

Assay of ecto-adenosine deaminase activity. Cells were washed with NaCl/P_i then suspended in RPMI-1640 medium containing 200 nM of adenosine deaminase (bovine adenosine deaminase, type VIII; Sigma). After incubation (1 h, 37°C) cells were washed in NaCl/P_i and the ecto-adenosine deaminase activity was assayed in control and adenosine deaminase treated cells by incubating 2×10^5 cells in buffer ADA. Incubations were performed at 37°C for 15 min or 30 min before centrifugation to pellet cells. Deamination of adenosine was then determined by

measuring the absorbance at 265 nm of the cell-free incubation media as described (Blanco et al., 1996).

Binding of ^{125}I-labeled adenosine deaminase to cells. Bovine adenosine deaminase (Sigma, type VIII) was iodinated using the Bolton-Hunter reagent (DuPont, NEN) by a procedure as recommended by the manufacturer. Cells (2×10^6) were washed in NaCl/P_i, suspended in 100 µl of RPMI-1640 medium containing 50 nM of ^{125}I-labeled adenosine deaminase, and incubated at 37°C for 1 h. Cells were then washed twice in cold NaCl/P_i and lysed in SDS/PAGE sample buffer (125 mM Tris/HCl, 2% SDS, 20% glycerol and 2% 2-mercaptoethanol, 0.002% bromophenol blue). Following SDS/PAGE analysis, gels were fixed, dried and the radioactivity associated with the 43 kDa ^{125}I-labeled adenosine deaminase band was quantified using a Phosphorimager (Molecular Dynamics).

Immunoblot assay. Crude cell extracts were diluted (1:1, by vol.) in the electrophoresis sample buffer and analyzed on an SDS/polyacrylamide gel (10%) electrophoresis before processing for immunoblot assay (Laurent et al., 1990) using mAb 1F7 specific for human CD26. The mAb was revealed with goat anti-mouse immunoglobulin labeled with horseradish peroxidase (ECL; Amersham).

Thin-layer chromatography. MOLT4, C8166 and M8166 cells were incubated in the buffer PB with Gly-Pro-NH-Np for 4 h at 37°C. Cells were then centrifuged and the supernatants were analyzed for the cleavage products to verify that the substrate was cleaved as a dipeptide. The samples were first desalted on a RP18 cartridge before analysis for cleavage products of Gly-Pro-NH-Np by TLC on silica gel plates (Merck, silica gel 60 F_{254}), with $CHCl_3$/CH_3OH/(17%) NH_4OH (4:4:2, by vol.) as solvent system.

Protease treatment of cells. Protease treatment of MOLT4 and C8166 cells was essentially as described previously (Borrow and Oldstone, 1992) with slight modifications. Briefly, cells were washed in NaCl/P_i and in RPMI-1640 medium containing 1 mM EDTA before treatment with trypsin (Sigma; 2.5 mg/ml at 20°C for 5 min) or proteinase K (Boehringer Mannheim GmbH, 0.2 mg/ml at 37°C for 30 min). The reactions were stopped by 10-fold dilutions in RPMI-1640 containing 10% fetal calf serum. Cells were then washed in NaCl/P_i or in NaCl/P_i containing 1% BSA and 0.01% sodium azide, and processed either for cell-surface peptidase assay or FACS analysis, respectively.

Gel-filtration chromatography. A Superose 6 column (1.6 cm×50 cm) from Pharmacia was equilibrated in buffer GF containing 20 mM Tris/HCl, pH 7.6, 50 mM NaCl and 0.1% Triton X-100. The bed volume was 100 ml. The column was calibrated using extracts from CD26 expressing MOLT4 cells (prepared in buffer E) supplemented with molecular-mass markers: catalase, 232 kDa, and BSA, 67 kDa. Elution was in buffer GF with collection of 1-ml fractions/2 min; with the void volume (Vo) and total column elution volume (Vc) at 36 ml and 114 ml, respectively. Aliquots (50 µl) from each fraction were diluted threefold in PB buffer, and assayed for DPP IV activity by the cleavage of Gly-Pro-NH-Np at pH 7.6. To differentiate between DPP IV and DPP II activity, the cleavage of Gly-Pro-NH-Np was assayed at pH 5.5 which is the reported pH optimum for DPP II (Yaron and Naider, 1993). In some experiments the DPP II-specific substrate Ala-Ala-NH-Np was used. To confirm the identity of DPP IV/CD26, the fractions containing DPP IV activity were assayed again for the cleavage of Gly-Pro-NH-Np after immunoprecipitation using mAb 1F7 (see above).

Plasma membrane preparation from C8166 cells. C8166 cells (300×10^6) were washed in NaCl/P_i and suspended in the homogenization buffer containing 10 mM Hepes, pH 7.6, and 0.5 mM $MgCl_2$. After 30 min incubation on ice, cells were homogenized with a Dounce homogenizer and the cell lysates were diluted 1:1 with 100 mM Hepes, pH 7.6, containing 1% BSA. This suspension was centrifuged at 40000 *g* for 30 min at 4°C and the pellet was then resuspended in 0.25 M sucrose solution and layered on three volumes of 1 M sucrose solution in NaCl/P_i containing 0.2 mM $MgCl_2$ and 1% BSA as described previously (Bauvois et al., 1992). The gradient was established by centrifugation at 100000 *g* for 1 h at 4°C. The membrane fraction was collected with NaCl/P_i containing 0.2 mM $MgCl_2$ and 1% BSA, and centrifuged at 40000 *g* for 30 min at 4°C. Finally, the pellet was suspended in buffer PB and aliquots were stored at −80°C. The activity of DPP IV-β was assayed after fourfold dilution in buffer PB by the cleavage of Gly-Pro-NH-Np at pH 7.6.

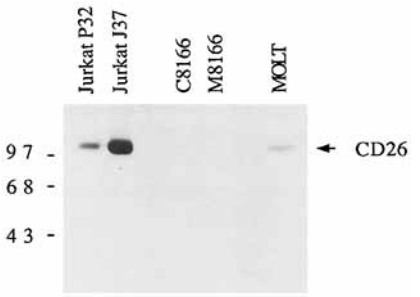

Fig. 1. The lack of expression of CD26 in C8166 cells. Cell extracts from different types of cells (as indicated at the top) were analyzed by polyacrylamide slab gel electrophoresis and processed by immunoblotting using mAb 1F7 specific for human CD26. An autoradiograph is presented. The numbers on the left show the position of molecular-mass (in kDa) protein markers. The cells were as follows: a parental Jurkat cell line (Jurkat P32), a transfected Jurkat cell line expressing enhanced recombinant form of CD26 (Jurkat J37), C8166 cells and a clone derived from these cells (M8166); MOLT4 cells. Material corresponding to 1×10^6 (P32, J37, C8166, M8166) and 0.5×10^6 (MOLT4) cells were analyzed.

RESULTS

The lack of CD26 expression in C8166 and M8166 cells. The expression of CD26 in different $CD4^+$ T-cell lines was investigated by immunoblotting using mAb 1F7 specific for human CD26. Among several $CD4^+$ cell lines tested, only C8166 and M8166 cells were found not to express CD26. A typical result is shown in Fig. 1, with Jurkat parental cells (clone P32), Jurkat cells transfected to express enhanced levels of CD26 (clone J37), C8166 and its clonal derivative M8166 cells, and MOLT4 cells. Both of the Jurkat cell lines and MOLT4 cells expressed the 110-kDa CD26 protein, and, as expected, the level of CD26 was high in the transfected Jurkat cell line J37. However, the expression of CD26 was not detectable in C8166 and M8166 cells, even after overexposure of the autoradiograph. The latter result was in accord with the lack of CD26 mRNA expression in these cells investigated by northern blot analysis (data not shown) or by the reverse-transcriptase/polymerase-chain-reaction technique (RT/PCR; Werner et al., 1994).

In the literature Jurkat cells are considered not to express CD26. However, our results clearly demonstrate that this is not the case. The expression of CD26 in Jurkat cells from different sources was found to express low but reproducibly detectable levels of CD26 (Figs 1 and 2).

Cell-surface staining of CD26 by FACS analysis. The cell-surface expression of CD26 was investigated in different T-cell lines using five anti-CD26 mAbs: BA5, Ta1, 1F7, TA5.9 and 4H12. Consistent with the expression of the 110-kDa protein in the Jurkat cell lines (parental and clone J37) and in MOLT4

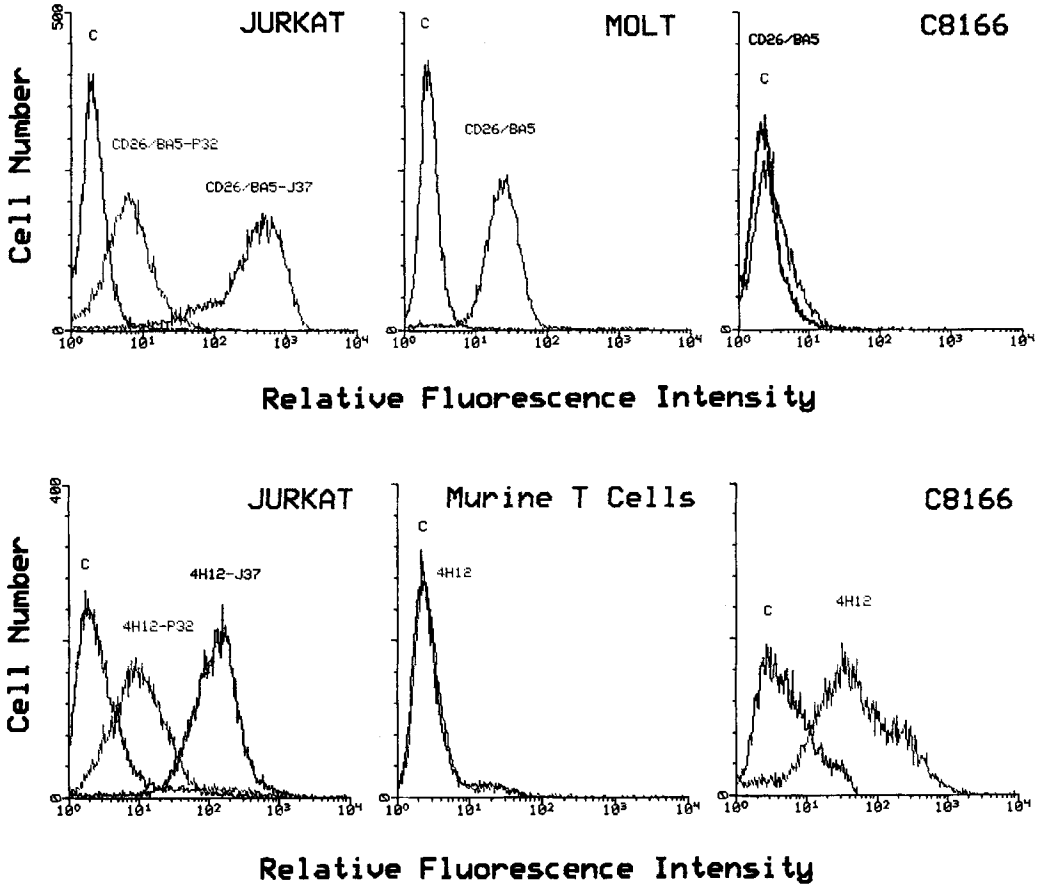

Fig. 2. The expression of CD26 in different types of human T cells by FACS analysis. (A) FACS analysis in Jurkat, MOLT 4 and C8166 cells using PE-labeled mAb BA5 specific for CD26. The peak C (for control) in each section represents the background fluorescence observed for each cell type using PE-labeled mAb B4 specific for CD19 (expressed in B cells). P32 and J37 represent two Jurkat cell lines, parental and transfected to express enhanced levels of CD26, respectively. (B) FACS analysis in Jurkat P32, J37, murine T and C8166 cells using mAb 4H12 by indirect immunofluorescence. The second antibody was FITC-labeled hamster anti-mouse IgG from BIOSYS. The peak C in each section represents the background fluorescence with the second antibody alone.

cells, the expression of the cell-surface CD26 was observed using either one of the five mAbs. A typical result is shown with mAb BA5 (Fig. 2A). Among these five anti-CD26 mAbs, only mAb 4H12 gave a specific fluorescence signal with the $CD26^-$ C8166 cells (Fig. 2B).

In our experiments, evidence that mAb 4H12 reacted with the cell-surface CD26 was illustrated by the increased staining of the high-CD26-expressing cells (Jurkat clone J37) compared with the low-CD26-expressing parental Jurkat cells (Fig. 2B). Furthermore, using extracts from CD26 cells, mAb 4H12 immunoprecipitated CD26 which could be shown either by assaying for DPP IV activity or by revealing the 110-kDa protein in the immune complex preparation. In an immunoblot analysis however, mAb 4H12 did not react with the SDS-denatured 110-kDa protein (data not shown), thus indicating that 4H12 is an antibody which recognizes the native CD26 only. The cell-surface staining of the C8166 and M8166 cells was routinely revealed with mAb 4H12. The latter result was probably the consequence of specific binding of mAb 4H12 to the surface of these $CD26^-$ C8166 and M8166 cells, since no signal was observed with the murine T cell line (Fig. 2B). These results therefore suggest that mAb 4H12 cross-reacts with a cell-surface human antigen on C8166 and M8166 cells which might share a common epitope with CD26.

CD26-like DPP IV activity on the surface of C8166 cells. Preliminary results suggested to us the presence of a CD26-like DPP IV activity on the surface of C8166 and M8166 cells. For this reason, we investigated the expression of different cell-surface peptidases by $CD26^-$ C8166 cells in comparison with $CD26^+$ MOLT4 cells. These experiments were carried out in the absence or presence of 25 μM Lys-[$Z(NO_2)$]-pyrrolidide [$Z(NO_2)$, 4-nitrobenzyloxycarbonyl], a potent specific inhibitor of the DPP IV activity (Schön et al., 1991; Born et al., 1994). The peptidases tested were as follows: DPP IV by the cleavage of Gly-Pro-NH-Np and Ala-Pro-NH-Np, DPP I by the cleavage of Gly-Arg-NH-Np, DPP II by the cleavage of Lys-Ala-NH-Np and Ala-Ala-NH-Np, proline endopeptidase by the cleavage of succinyl-Ala-Pro-NH-Np, and Ala-, Pro- and Arg-peptidases by the cleavage of Ala-, Pro- and Arg-NH-Np. It should be noted that, the DPP II substrates Lys-Ala-NH-Np and Ala-Ala-NH-Np can also be cleaved by DPP IV but at a much lower efficiency (Heins et al., 1988; Rahfeld et al., 1991).

In contrast to the lack of expression of CD26 in C8166 cells (Figs 1 and 2), a CD26-like peptidase was detectable with intact cells using classical substrates, Gly-Pro-NH-Np or Ala-Pro-NH-Np, for DPP IV activity (Table 1). As the cleavage of these dipeptides was inhibited by more than 85% at 25 μM of Lys-[$Z(NO_2)$]-pyrrolidide, it is possible that C8166 cells express a cell-surface protein which has DPP IV activity. For convenience we will refer to this protein as DPP IV-β. The specific nature of Lys-[$Z(NO_2)$]-pyrrolidide was demonstrated by the lack of its action on other cell-surface peptidases such as Ala-, Pro-, Arg- and Gly-Arg-peptidase (Table 1). Lys-Ala-NH-Np which is a

Table 1. Assay for the presence of different peptidases on the surface of CD26⁻ C8166 and CD26⁺ MOLT4 cells. C8166 and MOLT4 cells (2×10^6) were assayed for cell surface peptidase activities in the absence or presence (− or +) of 25 μM Lys-[Z(NO_2)]-pyrrolidide using different substrates (at 0.5 mM) as indicated. A purified preparation of CD26 from MOLT4 cells was also used to show the specificity of CD26 for the proline-containing dipeptides. The mean of duplicate samples is given. Similar results were obtained with three independent experiments. n.d., not determined.

Substrate	Lys-[Z(NO_2)]-pyrrolidide	Peptidase activity from		
		C8166 cells	MOLT4 cells	immunoprecipitated CD26
		nmol of substrate hydrolyzed		
Gly-Pro-NH-Np	−	52.6	53.9	85.6
	+	6.7	6.1	9.8
Ala-Pro-NH-Np	−	74.3	62.8	75.3
	+	13.2	12.2	8.5
Lys-Ala-NH-Np	−	29.7	16.3	15.6
	+	22.5	10.9	9.7
Ala-Ala-NH-Np	−	5.5	7.5	5.5
	+	4.6	5.2	3.8
Ala-NH-Np	−	7.5	45.1	<1
	+	7.7	46.4	n.d.
Pro-NH-Np	−	18.2	17.5	<1
	+	17.8	17.1	n.d.
Succinyl-Ala-Pro-NH-Np	−	<1	3.1	<1
	+	<1	3.0	<1
Gly-Arg-NH-Np	−	15.6	14.7	<1
	+	14.5	14.8	<1
Arg-NH-Np	−	50.5	78.7	<1
	+	52.7	78.9	n.d.

substrate of DPP II can also be cleaved by DPP IV. However the degree of cleavage of Lys-Ala-NH-Np by CD26 is at a much lower efficiency than that of Gly-Pro-NH-Np or Ala-Pro-NH-Np. This was clearly demonstrated by the immunoprecipitated CD26 preparation (Table 1). The degree of cleavage of Lys-Ala-NH-Np compared with Gly-Pro-NH-Np or Ala-Pro-NH-Np was also lower by the cell-surface peptidase activity on C8166 cells. This result suggests that DPP IV-β might manifest catalytic properties similar to those of CD26. Consistent with this, the cleavage of Lys-Ala-NH-Np was inhibited only partially by Lys-[Z(NO_2)]-pyrrolidide in both cases (Table 1).

Previously, several reports have shown that DPP II and DPP IV could be differentiated by their subcellular localization: DPP IV is associated with the cell membranes and is expressed on the cell-surface, whereas DPP II is localized intracellularly and mainly in the lysosomal compartment (Yaron and Naider, 1993; McDonald and Schwabe, 1980). To demonstrate that the cleavage of Gly-Pro-NH-Np by intact C8166 cells is due to the cell-surface DPP IV-β, we assayed the cleavage of the DPP II specific substrate Ala-Ala-NH-Np which is a very poor substrate of DPP IV/CD26 (Table 1). Under experimental conditions in which the DPP IV substrate Gly-Pro-NH-Np was cleaved efficiently by intact C8166 cells, very little cleavage of Ala-Ala-NH-Np was observed, thus favoring the suggestion that the cleavage of Gly-Pro-NH-Np was catalyzed mostly by the cell-surface DPP IV-β.

The pH profile of the DPP IV-β activity on C8166 cells is similar to that of CD26. Although DPP IV and DPP II could cleave Gly-Pro-NH-Np, DPP IV has a basic pH optimum, whereas DPP II has a pH optimum at 5.5−6.0 (Yaron and Naider, 1993; McDonald and Schwabe, 1980). The cleavage of Gly-Pro-NH-Np therefore was investigated at different pH values with intact C8166 and MOLT4 cells, and also in a partially purified preparation of CD26 obtained by immunoprecipitation using mAb 1F7 and extracts from MOLT4 cells (see Materials and Methods). During these experiments, we noticed that at pH values greater than or equal to 8, there was a considerable cleavage of Gly-Pro-NH-Np in control samples containing only buffer PB, thus indicating that there was a spontaneous cleavage of the substrate at elevated pH values. Accordingly, the net dipeptidyl-peptidase activity at each pH (Fig. 3) was calculated by subtracting the spontaneous cleavage value (background) from the total value.

Assay for the cleavage of Gly-Pro-NH-Np in cell extracts from MOLT4 and C8166 cells, illustrated the presence of high levels of dipeptidyl-peptidase activity within the range pH 6−8, which probably corresponded to the additive activities of DPP IV at high pH and DPP II at low pH (Fig. 3A). Accordingly, the results observed with the immunoprecipitated CD26 from MOLT4 cells, confirmed that optimum activity of DPP IV is at pH 7.5 with very little activity at pH 6.0 (Fig. 3B). Very similar pH/activity curves were obtained for the cell-surface dipeptidyl-peptidase activity on MOLT4 and C8166 cells compared with the immunoprecipitated CD26. These data are consistent with the suggestion that the cell-surface expressed dipeptidyl-peptidase on C8166 cells is of DPP IV type. C8166, M8166 and MOLT4 cells were assayed for the cell-surface cleavage of Gly-Pro-NH-Np at 7.6, and the digestion products were analyzed by TLC (see Materials and Methods). Consistent with the catalytic activity of a DPP IV-like dipeptidyl-peptidase, no single amino acids were apparent but the dipeptide Gly-Pro was detected by TLC (data not shown).

The DPP IV activity assayed by using the intact cells manifested very little activity at pH 6 or below for the cleavage of Gly-Pro-NH-Np (Fig. 3C), thus indicating that intracellular DPP II is not functional under the experimental conditions using intact cells. Similarly, very little cleavage of the DPP II-specific

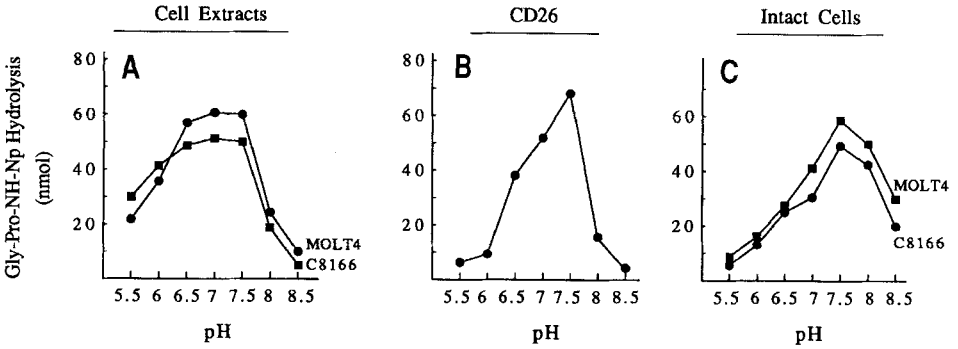

Fig. 3. Optimum cleavage of Gly-Pro-NH-Np by the cell-surface enzyme and by the immunoprecipitated CD26 is at pH 7.5. The cleavage of Gly-Pro-NH-Np was assayed in buffer PB which was adjusted at different pH values: 5.5, 6, 6.5, 7, 7.5, 8 and 8.5. Peptidase sources were (A) crude cell extracts from C8166 and MOLT4 cells; (B) a partially purified preparation of CD26 obtained by immunoprecipitation using extracts from MOLT4 cells; (C) intact C8166 and MOLT4 cells. Each point represents the mean of duplicate samples. Similar results were observed in three independent experiments. It should be noted that the results represent the net values calculated by subtracting the background value (spontaneous cleavage) from the total value at each pH.

Table 2. Cell.surface cleavage of Gly-Pro-NH-Np in CD26$^-$ C8166 cells is blocked by specific inhibitors of DPP IV activity. DPP IV activity of the immunoprecipitated CD26 from MOLT4 cells, and the cell-surface DPP IV activity on C8166, M8166 and MOLT4 cells were assayed by the cleavage of Gly-Pro-NH-Np (at 0.5 mM) at pH 7.5. In case of inhibition, the results are given in IC_{50} values. The cleavage products of Gly-Pro-NH-Np by C8166, M8166 and MOLT4 cells were monitored by TLC (Materials and Methods) to confirm that the digestion product was the dipeptide Gly-Pro. The inhibitors Lys-[Z(NO_2)]-pyrrolidide, Lys-[Z(NO_2)]-thiazolide and Ile-pyrrolidide were tested at 50, 25, 10, 5, 2, 1 and 0.5 μM.

Compound	IC_{50} of Gly-Pro-NH-Np cleavage			
	C8166 cells	M8166 cells	MOLT4 cells	immuno-precipitated CD26
	μM			
EDTA	none[a]	none[a]	none[a]	none[a]
Aprotinin	none[b]	none[b]	none[b]	none[b]
Pefabloc	1000	1000	1000	2000
$PhMeSO_2F$	none[c]	none[c]	none[c]	none[c]
Lys-[Z(NO_2)]-pyrrolidide	1.9	2.8	4.8	2.5
Lys-[Z(NO_2)]-thiazolidide	1.6	2.2	2.3	1.6
Ile-pyrrolidide	5.5	5.1	7.8	0.3

[a] No effect at 100 mM EDTA.
[b] No effect at 10 kU/ml aprotinin.
[c] No effect at 1 mM $PhMeSO_2F$.

substrate Ala-Ala-NH-NP occurred using intact C8166 and MOLT4 cells, whether at pH 5.5 or 7.5 (Table 1 and data not shown). Taken together, our observations are consistent with the hypothesis that the cleavage of Gly-Pro-NH-Np by intact cells is due to the activity of cell-surface-expressed DPP IV and DPP IV-β in MOLT4 and C8166 cells, respectively.

Several groups have reported that the pH optimum of the DPP IV activity is in the range pH 8–9 (Nagatsu et al., 1976; Demuth et al., 1987; Yaron and Naider, 1993). We believe that this result is an artifact due to the consequence of the spontaneous cleavage of Gly-Pro-NH-Np at pH values of 8 or more. Indeed, if one considers the total values, i.e., net DPP IV activity plus the spontaneous cleavage at each pH, then the optimum pH of the DPP IV activity on the cell-surface and in the immunoprecipitated CD26 would be artifactually around 8.5.

Characterization of DPP IV-β activity on C8166 cells. For further characterization of DPP IV-β in comparison with CD26, we investigated the effect of different agents on the cleavage of Gly-Pro-NH-Np at pH 7.6 (Table 2). The DPP IV-β activity on the surface of C8166 and M8166 cells manifested a very similar profile of inhibition by these agents, compared with the DPP IV activity on the surface of MOLT4 cells and the partially purified preparation of CD26. In all cases, the cleavage of Gly-Pro-NH-Np was not affected by the cation-chelator EDTA, the trypsin inhibitor aprotinin, and the serine protease inhibitor $PhMeSO_2F$. However, another serine protease inhibitor Pefabloc which is a newly commercialized substitute of diisopropylfluorophosphate (iPr_2P-F, also referred to as DFP in the literature) was partially inhibitory with an IC_{50} value in the range of 1–2 mM. The most significant inhibition of Gly-Pro-NH-Np cleavage was observed by three reversible inhibitors of DPP IV: Lys-[Z(NO_2)]-pyrrolidide, Lys-[Z(NO_2)]-thiazolidide and Ile-pyrrolidide (Schön et al., 1991; Born et al., 1994), with IC_{50} values at micromolar concentrations. The IC_{50} values of these three inhibitors for the cleavage of Gly-Pro-NH-Np by the cell-surface dipeptidyl-peptidase activity were comparable in C8166, M8166 and MOLT4 cells (Table 2), thus indicating that there is no significant difference between the DPP IV activity expressed in CD26$^-$ and CD26$^+$ cells. Previously, Werner et al, (1994) have reported that C8166 cell express a Gly-Pro-NH-Np cleaving peptidase activity which is not inhibited by Ala-thiazolidide, another inhibitor of the DPP IV activity. It should be emphasized that Ala-thiazolidide is a less effective inhibitor of DPP IV than the three inhibitors used in our studies (Table 2).

DPP IV-β like CD26 resists proteolysis by trypsin and proteinase K. Preliminary results indicated that the cell-surface CD26 in MOLT4 cells is resistant to degradation by trypsin and proteinase K. FACS analysis of MOLT4 cells before and after treatment with these proteolytic enzymes demonstrated that the detection of CD26 was not modified whereas the detection of CD4 was drastically reduced (Table 3). Similarly, the detection of CD4 on the surface of C8166 cells was reduced by 48% and 91% following treatment with trypsin and proteinase K, respectively (Table 3). MOLT4 and C8166 cells were also assayed for cell-surface DPP IV (by the cleavage of Gly-Pro-NH-Np) and Arg-peptidase (by the cleavage of Arg-NH-Np) activities. The results given in Table 4, indicate that there was no significant effect on the cleavage of Gly-Pro-NH-Np in C8166 and MOLT4 cells after treatment with trypsin or proteinase K. Once again, the resistance of CD26 and DPP IV-β to trypsin and proteinase

Table 3. Resistance of cell-surface CD26 but not CD4 to digestion by trypsin and proteinase K. MOLT4 and C8166 cells (10^7) were treated as described in Materials and Methods with trypsin (2.5 mg/ml, 5 min at 20°C) or proteinase K (0.2 mg/ml, 30 min at 37°C) before FACS analysis using PE-labeled mAb BA5 against CD26 and FITC-labeled mAb B-F5 against CD4. The expression of CD4 or CD26 in the control cells (not treated with trypsin or proteinase K) was considered as 100%. Consequently, the percentages of positive cells after protease treatment were estimated by comparison with the untreated control cells.

Cells	Treatment	Positive cells by FACS analysis	
		CD26	CD4
		%	
MOLT4	none	100	100
	trypsin	100	22
	proteinase K	98	5
C8166	none	0	100
	trypsin	0	52
	proteinase K	0	9

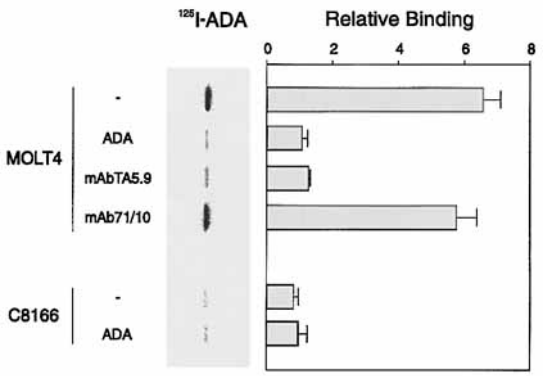

Fig. 4. Adenosine deaminase binds MOLT4 but not C8166 cells. The binding of ^{125}I-labeled adenosine deaminase to MOLT4 and C8166 cells (2×10^6) was assayed in the absence (lanes −) or presence of unlabeled adenosine deaminase (ADA, 2 μM), mAb TA5.9 (10 μg/ml) against adenosine deaminase binding site in CD26 and control mAb 71/10 (10 μg/ml). After PAGE of the cell extracts from the different samples, the 43-kDa ^{125}I-labeled adenosine deaminase (^{125}I-ADA) band was quantitated by phosphorimager.

Table 4. Resistance of cell-surface DPP IV and DPP IV-β to digestion by trypsin and proteinase K. MOLT4 and C8166 cells were treated with trypsin or proteinase K as described in Table 3 and in Materials and Methods. Cells were washed with NaCl/P_i and then suspended in buffer PB and aliquots of each control and protease treated cells (2×10^6) were assayed for the cleavage of Gly-Pro-NH-Np and Arg-NH-Np to monitor for cell-surface expression of DPP IV and Arg-peptidase activities, respectively. The values represent the peptidase activity in nanomoles of NH-Np hydrolyzed during each respective reaction. Similar results were obtained in two independent experiments. Note the significant reduction of the Arg-peptidase activity.

Cells	Treatment	Peptidase activity with	
		Gly-Pro-NH-Np	Arg-NH-Np
		nmol of NH-Np produced	
MOLT4	none	65.9	72.2
	trypsin	60.1	24.2
	proteinase K	63.8	22.4
C8166	none	56.7	60.5
	trypsin	51.3	28.7
	proteinase K	48.7	23.2

K was specific, since the cleavage of Arg-NH-Np was reduced by more than 50% in both cell types after trypsin or proteinase K treatment (Table 4).

It should be noted that proteinase K treatment results in the elimination of most of cell-surface proteins (Dragic et al., 1995). Therefore, the proteinase K-resistance of CD26 and DPP IV-β illustrates an intriguing but somewhat unique nature of these proteins.

Adenosine deaminase binds MOLT4 but not C8166 cells. As CD26 is the adenosine-deaminase-binding protein (Kameoka et al., 1993; De Meester et al., 1994), it was essential to evaluate the capacity of adenosine deaminase to bind C8166 cells through DPP IV-β.

We first investigated the presence of adenosine deaminase on the surface of C8166 and MOLT4 cells by assaying for ecto-adenosine deaminase activity (Materials and Methods). Such endogenous adenosine deaminase activity was found in MOLT4 but not in C8166 cells (data not shown). We estimated that 35% to 65% of adenosine-deaminase binding sites in CD26 on the cell-surface of MOLT4 cells were occupied by endogenous adenosine deaminase. Iodine-labeled adenosine deaminase was then used to demonstrate the direct binding of adenosine deaminase to CD26. ^{125}I-labeled adenosine deaminase was found to bind specifically to the surface of MOLT4 cells, since such binding was abolished in the presence of unlabeled excess adenosine deaminase or by mAb TA5.9 against the adenosine deaminase binding site in CD26 (Fig. 4). Under similar experimental conditions, ^{125}I-labeled adenosine deaminase did not bind C8166 cells. These data indicate that in spite of very close similaries in the catalytic activity between CD26 and DPP IV-β, they could be differentiated by the lack of an adenosine-deaminase-binding-site in the DPP IV-β molecule.

Gel-filtration chromotography to isolate DPP IV and DPP II from MOLT4 cells. MOLT4 cells extracts supplemented with catalase (232 kDa) and BSA (67 kDa) were subjected to gel-filtration chromatography (Fig. 5) under the experimental conditions described in Materials and Methods. Aliquots from each fraction were assayed for DPP IV and DPP II activity by the cleavage of Gly-Pro-NH-Np and Ala-Ala-NH-Np, respectively. To confirm the identity of DPP IV as CD26, aliquots from each fraction were also immunoprecipitated using anti-CD26 mAb 1F7 and assayed for the cleavage of Gly-Pro-NH-Np in the immune complex preparations.

The cleavage of Gly-Pro-NH-Np in the column fractions revealed two overlapping peaks located within fractions 22−42 (Fig. 5A). The first peak was colocalized with the unique peak obtained after immunoprecipitation with mAb 1F7, thus indicating that it is the peak of CD26 which corresponded to a protein of 110 kDa, in accord with the molecular mass of CD26 after PAGE analysis. The identity of the second peak was not clear; it might contain a CD26-like dipeptidyl-peptidase, such as the DPP IV-β. Whatever the case, the tail of this peak contained DPP II, the presence of which was clearly illustrated by the cleavage of Ala-Ala-NH-Np (Fig. 5B). The DPP IV/CD26 and DPP II were recovered as proteins with molecular masses of 110 kDa and 70 kDa, respectively. Under our experimental con-

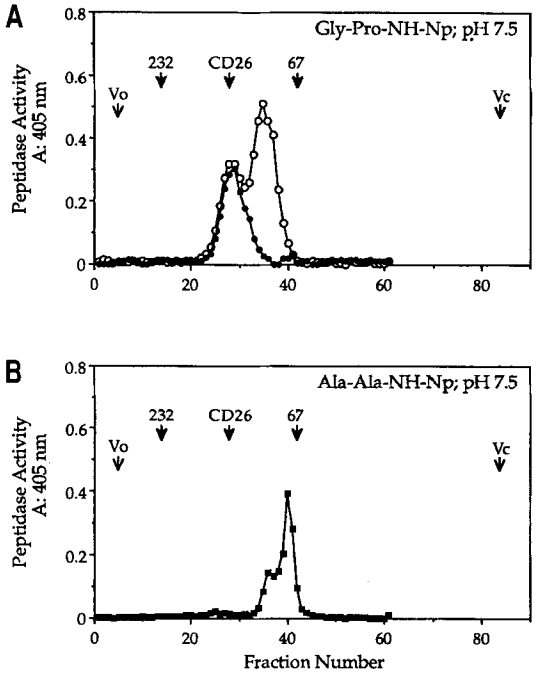

Fig. 5. Gel filtration of extracts from MOLT4 cells. CD26 elutes as a monomer. Extracts (500 µl; material corresponding to 10^8 cells) from MOLT4 cells were loaded on the gel-filtration column and 1 ml fractions were collected as described in Materials and Methods. Aliquots (50 µl) from each fraction were diluted threefold in buffer PB at pH 7.6 and assayed for the cleavage of Gly-Pro-NH-Np (A; open circles) or Ala-Ala-NH-Np (B; closed squares). Aliqouts of each fraction were also immunoprecipitated using mAb 1F7, and the immune complex preparations were assayed for the cleavage of Gly-Pro-NH-Np (A; closed circles). Abbreviations Vo and Vc refer to the fractions corresponding to the void volume and the total column elution volume, respectively. The molecular mass protein markers of 232 kDa and 67 kDa corresponded to the peaks of catalase and BSA. The arrow CD26 indicates the peak of DPP IV activity observed in the immunoprecipitated samples; this peak corresponded to a 110-kDa monomeric form of CD26. The ordinates give the values of absorbance at 405 nm.

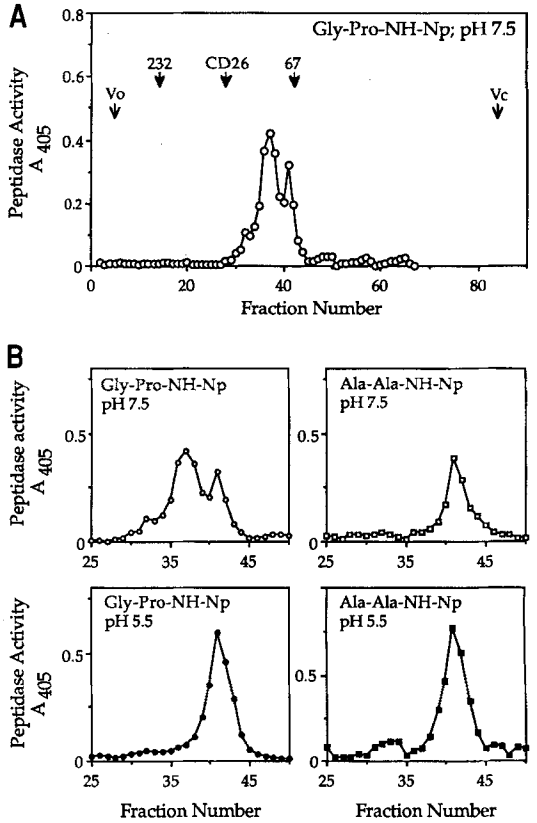

Fig. 6. DPP IV-β elutes from the gel-filtration column as an 82-kDa protein. Extracts (500 µl; material corresponding to 10^8 cells) from C8166 cells were loaded on the gel filtration column and 1 ml fractions were collected as described in Fig. 5 and in Materials and Methods. (A) The cleavage of Gly-Pro-NH-Np was carried out at pH 7.5 using 50-µl aliquots of each fraction. Abbreviations Vo and Vc and the position of the molecular mass protein markers of 232 kDa and 67 kDa were as in Fig. 5. (B) 50-µl aliquots from fractions 25–55 were assayed for the cleavage of Gly-Pro-NH-Np and Ala-Ala-NH-Np at pH values of 7.5 and 5.5 as indicated. The ordinates in (A) and (B) give the values of absorbance at 405 nm.

ditions therefore, DPP IV/CD26 and DPP II eluted as monomers in contrast to previously reported observations which have emphasized that these enzymes exist as dimers. As the gel filtration experiments were carried out in the presence of molecular mass protein markers of 232 kDa and 67 kDa, we have no doubt that DPP IV and DPP II were recovered as monomers. It might be possible that the dimeric forms were dissociated in the presence of 0.5% Triton X-100 in buffer E which was used for the preparation of cell extracts.

Estimation of the apparent molecular mass of DPP IV-β. As C8166 cells are CD26 negative cells, they provide a suitable source to characterize the DPP IV-β free of CD26. Therefore, extracts from such cells were used in gel-filtration experiments under conditions as described above. Assay for the DPP IV activity by the cleavage of Gly-Pro-NH-Np at pH 7.6 revealed two overlaping peaks in fractions 37 and 42, corresponding to proteins with a molecular mass of 82 kDa and and 70 kDa, respectively (Fig. 6A). Further experiments were performed in fractions 25–50, using two substrates Gly-Pro-NH-Np and Ala-Ala-NH-Np, each at pH 7.6 and 5.5 (Fig. 6B).

With Gly-Pro-NH-Np as a substrate, the two peaks corresponding to the 82-kDa and 70-kDa proteins were observed once again at pH 7.5 in column fractions 25–50. However, only the 70-kDa peak was detected at pH 5.5, thus suggesting that the 82-kDa and 70-kDa peaks should correspond to enzymes with DPP IV and DPP II activity, respectively. Consistent with this, the cleavage of Ala-Ala-NH-Np at pH values of 7.5 or 5.5 was observed only in fractions corresponding to the 70-kDa peak. As C8166 cells are CD26-negative cells, then the DPP IV activity in fractions 34–39 (i.e. the 82-kDa peak) should correspond to that of DPP IV-β.

DPP IV-β is associated with plasma membrane. Plasma membranes from C8166 cells were assayed for DPP IV and DPP II activity by the cleavage of Gly-Pro-NH-Np and Ala-Ala-NH-Np, respectively (see Materials and Methods). This membrane preparation contained fivefold higher levels of DPP IV activity/mg protein compared with that found in crude cell extracts, whereas no significant level of DPP II activity was detectable.

DISCUSSION

By the use of CD26-negative T-lymphoblastoid C8166 cells, here we were able to demonstrate the existence of a CD26-like ecto-peptidase manifesting a typical DPP IV activity. For convenience, this novel CD26 homologue is referred to as DPP IV-β. The profiles for substrate and inhibitory molecules were found to be indistinguishable for both CD26 and DPP IV-β. In addi-

tion, both proteins manifested optimum dipeptidyl-peptidase activity at pH 7.5, and resisted proteinase K digestion. The only distinct property that we found, was the binding of adenosine deaminase to CD26 but not to DPP IV-β. Among five anti-CD26 mAbs, only mAb 4H12 stained C8166 cells, suggesting that it cross-reacts with a cell-surface antigen which shares a common epitope with CD26. The cross-reactivity of mAb 4H12 with C8166 cells was found to persist after proteinase K digestion of cells (data not shown). It is most likely therefore, that this antigen is DPP IV-β. However, we have no direct evidence for the binding of this anti-CD26 mAb 4H12 to DPP IV-β.

Recently, CD26-like proteins have been described by several investigators in human cells and serum. Scanlan et al. (1994) have described a 95-kDa cell-surface antigen referred to as fibroblast-activation protein α (FAPα), which shows 48% amino acid sequence identity to CD26. In fibroblasts, FAPα could exist in an oligomeric form with CD26 at the cell membrane. However, FAPα has been reported to manifest very little DPP IV activity (Rettig et al., 1994). Another CD26-like protein secreted by activated T lymphocytes has been described by Duke-Cohan et al. (1995, 1996). This latter is a soluble protein of 175 kDa which cross-reacts with some mAbs against CD26, and also manifests DPP IV activity. This 175-kDa protein does not bind adenosine deaminase. However, it is unlikely that DPP IV-β is this 175-kDa protein, since gel-filtration experiments showed that DPP IV-β is a protein of 82 kDa (Fig. 6). Furthermore, DPP IV-β as DPP IV/CD26, is not affected by EDTA even at 100 mM (Table 2), whereas the DPP IV activity of the 175-kDa protein is inhibited by EDTA with an IC_{50} value of 8.9 mM (Duke-Cohan et al., 1996). No detectable DPP IV activity was observed in the supernatant of C8166 cells incubated several hours in NaCl/P_i containing 1% BSA. Thus the DPP IV-β is membrane associated and does not appear to be liberated in the culture medium. The cell-surface DPP IV activity in intact C8166 cells was not reduced in cells extensively washed with NaCl/P_i containing 100 mM EDTA or after several hours of incubation of washed cells in NaCl/P_i. C8166 cells provide a natural source of DPP IV-β, free of any potential contamination by CD26. Therefore, these cells should be used for the purification of DPP IV-β and for further characterization of this CD26-like protein.

The DPP IV activity of CD26 has been suggested to be essential for determining the half-life and activity of some natural substrates, such as substance P, chorionic gonadotropin hormone, monomeric fibrin and glucose-dependent insulinotropic polypeptide (reviewed by Yaron and Naider, 1993; Kieffer et al., 1995). Other N-terminus Xaa-Pro-containing substrates might represent some cytokines, such as IL-1β, IL-2, tumour-necrosis factor β (TNF-β) and granulocyte colony-stimulating factor (G-CSF). However, there is no evidence for the interaction of these cytokines with DPP IV under physiological conditions (for a review see Fleischer, 1994). However, the most documented function of CD26 appears to be in the T-cell-activation process (discussed above).

In relation to HIV infection, T lymphocytes of HIV-infected individuals have been shown to have an intrinsic defect in their recognition and response to recall antigens (Lane et al., 1985; Van Noesel et al., 1990), which is a specific property of $CD4^+$/$CD26^+$ T cells (Morimoto et al., 1989). Accordingly, several groups have reported a selective decrease in CD26-expressing $CD4^+$ T lymphocytes in HIV-1-infected individuals (De Pasquale et al., 1989; Valle-Blasquez et al., 1992; Vanham et al., 1993; Gougeon et al., 1996). In addition to these, memory $CD4^+$ T cells which are also characterized by the enhanced expression of CD26, have been shown to be preferentially infected by HIV-1 *in vitro*, and appear to be the principal *in vivo* reservoir for HIV within the $CD4^+$ T-cell population of HIV-infected individuals (Schnittman et al., 1990). Consistent with these, recent observations further suggest that CD26 might be implicated in HIV entry and its cytopathic effect in $CD4^+$ T cells (Callebaut et al., 1993, 1994; Oravecz et al., 1995; Callebaut and Hovanessian, 1996). Moreover, the defective *in vitro* recall antigen response in HIV-1-infected individuals was reported to be restored by the addition of soluble CD26 (Schmitz et al., 1996). Thus, these observations *in vivo* and *in vitro* suggest that CD26 might play an important role in HIV infection. Using various $CD4^+$ human T-cell lines expressing different levels of CD26, several authors have argued against the involvement of CD26 in HIV infection because they could not demonstrate a strict correlation between the level of CD26 expression and their permissivity to HIV infection of *in vitro* cell cultures (Lazaro et al., 1994; Morimoto et al., 1994). The lack of detection of CD26 in some reports, may in part be due to the sensitivity of the assay used. For example, $CD4^+$ Jurkat cells which are considered not to express CD26, do express low levels of CD26 which might be sufficient for HIV infection. Independently, DPP IV-β might compensate the lack of CD26 in some cells which do not express CD26.

In view of the different functions of CD26 involving different biochemical, immunological and viral processes, it remains essential to characterize the DPP IV-β, determine its expression in different types of cells and define its role in comparison with CD26.

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