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Cut to the chase: a review of CD26/dipeptidyl peptidase-4's (DPP4) entanglement in the immune system

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Summary

CD26/DPP4 (dipeptidyl peptidase 4/DP4/DPPIV) is a surface T cell activation antigen and has been shown to have DPP4 enzymatic activity, cleaving-off amino-terminal dipeptides with either L-proline or L-alanine at the penultimate position. It plays a major role in glucose metabolism by N-terminal truncation and inactivation of the incretins glucagon-like peptide-1 (GLP) and gastric inhibitory protein (GIP). In 2006, DPP4 inhibitors have been introduced to clinics and have been demonstrated to efficiently enhance the endogenous insulin secretion via prolongation of the half-life of GLP-1 and GIP in patients. However, a large number of studies demonstrate clearly that CD26/DPP4 also plays an integral role in the immune system, particularly in T cell activation. Therefore, inhibition of DPP4 might represent a double-edged sword. Apart from the metabolic benefit, the associated immunological effects of long term DPP4 inhibition on regulatory processes such as T cell homeostasis, maturation and activation are not understood fully at this stage. The current data point to an important role for CD26/DPP4 in maintaining lymphocyte composition and function, T cell activation and co-stimulation, memory T cell generation and thymic emigration patterns during immune-senescence. In rodents, critical immune changes occur at baseline levels as well as after *in-vitro* and *in-vivo* challenge. In patients receiving DPP4 inhibitors, evidence of immunological side effects also became apparent. The scope of this review is to recapitulate the role of CD26/DPP4 in the immune system regarding its pharmacological inhibition and T cell-dependent immune regulation.

Keywords: autoimmunity, B cell, cell activation, chemokines, T cells

Structure and characterization of CD26

Originally described 50 years ago [1], the lymphocyte cell surface protein CD26 possess a dipeptidyl peptidase-4 (DPP4) activity. It cleaves dipeptides from the N-termini of oligopeptides and smaller peptides with proline or alanine at the penultimate position, as illustrated in Fig. 1b [International Union of Biochemistry and Molecular Biology (IUBMB) Enzyme Nomenclature EC 3.4.14.5].

CD26/DPP4 is a homodimer and an integral type II glycoprotein anchored to the membrane by its signal peptide. The primary structure consists of a short six amino acid cytoplasmic tail, a 22 amino acid transmembrane, a 738 amino acid extracellular portion comprised of a flexible stalk, glycosylation-rich region, cysteine-rich region and catalytic region with the catalytic triad Ser_{630}, Asp_{708} and His_{740} (Fig. 1e). Recent studies have revealed that the transmembrane region contributes to enzyme activity and quaternary structure by dimerization [2]. The crystal structure of human CD26/DPP4 has been elucidated to reveal two domains: an eight-bladed propeller and an α/β-hydrolase domain. The propeller is open and consists of two subdomains made up of blades II–V and VI–VIII for the glycosylation-rich and cysteine-rich regions, respectively (Fig. 1d). Most monoclonal anti-CD26/DPP4 antibodies, as well as adenosine deaminase (ADA) and caveolin-1, bind to the glycosylation-rich domain of human CD26/DPP4, whereas collagen, fibronectin, plasminogen and streptokinase bind to the cysteine-rich region (Fig. 1a) [3–5]. There are two openings: a side opening and a propeller tunnel (Fig. 1a) [6,7]. The DPP4 substrate neuropeptide Y (NPY) was found to enter DPP4 at the side opening [8].

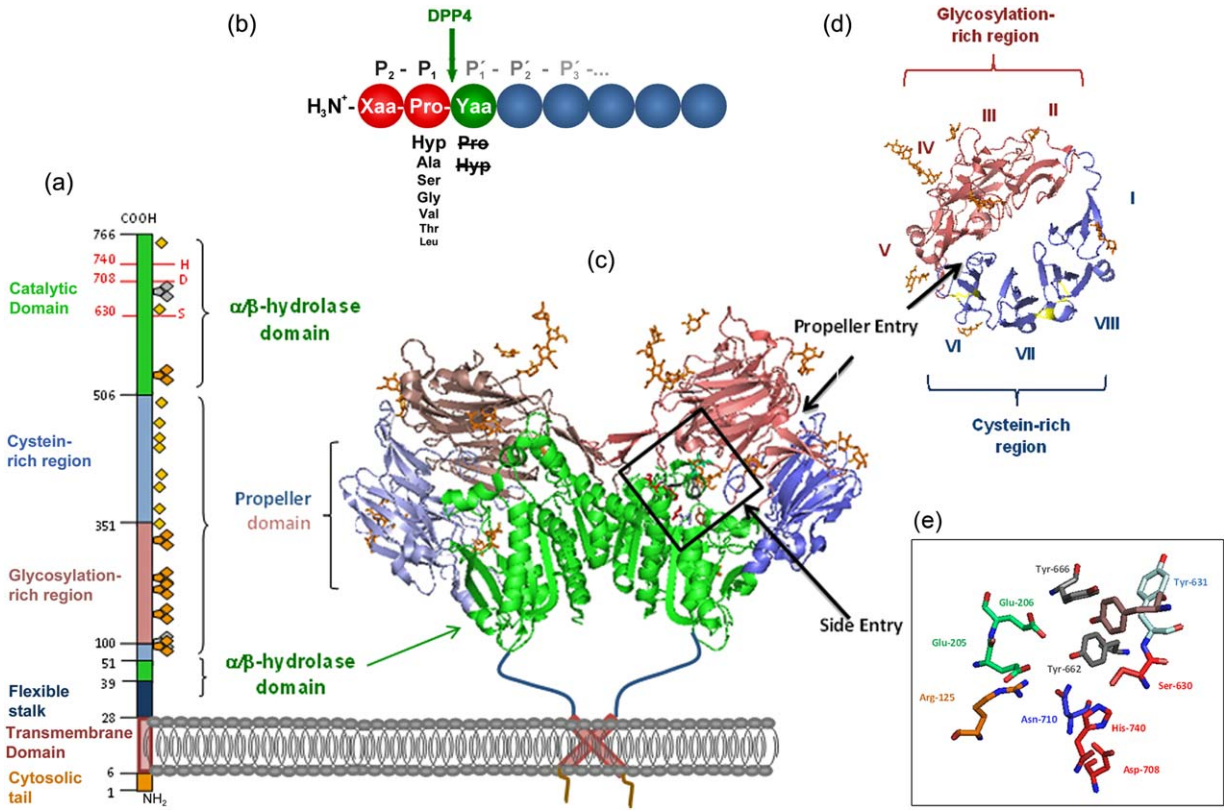


Fig. 1. Primary and quaternary structure of human dipeptidyl peptidase 4 (DPP4), based on Protein Data Bank: 1W1I. (a) Primary structure of DPP4 subunit, consisting of an intracellular tail (aa 1–6), transmembrane region (aa 7–28), flexible stalk (aa 29–39), glycosylated region (aa 101–350), cysteine-rich region (aa 55–100, 351–497), and catalytic region (aa 506–766). , *N*-glycosylation; , potential unoccupied *N*-glycosylation; , cysteine residues involved in S-bridges; red numbers and letters indicate the catalytic triad. (b) Substrate specificity of DPP4. X_{aa} and Y_{aa} indicate any amino acid. Decreasing font of amino acid at P_1 position represents declining rate of hydrolysis. Amino acids crossed out must not occupy P_1'. Arrow indicates site of cleavage. (c) quaternary structure of homodimeric human recombinant DPP4 as determined by Weihofen *et al.*, 2004, showing the α/β-hydrolase domain (aa 39–51 and 506–766) in green and propeller domain (aa 55–497) with the glycosylation-rich subdomain (red) and the cystein-rich subdomain (blue). (d) Propeller domain viewed from the top, illustrating the eight propeller blades designated with roman numbers and two subdomains. (e) Active site zoomed in, depicting the residues involved in catalysis, catalytic triad Ser_{630}, Asp_{708}, His_{740} are shown in red, Tyr_{547} responsible for oxyanion hole in brown, Tyr_{662} and Tyr_{666} forming the hydrophobic pocket in grey, Arg_{125} and Asn_{710}, contributing to an electrostatic sink in orange and blue, respectively, and Glu_{205} and Glu_{206} ensuring *N*-terminal anchoring in pale green. S–S bridges are illustrated in yellow and carbohydrates in orange. Structures were drawn with PyMOL™ 2008 DeLano Scientific LLC, using Protein Data Base: 1W1I [7].

Post-translational modification

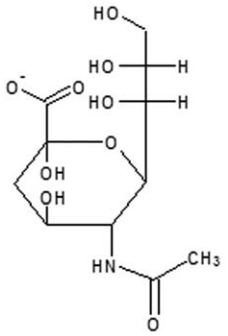
Glycosylation-based heterogeneity

Carbohydrates contribute approximately 18–25% of the total molecular weight, and human DPP4 contains nine potential *N*-glycosylation sites [4]. Analysis of oligosaccharides revealed extensive heterogeneity composed of one high mannose type and several mono-, bi-, tri- and tetra-antennary complex types of *N*-glycans [9,10]. Thus, DPP4 is comprised of several isoforms differing in sialylation and being dependent upon species, tissue, epitope and differentiation status [11,12]. While co-translational core *N*-glycosylation is responsible for the folding and stability of DPP4 [13–15], *N*-terminal sialylation appears to play a more (patho-)physiological role (summarized in Fig. 2).

Resting T cells were determined to be more sialylated than activated cells [16]. Hypersialylation has been associated with HIV-infection, rheumatoid arthritis, systemic lupus erythematosus and ageing [16,17], whereas decreased sialylation has been observed in lung cancer [18]. The process of sialylation seems to be dynamic, as de- and re-sialylation has been detected in rat hepatocytes [19,20]. Furthermore, trafficking of DPP4 to the apical surfaces has been shown to be influenced greatly by terminal sialylation [21,22].

Tyrosine phosphorylation

Tyrosine phosphorylation of DPP4 has been described recently in association with cellular c-Scr, HIV-Tat and mannose 6-phosphate binding [23–25].



Properties and Functions of DPP-4 Sialylation

- Bisialylation > monosialylation = trisialylation > tetrasialylation >> pentasialylation
- Species specific
- Tissue specific
- Epitope specific
- Dependent on cell differentiation state
- Age dependent
- Dynamic: De- and resialylation
- Facilitates trafficking to apical membrane

Increased Sialylation:

- HIV infection
- Rheumatoid arthritis
- Systemic Lupus erythematosus
- Old age
- Resting T-cells

Decreased Sialylation:

- Lung cancer
- Activated T-cells
- Young age

Fig. 2. Structure, properties and functions of dipeptidyl peptidase 4 (DPP4) sialylation [10–12,16–18,20].

Soluble CD26/DPP4 (sCD26)

CD26/DPP4 exists in a soluble form, thought to be shed from the membrane into plasma, which still maintains its enzymatic activity (for review see [26,27]). Recently, the bone marrow – but not the kidney – could be determined as one of the sources of soluble serum CD26/DPP4 by transplantation studies in DPP4-deficient rats [28]. Standard concentrations of serum and cerebrospinal fluid (CSF) levels for healthy children and adults have been assessed [4,26,27,29,30]. The alterations of human DPP4 activity in the serum and CD26/DPP4 expression in numerous diseases will be discussed in more detail below and are summarized in Table 3.

Gene

The gene structures of human and mouse *DPP4* show great homology, with some minor variation in gene and exon size [31,32]. In humans, the gene is located on chromosome 2q24.3, spans 81.8 kb and contains 26 exons. The nucleotides encoding the sequence around the active site serine (Gly-X-Ser-X-Gly) are split between exons 21 and 22. Similarly, the exons of the catalytic triad are 22 for Ser, 24 for Asp and 26 for His [31]. In F344/DuCrj(DPP4neg) rats, among other point mutations, a G to A transition at nucleotide 1897 in the *Dpp4* cDNA sequence leads to a substitution of Gly^{633} to Arg in the catalytic centre of the enzyme (Gly^{629}–Trp–Ser–Tyr–Gly^{633}) [33] and a retention of the mutant protein in endoplasmatic reticulum largely abrogating expression of the mutant CD26/DPP4 protein [34,35]. The Ser^{631} is the active serine of rat DPP4 and the same point mutations were reconfirmed in otherwise independent substrains of F344 rats [36,37], and were also used to generate DPP4-deficient congenic DA.F344-$Dpp4^{m}$/SvH rats [38].

DPP4 contains neither a TATAA nor a CCAAT box as a promoter, but has a C- and G-rich region containing several consensus binding sites for transcriptional factors [39,40]. The expression is regulated at RNA level and is organ-specific [41–44]. Within an organ, it is dependent upon cell type, differentiation state and activation state. Several cytokines are known to regulate DPP4 expression in a cell-type-specific manner such as interferon (IFN)-γ, tumour necrosis factor (TNF)-α and lipopolysaccharide (LPS) in human umbilical vein endothelial cells (HUVEC) [42,45–49]. In some tumours, binding of the transcription factors is enhanced by certain cytokines also modifying the expression of CD26/DPP4 [50].

Expression of CD26/DPP4

CD26/DPP4 is expressed ubiquitously in many tissues – endothelia and epithelia – including but not limited to kidney, liver, lung, intestine and, interestingly, also on immune cells (e.g. T cells, activated B, activated natural killer (NK) cells and myeloid cells) [31,35,41,51–55].

T cells

CD26/DPP4 is expressed on only a fraction of resting T cells, mainly $CD4^{+}CD45RO^{+}$ memory T cells, but is upregulated strongly following T cell activation [54]. Detailed expression patterns have been recently reviewed elsewhere [56]. Altogether, up to 70% of peripheral blood lymphocytes can express detectable CD26/DPP4 protein levels [55]. Importantly, CD26/DPP4 has been described as a negative selection marker for human regulatory T cells (T_{regs}) [57,58]. In contrast, human T helper type 17 (Th17) cells showed very high expression of enzymatically active CD26/DPP4 [59]. Recently, mucosal-associated invariant T cells (MAITs) have also been shown to express high levels of CD26/DPP4 in humans [60].

NK cells

NK cells usually express only low amounts of CD26/DPP4, but surface expression increases significantly up to 30% after interleukin (IL)-2 stimulation as well as IL-12 or IL-

15 stimulation [61–63]. A functional aspect of this up-regulated expression of CD26/DPP4 on NK cells might be an increased CD16-dependent lysis. This may be caused by the mediation of protein tyrosine phosphorylation and an involvement of CD26/DPP4 in the production of cytokines by NK cells [35,64,65]. In a model of lung metastasis, NK cell cytotoxicity against tumour (MADB106) cells proved to be diminished in a CD26/DPP4-deficient F344 rat substrain. Additionally, the absolute capacity of single NK cells to lyse tumour target cells is reduced in a congenic rat model, suggesting that CD26/DPP4 enzymatic activity sustains NK cytotoxicity [35,38]. NK cells exert their cytotoxicity via secretory lysosomes, and CD26/DPP4 was identified on the membrane of secretory lysosomes in NK cells by proteomic analysis [66,67]. Concerning the NK cell maturation, the percentage of NK cells in DPP4-deficient animals was increased significantly, while total leucocyte numbers were decreased in a congenic DPP4-deficient rat model, as well as in knock-out mice [38,68].

B cells

Upon activation, up to 50% of human B cells express CD26/DPP4 [53]. Specific suppression of DPP4 activity reduces the B cell activation and synthesis of DNA in a dose-dependent manner [53,69]. In mice, an impaired immunoglobulin isotype switching of B cells in CD26-deficient mice became apparent in one study [68], while another could not show any differences [70]. Another *in-vitro* study showed no effect of CD26/DPP4 deficiency on B cells in rats expressing a truncated CD26 molecule lacking the DPP4 activity [71]. However, monitoring the long-term effect of DPP4 deficiency *in vivo,* we found B cell numbers to be decreased markedly in later life [72]. One of the best substrates of DPP4, neuropeptide Y (NPY), has been shown to mobilize B1-like B cells selectively [73]. Hence, a pharmacologically induced lack of DPP4 function may, indirectly, modulate 'stress-induced' B cell redistribution and composition of B cell reservoirs. In humans, CD26/DPP4 is currently under investigation as a possible prognostic marker in B cell carcinoma [74].

Myeloid cells

CD26/DPP4 was shown to be chemorepellent for human and murine neutrophils, whereas DPP4 truncation affected recruitment of eosinophils via its substrate eotaxin (CCL11) [38]. CD26/DPP4 has also been shown to be expressed on dendritic cells [75–77] and, in rodents, on monocytes/macrophages [78]. In rats, DPP4 could be shown in Küpffer and microglia cells, respectively, with DPP4 being expressed in lysosomes and increased upon activation [79–81]. Data on the role of CD26/DPP4 on monocytes/macrophages in humans are scarce. Nevertheless, a special interest arises from the fact that long-term DPP4 inhibition influences atherosclerosis positively by inhibiting inflammation mediated by myeloid cells [82]. The detailed involvement of CD26/DPP4 in atherosclerosis has been reviewed recently elsewhere [56].

Substrates of DPP4

Many gastrointestinal hormones, growth factors, neuropeptides and chemokines share either the X-Pro or -Ala motif at their N-terminus and have been shown to be cleaved by DPP4, as summarized in Table 1 (for a review, see [4]). Substrates of DPP4 are involved in neuroendocrine system, nociception, metabolism/nutrition, cardiovascular functions, immune regulation such as chemotaxis, and in infection (Table 1; Fig. 3) [4]. Structure–activity relationships have shown that truncation by DPP4 either results in modulation of receptor selectivity with different physiological responses such as in NPY or ablation of receptor selectivity with additional but lower physiological outputs, such as in substance P, or inactivation towards receptor response such as in glucagon-like peptide 1 (GLP-1), pituitary adenylate cyclase-activating polypeptide (PACAP), eotaxin and stromal-derived factor (SDF)-α [4,56,83]. However, the regulation of chemokines with regard to immune response and receptor selectivity is extremely diverse (for a review, see [84]). After truncation, most DPP4 substrates, being devoid of the X-proline N-terminal dipeptide, are degraded more rapidly by additional peptidases [85]. This is the case for substrates such as substance P being degraded further by aminopeptidase N, or GLP-1 being degraded by neprilysin [85,86]. Intriguingly, many cytokines also contain an X-Pro N-terminal motif, but DPP4 could only truncate their fragments [87].

Binding partners

Several molecules have been shown to bind to DPP4, thereby triggering various physiological responses and modulation immune responsiveness [4]. These can be subdivided into four categories: immune regulation, cell adhesion, cell–cell communication and peptide transport (Table 2).

Physiological role of DPP4

DPP4 has been described as a 'moonlighting' protein due to its multiple functions. DPP4 exerts its physiological roles either via its enzymatic activity by regulating many peptides or via its interactions with a variety of binding partners [88]. It is involved in processes such as nutrition, nociception, cell-adhesion, psychoneuroendocrine regulation, immune response and cardiovascular adaptation, as reviewed recently [4,5,27,88–92] and summarized in Fig. 3a.

Function of CD26/DPP4 in the immune system

T cell development

Bone marrow-derived T progenitor cells undergo maturation in the thymus [93]. The vast majority of cells in the

Table 1. Selection of known dipeptidyl peptidase 4 (DPP4) substrates [4].

	Peptide	N-terminus	# Amino acids	Selectivity‡	Physiological effect
Inactivation/Alteration *in vivo*	*Pancreatic polypeptides:*				
and *in vitro*	Peptide YY	YP↓IKPE...	36	+ (++)*	M/N
	Neuropeptide Y	YP↓SKPD...	36	+++	Ne, No, C, Im
	Chemokines:				
	SDF-1α	KP↓VSLS...	68	++++	Im
	MDC	GP↓YG↓AN	69	++++ (++)†	Im
	I-TAC	FP↓MFKR...	73	+++	Im
	IP-10	VP↓LSRT...	77	++	Im
	Mig	TP↓VVRK...	10	++	Im
	RANTES	SP↓YSSD...	68	+	Im
	Eotaxin	GP↓ASVP...	74	+	Im
	LD78β	AP↓LAAD...	70	+	Imm
	PACAP/glucagon family:				
	GLP-1	HA↓EG↓TF...	30	++ (+++)*	M/N
	GIP	YA↓EGTF...	42	++	M/N
	PACAP38/PACAP27	HS↓EG↓IF...	38/27	++ (+)†/+ (+)†	M/N, Ne
	Glucagon	HS↓QGTF...	29	++	M/N
	GLP-2	HA↓DG↓SF...	33	+	M/N
	Neuropeptides/Peptides:				
	Substance P	RP↓KP↓Q...	11	++ (++)†	No, Ne, C, Im
	Endomorphin-2	YP↓WF-NH_2	4	+	No
	GRP	VP↓LP↓AG...	27	+++ (+++)†	M/N
	Procalcitonin	AP↓FRSA...	116	n.d.	Inf
Inactivation/Alteration	*Chemokines:*				
shown *in vitro* only	SDF-1β	KP↓VSLS...	72	n.d.	Im
	PACAP/Glucagon family:				
	GHRH44/GHRH29-NH_2	YA↓DAIF...	44/29	+++	Ne
	Oxyntomodulin	HS↓QGTF...	37	++	Ne
	PHM	HA↓DGVF...	27	++	Ne
	VIP	HS↓DA↓VF...	59	+ (+)†	Ne, M/N
	Secretin	HS↓DGTF...	27	+	M/N
	Neuropeptides/peptides:				
	BNP-32	SP↓KMVQG...	32	++	C
	IGF-I	GP↓ETLCGA...	105	+	Ne, M/N
	Haemorphin-7	LV↓VYPW...	10	++	C
	β-casomorphin	YP↓FVEPI	7	++	Ne, M/N
	Endomorphin-1	YP↓FF-NH_2	4	+	No
	Enterostatin	VP↓DP↓R	5	+	M/N
	Tyr-MIF-1	YP↓LG-NH2	4	+	No
	Morphiceptin	YP↓FP-NH2	4	n.d.	No
	Kentsin	TP↓RK	4	n.d.	No
	Vasostatin-1 (chromogranin $A_{1–76}$)	LP↓VNSPM...	76	+++	C
	SR-17 (chromogranin $B_{586–602}$)	SA↓EFPDFY...	17	+	C
	Pro-colipase	VP↓DP↓R...	101	+	M/N
	CLIP	RP↓V...	22	+	Ne
	Trypsinogen pro-peptide	FP↓T...	8	+	M/N
	Trypsinogen (pig)	FP↓T...	231	+	M/N
	Prolactin (sheep)	TP↓V...	198	+	Ne, M/N
	Aprotinin (bovine)	RP↓D...	58	+	Trypsin inhibitor
	Chorionic gonadotrophin	AP↓D...	243	+	Ne
	Promellitin	AP↓EP↓EP↓	50	n.d.	Bee venom
	Chemokine:				
	GCP-2	GP↓VS...	75	n.d.	Im

*Different values obtained by various laboratories. †Selectivity of second cleavage of the same substrate. ‡*In-vitro* values. k_{cat}/K_M values: + = 0 – 1 × 10^5 M^{-1} s^{-1}, ++ = 1 – 10 × 10^5 M^{-1} s^{-1} +++ = 10 – 30 × 10^5 M^{-1} s^{-1}, ++++ = 30 – 50 × 10^5 M^{-1} s^{-1}. Ne = neuroendocrine; No = nociception; M/N = metabolic/nutrition; C = cardiovascular; Im = immunology; Inf = infection; n.d. = not determined.

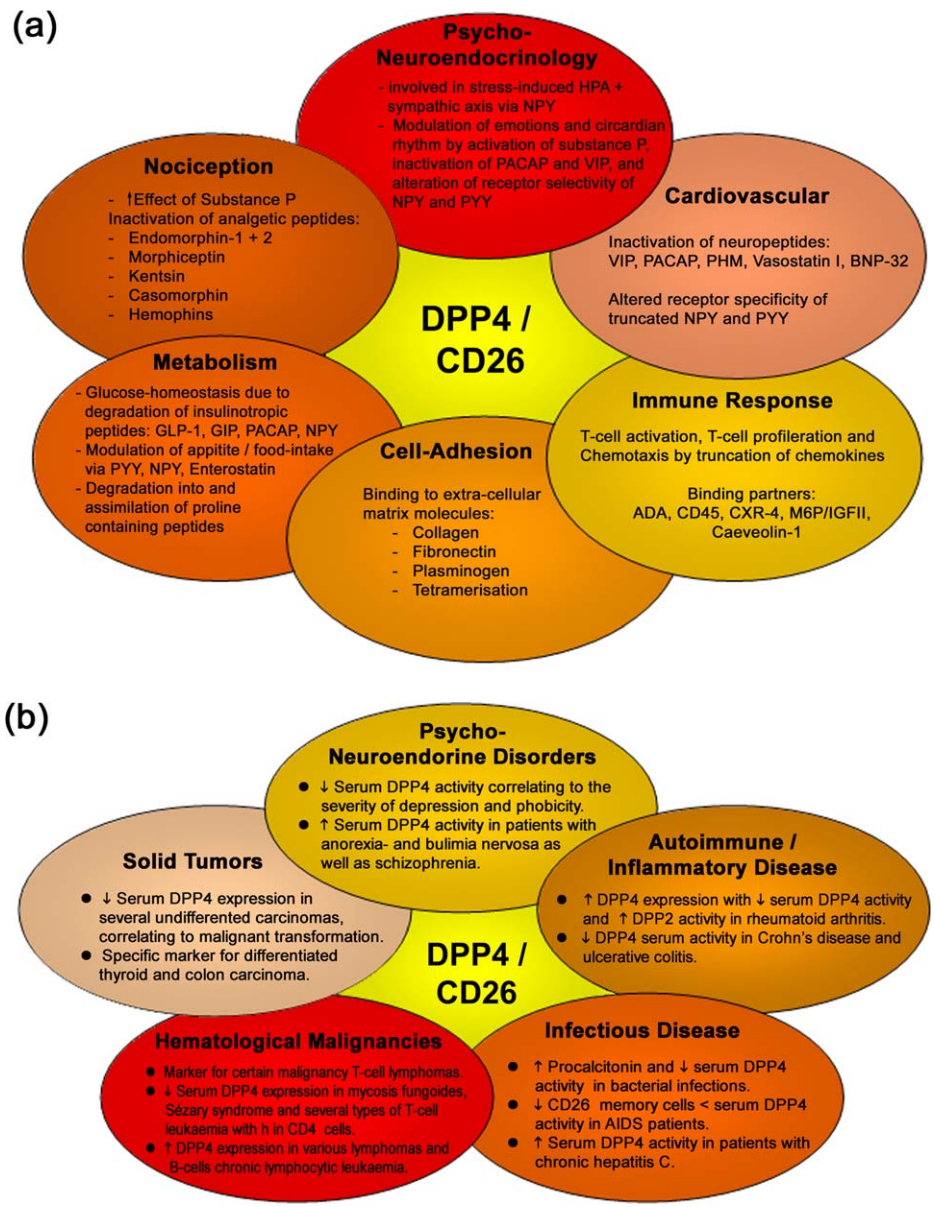


Fig. 3. Physiological and pathological processes influenced by dipeptidyl peptidase 4 (DPP4) [4,92,93]. (a) Summary of physiological roles of DPP4; (b) pathophysiological role of DPP4 with either altered expression and/or activity.

thymus express CD26/DPP4 and, therefore, it is thought to be a thymic maturation marker in rodents as well as humans [55,94]. CD26/DPP4 has been described as a mediator of lymphocyte migration through the thymus. It is down-regulated on cells that undergo apoptosis and up-regulated on maturing thymocytes, reaching the highest level of CD26/DPP4 expression in mature CD4 or CD8 single-positive T cells within the thymus [94–96]. Findings are conflicted in the periphery, but describe the expression of CD26/DPP4 favourably as a characteristic of memory T cells, with CD26/DPP4 bright cells responding maximally to recall antigens [97–100]. CD26/DPP4 has shown the ability to act as a non-integrin receptor, being able to bind fibronectin and collagen [101,102]. Another study indicated that CD26/DPP4 acts as an endogenous inhibitor of T cell motility regulated by a cascade of interacting cell surface molecules [103]. Proper adhesion is of great importance: first for progenitor cells entering the thymus; secondly, for thymocytes trafficking from cortex to medulla during their maturation; and thirdly, egressing as mature T cells [93]. Apparently, CD26/DPP4-associated enzymatic activity is controlled ontogenetically during T cell maturation and may be involved in thymic deletion of emerging clones [95,96]. However, the precise functional role of CD26/DPP4 expressed on maturing thymocytes remains unclear.

The thymus undergoes an age-dependent involution but remains active up to a high age, playing a central role in replenishing the peripheral T cell pool [104]. Impairment of CD26/DPP4 function under long-term conditions had a remarkable effect on T cell subpopulations in a Fischer-344 (F344) rat model. In CD26/DPP4-deficient F344 rats the

Table 2. Summary of molecules known to associate with dipeptidyl peptidase 4 (DPP4).

	Binding partner	Binding site	Function	Refs
Immunology	ADA	α1 and α2 of ADA bind to DPP4 via loop A between blades IV and V, and loop B between β 3 and β 4 of blade V, respectively. Glycosylation of DPP4-Asn-229 involved, as observed in crystal structure Ternary complex between A_{2B}R-ADA of dentritic APC to lymphocytic CD26. ADA binding only in higher mammalian and species-dependent: human > porcine ≠ rat ≠ mouse	Binding of extracellular ADA to A_{B2} receptor on dentritic APC cells and CD26 on T cells to form a ternary complex, resulting in: co-stimulation of T cells, T cell proliferation, T cell protection	[7,193] [24]
	CD45	Binding of DPP4 at the intracellular PTP2 domain of CD45 causes recruitment of both enzymes on lipid rafts	Signal transduction resulting in phosphorylation of Erk1/2TCR-zeta, ZAP70 by p56lck	[191]
	M6P	Carbohydrate moiety of DPP4	Induces association of M6P/IGFRII and DPP4	[190]
	Caveolin-1	Binding of caveolin-1 on APC cells to soluble CD26 at aa 201–210 and Ser_{630} leading to: T cell proliferation + ⇑ CD26 on T cells ⇒ binding of CARMA-1 on cytoplasmic tail of CD26 ⇒ phosphorylation of caveolin-1 ⇒ dissociation of Tollip and IRAK-1 ⇒ phosphorylation of IRAK-1 ⇒ activation of NF-κB ⇒ ⇑ CD86	Causes up-regulation of CD86 on TT-loaded dentritic monocytes, thus leading to the association of APC with CD28 on T cells and subsequently to T cell activation	[189] [114] [112]
	CARMA1 (CARD11)	Binding of CARMA-1 on cytoplasmic tail of CD26 ⇒ recruitment of CARMA-1, CD26, Bcl10 and IkappaB kinase complex to lipid rafts ⇒ signal transduction	Leading to activation of ZAP70, PLC, MAPK, phosphatyl inositol and ⇑ IL-2	[112] [112]
	M6P/IGFRII	Needs M6P bound on DPP4	T cell activation, internalization of DPP4, transendothelial migration by binding of lymphocytes to endothelial DPP4	[25] [190]
	CXCR4 receptor	?	Reduction of chemoattraction, co-internalization in presence of SDF-α, formation of invadopodia in presence of SDF-α and gp120	[194]
	Tromoxane A_2 receptor	?	Natural DPP4 inhibitor, T cell suppression	[195]
	HIV-TAT	2 binding sites, sialic acid moiety and active site of DPP4 Crystal structures shows P_2 and P_1 of $Tat_{1–9}$ bind to S_1 and S_2 of DPP4, respectively	HIV-entry, inhibitor of DPP4 due to reverse binding at the active site	[16,24]
	HIV-gp120	Cysteine-rich region, HIV-gp 120 interacts via its C3 region with DPP4 on lymphocytes	HIV-entry and subsequent apoptosis; inhibits ADA binding to DPP4 in presence of CXCR4, although binding site distinct to ADA	[194]

Table 2. *Continued*

	Binding partner	Binding site	Function	Refs
Cell adhesion/cell-cell communication	Collagen	Cysteine-rich region between aa 238 and 495	Extracellular adhesion? Cancer? Metastasis?	[196]
	Fibronectin (FN)	Cysteine-rich region of DPP4 between aa 469–479 via aa LTSRPA motif (FN)	Fibronectin-mediated spreading of fibroblasts, lung metastasis, dissociates in presence of soluble DPP4	[197]
	Plasminogen receptor (PgR) Plasminogen/ Plasmin (Pg/Pl)	Cysteine-rich region of DPP4 close to ADA binding site, sialic acid carbohydrate moieties of plasminogen binds to Pg-receptor/ DPP4 complex (aa 313–319)/ $\alpha_{IIb}\beta_3$ and urinary/tissue plasminogen activator uPA/tPA. Activated plasmin (Pl) changes conformation and binds to DPP4. Quintary complex abolished by angiostatin binding to DPP4	⇑ Ca^{2+} response in synovial fibroblasts, activation of synovial fibroblasts, signal transduction in prostate cancer cells resulting in ⇑ MMP 9. Quintary complex of ADA, Pg 2, DPP4 and urinary plasminogen activator (uPA/tPA) and PgR ⇒ ⇑ Pg 2 to plasmin	[198] [199] [5]
	Streptokinase (SK)	Cysteine-rich region of DPP4 only from rheumatoid synovial fibroblasts via aa LTSRPA motif (SK)	Ca^{2+} response in synovial fibroblasts, ⇑ DPP4 autoantibodies, SK bound to DPP4 hydrolysis FN	[188] [198]
	Vitronectin	Sialic acid moiety of DPP4	Extracellular adhesion? Metastasis? Complement system? Coagulation?	[16]
	Glypican 3	Both glycosylated and unglycosylated glypican 3 bind to DPP4	Natural DPP4 inhibitor. Binding of soluble glypican 3 to CD26 ⇓ cell-proliferation and induces apoptosis	[200]
	FAP	Heteromeric complex	Heteromeric complex on invadopodia causing metastasis, tumor invasion, angiogenesis, wound healing and fibroblast migration	[201]
	DPP4	Blades IV of each subunit align to form an eight-stranded antiparallel sheet, possibly Asn229 involved	Tetramerization, cell-adhesion, cell–cell communication?, chemotaxis?	[202]
Peptide transport	Na^+/H^+ exchanger isoform NH3	?	Peptide transporter on microvilli membrane of renal proximal tubule, reabsorption of dipeptides with proline. In prostate cancer, association of DPP4, Pg 2 and NH3 results in Ca^{2+} signal cascade and in intracellular pH ⇓ tumour cell-proliferation + invasiveness	[203] [204]

$CD4^+$ T cell pool showed decreased numbers of memory T cells, as well as rat tracheal epithelial (RTE) and increased numbers of naive T cells instead [72]. Also, thymus architecture appears to be altered in this model of chronic genetic CD26/DPP4-deficiency. Again, in CD26/DPP4-deficient mice, the percentage of $CD4^+$ T cells is lower among the splenic lymphocyte population [68]. In another congenic CD26/DPP4 rat model, the overall number of leucocytes proved to be decreased in CD26/DPP4-deficient animals [38]. Similar observations were made in humans, as (reversible) dose-dependent decreases in absolute lymphocyte numbers were observed in patients receiving DPP4-inhibitors [105]. One case of severe leucopenia associated with DPP4 inhibition has been reported, but causality has not been proven [106]. In contrast, a current meta-analysis, including 16 papers with randomized trials

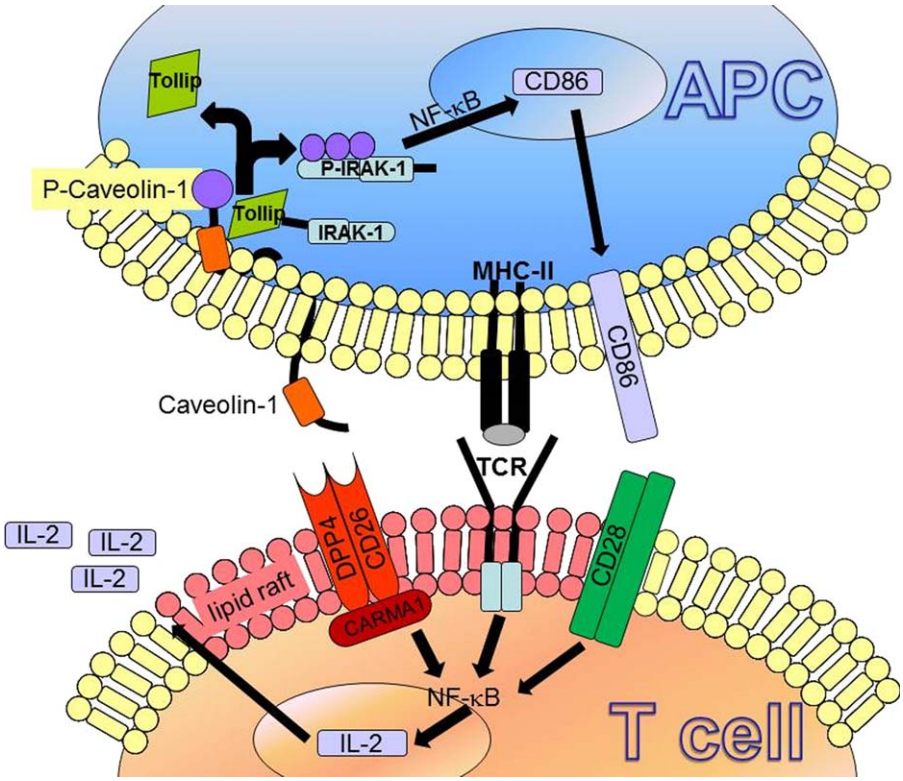


Fig. 4. A model of CD26 interacting with caveolin-1 resulting in T cell co-stimulation and activation as proposed by Ohnuma *et al.* [188]: after antigen uptake via caveolae by antigen-presenting cells (APCs), caveolin-1 is exposed on the cell surface and aggregates in the immunological synaps in lipid rafts. Consequently, caveolin-1 binds to CD26 and is phosphorylated, leading dissociation of interleukin (IL)−1 receptor-associated kinase 1 (IRAK-1) and Tollip. This lead to activation of nuclear factor (NF)-κB and results in CD86 up-regulation, supporting the immunological synapse and thus T cell co-stimulation. In T cells, after caveolin-1 to CD26 binding, (CARD11) CARMA1 is recruited to the cytosolic portion of CD26. Activation of NF-κB lead to T cell proliferation and IL-2 production.

comparing DDP4 inhibitors in addition to sulphonylurea, could not identify a significantly increased risk of this potential side effect [107].

T cell stimulation

Early *in-vitro* studies showed that DPP4 inhibition decreases the induction and activation of cytokines controlling human T lymphocyte proliferation [108]. DPP4 inhibition on mitogen-stimulated thymocytes and splenocytes inhibited DNA synthesis as well as production of IL-2, IL-6 and IL-10, and increased secretion of the regulatory cytokine transforming growth factor (TGF)-β1 [109]. In congenic rats, the T cell proliferative response of CD26/DPP4-deficient rats upon stimulation with anti-T cell receptor (TCR) antibodies was decreased fivefold *in vitro* [38]. In the past, there has been a controversial debate as to what extent CD26/DPP4 and its catalytic region are important for T cell co-stimulation [110,111]. Recent *in-vitro* findings now demonstrate that CD26/DPP4 is able to trigger direct T cell activation and proliferation directly via (=CARD11) CARMA1-mediated nuclear factor (NF)-κB activation in T cells [112]. Additionally, CD26/DPP4 on T cells interacts directly with antigen-presenting cells (APCs) via caveolin-1. Upon linkage, Tollip and interleukin-1 receptor-associated kinase 1 (IRAK-1) disengage from caveolin-1 leading to subsequent IRAK-1 phosphorylation [113]. As illustrated in Fig. 4, this results in an up-regulation of the co-stimulatory molecule CD86, which enhances the bond of the immunological synapse [113]. One the other side of the immunological synapse, blocking CD26/DPP4-mediated T cell co-stimulation with soluble caveolin-1-immunoglobulin (Ig) fusion protein induces anergy in $CD4^+$ T cells [114]. A recent study demonstrates that CD26-mediated co-stimulation of $CD8^+$ T cells is enhanced compared to that obtained through CD28-mediated co-stimulation [115]. The clinical relevance of these findings remains to be determined, as one study showed intact T cell-dependent immune responses to antigenic challenge after specific DPP4-inhibition and in $CD26^{-/-}$ mice [70]. However, the clinical use of DPP4-inhibitors could prove to be critical, as the catalytic center of CD26/DPP4 is part of the linking site required for co-stimulation [113]. Besides co-stimulation, direct anti-inflammatory mechanisms of DPP4 inhibitors are discussed [116]. Yazbeck *et al.* propose a model of conformational change in the intracellular domain after binding an inhibitor to the catalytic center of CD26/DPP4. Subsequently, T cell proliferation and production of proinflammatory cytokines are suppressed [116–118].

Involvement of CD26/DPP4 in pathology

Due to its ubiquitous distribution and involvement in various physiological processes, a great number of pathological conditions are associated with either altered DPP4 expression and/or activity correlating with the severity of the respective condition. These can be subdivided into at least five categories, as illustrated in Fig. 4b:

Table 3. Summary of altered CD26/dipeptidyl peptidase 4 (DPP4) activity and expression in human sera [4,5,26,27,30,91]

	Disease	Serum CD26/DPP4	Serum CD26/ DPP4 activity	Remarks
Healthy	Male*		⇑	
	Female*		⇓	
	Age*		⇑	
Psychological diseases	Major depression	⇓	⇓	⇓ ADA activity
	Schizophrenia		⇑	
	Anxiety		⇓	
	Stress		⇓	
	Anorexia nervosa		⇑	⇓ $T^{+CD26/CD25}$ cells
	Bulimia		⇑	⇓ $T^{+CD26/CD25}$ cells
	Alcoholism		⇓	
Autoimmune diseases	Rheumathoid arthritis	⇓	⇓	⇑ sDPP-2 activity, ⇑ sCD30 ⇑ $Synoviocytes^{CD26}$
	Lupus erythematosus	⇓	⇓	⇑ DPP-2 ⇓ $PBMC^{CD26}$
	Sjögren syndrome	⇓	⇓	⇑ DPP-2 in leucocytes
	Psoriaris	⇓		⇑ ADA
	Scleroderma	⇓		
	ANCA-associated vasculitis	⇓		⇑ sCD30 ⇑ IL-10
	Coeliac disease		≈	⇑ intestinal CD26
	Allergic asthmatics	⇑	⇓	⇑ $T^{CD26/CD4}$ cell, ⇑ iNK ⇑ eosinophils
	Diabetes type 1	⇑	≈	⇓ $T^{CD26/CD4}$ ⇓ $T^{CD26/CD8}$
Inflammatory/infectious diseases	Pancreatitis		≈	
	Gastric ulcer		⇓	
	Acute hepatitis	⇑	⇑	
	Chronic hepatitis	⇓	⇑	
	Crohn's disease		⇓	⇑ DPP4 ⇑ FAP enterocyes
	HIV	≈	⇓	⇑ ADA, ⇓ T^{CD26} cell
	Sepsis		⇓	
Metabolic/cardiovascular	Diabetes type 2		⇓	
	Hypertension		⇑	
	Cirrhosis		⇑	
	Osteoporosis		⇑	
Cancer/tumour	Gastric cancer	≈	⇓	
	Bile duct cancer		⇑	
	Colorectum	⇓	⇓	
	Pancreatic cancer		⇓	
	Oral sqamous cell carcinoma	⇓	⇓	
	Hepatocellular carcinoma		⇑	
	Multiple myeloma		⇓	
	Hodgkin's disease		⇓	
	Lymphosarcoma		⇓	

*In healthy subjects, males show a higher baseline activity of CD26/DPP4 compared to females. In males as well in females DPPV activity is higher in older individuals compared to younger ones.

psychoneuroendocrine disorders, autoimmune and inflammatory diseases, infectious diseases, haematological malignancies, as well as solid tumors [4,91,92]. However, to the best of our knowledge, DPP4 expression or activity is not used routinely for diagnostic purposes in the clinic. Nevertheless, altered CD26/DPP4 activities or concentrations in serum have been associated with various pathogenic conditions involving psychological, autoimmune, inflammatory, infectious, metabolic and cardiovascular disorders, as well as tumor and cancer, as summarized in Table 3. Although, previously, several DPP4-like enzymes were described to contribute to the overall DPP4-like activity in serum such as attractin and β-DPP IV, it is now generally accepted that CD26/DPP4 constitute more than 90% of the overall DPP4-like activity in serum and plasma [119,120].

Role of CD26/DPP4 and its inhibition in human diseases and their animal model

CD26/DPP4 has been linked to a number of diseases as summarized in Table 3, including but not limited to

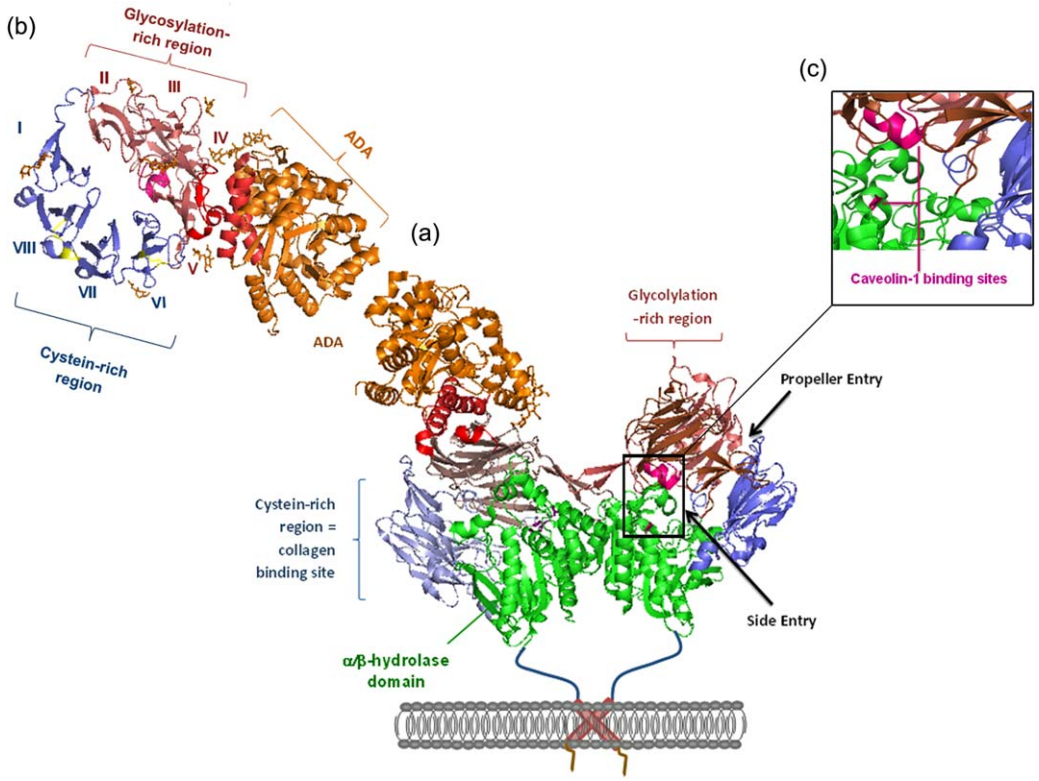


Fig. 5. Crystal structure of human dipeptidyl peptidase 4 (DPP4) and bovine adenosine deaminase (ADA) obtained from Protein Data Bank: 1W1I. (a) DPP4 crystal structure associated with bovine ADA at its glycosylation-rich region of the propeller domain. (b) Top view of propeller domain, showing ADA binding site at bladea 4 and 5 as well as ADA interactions with carbohydrates of N229. (c) Caveolin-1 binding site at aa 201–210 and Ser_{630}[7].

asthma, multiple sclerosis, arthritis and inflammatory bowel disease.

Asthma

Allergic asthma is one of the most common diseases, with its prevalence having increased dramatically in developed countries during the last two decades [121]. Its pathogenesis involves a complex series of reactions within the airways that is associated with allergen-specific airway hyperresponsiveness and inflammation, which can be studied in animal models [122]. The expression of CD26/DPP4 in the bronchi was described first by the group of van der Velden, showing a localization of CD26/DPP4 in serosal glands, blood vessels and on T cells [123], but there were no differences between asthmatics and healthy controls for the expression of CD26/DPP4 in the lamina propria determined by biopsies. However, investigating the effects of airway inflammation in rats, we found a significant increase of DPP4 enzymatic activity in the lung parenchyma. Also, strong immunohistochemical staining and high mRNA levels were detected in bronchial epithelium and trachea [124]. Furthermore, the expression of the soluble form of CD26/DPP4, in the blood as well as on T cells, increased in patients suffering from asthma [125]. Conflicting results arise from a mouse study indicating an enhanced ovalbumin-induced airway inflammation in CD26/DPP4-deficient mice [126].

Does CD26/DPP4 play a role in the pathogenesis of asthma or allergic-like airway inflammation? Using a model of ovalbumin-induced airway inflammation in rats, we found a CD26/DPP4-dependent T cell recruitment to the lungs, with reduced signs of inflammation in CD26/DPP4-deficient rats [127]. These results were confirmed additionally by a significant reduction of the airway-specific recruitment of T cells to bronchi and lung parenchyma in rats genetically lacking expression of CD26/DPP4. This site-specific recruitment appeared and was mediated by chemokines, rather than nerve–T cell interactions [128]. Furthermore, the amount of T cells expressing CD26/DPP4 was increased, and correlated with the severity of airway inflammation [129]. To address further the questions of the role of T cells expressing CD26/DPP4 in airway inflammation, we have transferred labelled T cells from CD26-expressing or CD26/DPP4-deficient F344 rat donors and subsequently cross-transferred to recipients of the other substrain [130]. Here, we found significantly more T cells in CD26/DPP4-deficient recipient lungs, regardless of the origin of the transferred T cells [130]. Additionally,

Table 4. Involvement of CD26/dipeptidyl peptidase 4 (DPP4) in T helper type 1 (Th1) and Th2 responses [3,17,49,67,81,88,112,116,188–192].

	Th1	Th2
CD26 expression	High expression on $CD26^{bright}$ memory T cell subset	Slight up-regulation of CD26 expression
	Binding of caveolin-1 on dentritic APC cells to CD26 on Th1 cells, results in binding of CARMA-1 cytoplasmic tail of CD26 ⇒ recruitment of CARMA-1, CD26, Bcl10 and IkappaB kinase complex to lipid rafts ⇒ signal transduction ⇑ up-regulation of CD26 expression upon induction of Th1 response	
Subcellular compartmentation	Association of CD26 to lipid rafts	Binding of CD26 to M6P/IGFIIR ⇒ internalization + T cell activation
	Binding of CD26 to $CD45R0^+$ on lipid rafts, resulting in signal transduction; followed by disassembly of CD45R0 from lipid rafts	Association of CD26 + CXCR4 ⇒ internalization
Chemokine	CXCR3 → IP-10	CXCR4 → SDF-$1\alpha_{1-68}$ > SDF-$1\alpha_{3-68}$
DPP4 substrates	CCR1 → $RANTES_{1-68}$ > $RANTES_{3-68}$	CCR3 → $eotaxin_{1-74}$ > $eotaxin_{3-74}$
	CCR5 → $RANTES_{3-68}$ > RANTES $_{1-68}$	CCR4 → $MDC_{1-67} \neq MDC_{3-67}$
		CCR1 → $RANTES_{1-68}$ > $RANTES_{3-68}$
		CCR3 → $RANTES_{3-68}$ > RANTES $_{1-68}$
Neuropeptide/peptide DPP4 substrates	NPY released from SNS involved in inflammatory response of macrophages, NK and T cells	Mast-cells: substanceP → allergy + asthma
		NPY released from SNS involved in inflammatory response of PMN and B cells
	GLP-2 involved in Crohn's disease	
Inflammatory response	Soluble DPP4 found in secretory lysosomes of T_C cells	T cell recruitment in asthma is DPP4-dependent
	Membrane-bound DPP4 in secretory lysosomes of NK cells	
	⇑ DPP4 on activated macrophages truncate NPY → loss of Y1-R binding → ⇓ IL-1β and IL-6 release	
	⇑ DPP4 on activated microglia	
Site of inflammation	Arthritis: ⇑ DPP4 on activated synoviocytes + CD26 internalized via caveolae ⇑ hypersialylation of DPP4 → ⇓ sDPP4 activity → ⇑ SDF-α → ⇑ inflammation ⇑ DPP4 on endothelial cells via IFN-γ, TNF-α and LPS stimulation	Asthma: ⇑ DPP4 in lung parenchyma
		Binding of CD26 to M6P/IGFIIR → transendothelial migration of lymphocytes
	Crohn's disease: ⇑ DPP4 in enterocytes → ⇓ GLP-2 ⇑ DPP4 on activated microglia + astrocytes after ischaemia	
Diseases associated with DPP4/CD26	Rheumatoid arthritis	Truncation of SDF-1α by DPP4 → ⇓ protection against HIV entry
	Multiple sclerosis	
	Truncation of RANTES by DPP4 → ⇑ protection against HIV entry	$CD26^+$ T cells involved in the pathogenesis of asthma correlating to IgE titre of antigen
	Ischaemia	

CD26-deficient rats exhibited a significantly increased influx of T_{regs} into the lungs *in vivo* and increased IL-10 production of draining lymph node cells *in vitro* [131].

These findings demonstrate a negative regulatory role of the bronchus-associated lymphatic tissue (BALT)-specific expression of CD26/DPP4 in T cell adhesion during an asthma-like inflammation. However, first data concerning studies targeting CD26/DPP4 by a pharmacological treatment regimens show differential effects depending on the route, dose and time of the application [132]. Additionally, inhibition of CD26/DPP4 enhances CCL11/eotaxin-mediated recruitment of eosinophils *in vivo* [133].

Multiple sclerosis/EAE

Multiple sclerosis (MS) and its corresponding animal model of experimental autoimmune encephaolomyelitis (EAE) are chronic inflammatory autoimmune diseases affecting the central nervous system (CNS) [134]. Patients suffering from MS exhibit increased numbers of $CD26^+$ T

cells, also showing higher expression levels of CD26/DPP4, which correlate with disease activity [135,136]. Compelling evidence has demonstrated that besides myelin specific T helper 1 (Th1) cells, IL-17-producing $CD4^+$ cells (Th17) are major contributors to the pathogenesis of autoimmune inflammation [137]. In line with these findings, human Th17 cells have been shown to express high amounts of enzymatically active CD26/DPP4 [59]. Pharmacological inhibition of DPP4 decreased incidence, onset of symptoms and overall disease severity in EAE significantly, while neither acting as generally immunosuppressive nor eliminating encephalitogenic T cells, and not inhibiting T cell priming [138]. In humans, inhibitors of CD26/DPP4 suppress activation of MBP-specific $CD4^+$ T cell clones [139]. Demonstrating the limitations of disease models and/or selectivity of pharmacological intervention, $CD26^{-/-}$ mice demonstrate a higher disease severity compared to wild-type (WT) controls, which the authors explained by a functional deregulation of Th1 immunity because of a reduced TGF-β production [117]. A possible involvement of other members of the DPP4 family or the encephalopathic role of Th17 cells has not been addressed at this point. Later, it has be shown conclusively that the combined suppression of DPP4 and aminopeptidase N (APN) results in decreased T cell-specific IL-17 production and thus disease amelioration [140].

Arthritis

Rheumatoid arthritis is a chronic, systemic inflammatory disease with progressive destruction of articular cartilage [141]. A number of studies show decreased levels of DPP4 activity in subjects suffering from this disease [142]. Furthermore, the expression of CD26/DPP4 on joint-infiltrating T cells has also been shown to be decreased [143]. Lower serum DPP4 activity in rheumatoid arthritis is caused by hypersialylation and DPP4 autoantibodies, as illustrated in Fig. 2 [17]. The involvement of CD26/DPP4 in arthritis has been reviewed recently, involving glycosylation and DPP4 autoantibodies on one hand and SDF-α on the other hand [17,144–146]. Additionally, one study summarizes three cases of DPP4 inhibitor-induced polyarthritis [147].

Again, $CD26^{-/-}$ mice showed a markedly increased severity of disease due to lower DPP4 activity in synovial fluids, resulting in increased levels of SDF-α [145].

Inflammatory bowel disease (IBD)

IBD, with is two major forms Crohn's disease and ulcerative colitis, is characterized by chronic, remittent or progressive inflammatory processes in the gastrointestinal tract [148]. T cells from patients with IBD have higher levels of CD26/DPP4 expression, while levels of circulating CD26/DPP4 are decreased [149,150]. This parallels the findings of colitis models in mice [151]. In one study, $CD26^{-/-}$ mice show a greater disease severity [152]. In another study, the acute phase of colitis, loss of body mass and disease activity in $CD26^{-/-}$ mice was less intensive than in the controls, while no pronounced histopathological differences could be found [151]. Interestingly, lack of CD26/DPP4 led to a twofold increase in the number of macrophages during the acute phase of disease, while an increased influx of dendritic cells became apparent in controls [151]. Another study focused on the gut–brain axis and the altered receptor specificity of neuropeptide Y after DPP4-mediated cleavage, finding that CD26/DPP4 deficiency affects the neuroimmune response at systemic and local levels during colitis development and resolution in mice [153]. Furthermore, higher familial adenomatous polyposis (FAP) levels were detected in patients with Crohn's disease [154].

Again, the pharmacological inhibition of DPP4 by two different inhibitors reduced disease activity significantly in Crohn's disease, due to increased levels of GLP-2 [155,156]. These findings suggest a pathophysiological role of CD26/DPP4 in the nature of immune responses activated during Crohn's disease.

Others

CD26/DPP4 appears to play a role in a number of other diseases (see Table 2). In atopic dermatitis, CD26/DPP4 expression was up-regulated in the skin biopsies of patients compared with healthy controls, as well as in both models of contact hypersensitivity [157]. In psoriasis, reduced expression of CD26/DPP4 on $CD8^+$ T cells has been observed [158]. In atherosclerosis, inhibition of DPP4 exerts anti-atherosclerotic effects and reduces inflammation via inhibition of monocyte activation/chemotaxis [82].

Clinical use of DPP4 inhibitors

DPP4 has been identified as a therapeutic target for T2DM due to its ability to cleave and inactivate insulinotrophic incretins such as GIP and GLP-1 [159]. These incretins are released upon glucose intake and enhance the insulin secretion with a half-life of a few minutes, strictly dependent upon DPP4-like enzymatic activity. Furthermore, incretins exhibit positive effects on pancreatic β cells in the islets, including stimulation of growth and replenishing insulin stores by stimulation gene transcription. Once released, GIP and GLP are degraded rapidly by DPP4 and thus the inhibition of DPP4 prolongs GIP/GLP half-life and insulinotrophic effect [159,160]. After the first DPP4 inhibitor sitagliptin (Januvia®) had been approved by the Food and Drug Administration (FDA) in 2006 [European Medicines Agency (EMA), 2007], numerous functionally related drugs, commonly called gliptins, were released [161]. Currently, there are nine DPP4 inhibitors commercially available on the market, with sitagliptin Januvia® (Merck & Co., Inc., Kenilworth, NJ, USA), saxagliptin Onglyza® (Bristol Myers Squibb, New York, NY, USA), linagliptin Tradjenta™ (Böhringer Ingelheim, Ingelheim, Germany) and alogliptin Nesina® (Takeda Pharmaceuticals, London, UK)

being approved by the FDA. Sitagliptin, vildagliptin Galvus® (Norvatis, Basel, Switzerland), saxagliptin and linagliptin were approved by the EMA; and anagliptin Suiny® (Sanwa Kagaku Kenkyusho Company Ltd and Kowa Company Ltd, Nagoya, Japan), teneligliptin Tenelia® (Mitsubishi Tanabe Pharma and Daiichi Sankyo, Dusseldorf, Germany), trelagliptin Zafatek® (Takeda Pharmaceuticals) and omarigliptin Marizev® (Merck & Co., Inc.) being approved in Japan. All of them are administered orally and taken daily, except for omarigliptin, which has weekly doses. To date, 125 meta-analyses have been reported in PubMed, focusing on the efficacy and drug safety of DPP4 inhibitors, as well as its effects on comorbidities such as renal impairment and cardiovascular outcome [160–173]. So far more than 500 clinical trials have been performed throughout the world, covering all ethnic population groups, and aproximately 250 further trials are currently ongoing (www.clinicaltrials.gov; 31 January 2016). Generally, DPP4 inhibitors reduce DPP4 activity at approximately 70–90% of baseline and also lower the haemoglobin A1c (HbA1c) 0.74%. All DPP4 inhibitors are excreted via the renal route except for linagliptin, which is eliminated via the biliary route [174].

Although demonstrating an overall favourable adverse side-effect profile, meta-analysis showed that infections (most common: upper respiratory tract infection and urinary tract infection) increased significantly after DPP4 inhibitor treatment [160–162,164–173]. Other side effects may include pancreatitis, headache, nausea, angioedema, hypersensitivity and skin reactions, as well as severe joint pain [160–162,164–173]. In response to a report of precancerous changes in transplanted pancreases of donors treated with the DPP IV inhibitor sitagliptin, the FDA and the EMA each undertook independent reviews of all clinical and preclinical data related to DPP4 inhibitors. These reviews revealed no association of DDP4 inhibition with pancreatic cancer [175,176]. Currently, gastrointestinal, cutaneous and mucosal side effects, atherosclerosis and cancer are also of special interest and have initiated extensive, ongoing research [165]. When considering the more recent findings, DPP4 inhibitors might be considered to represent even more of a double-edged sword. Apart from the metabolic benefit, the associated immunological effects induced by long-term DPP4 inhibition, in particular on T cells, are not understood fully at this stage. Further post-marketing surveillance will hopefully elucidate the potential risks of this class of drugs for immunological side effects.

Almost all anti-diabetic DPP4 inhibitors were designed to exhibit a long half-life, with 'one pill a day' facilitating both patients' compliance and marketing. The short-acting PSN-9301 appears to be the only exception [177]. A once-daily application is convenient from a patient viewpoint. However, long-acting inhibitors of DPP4 might compete with other natural substrates of DPP4 and their associated physiological functions, such as surfactant protein (SP) in rhinosinusitis and angioedema, SDF-α in arthritis and NPY/PYY, as well as substance P in blood pressure [80,178,179]. Recently, the FDA revised its prescribing information to include case reports on acute pancreatitis as well as polyarthritis in patients using sitagliptin [146,180]. Further case reports describe contradicting effects of sitagliptin in psoriasis: as sitagliptin was observed, on one hand, to trigger psoriasis, it was also claimed to ameliorate the disease on the other hand [181,182]. Interestingly, investigating NPY hydrolysis in serum and blood [80], a novel C-terminal truncation of NPY by an angiotensin-converting-enzyme (ACE)-like enzyme was detected. This finding strongly suggests a potential interaction within current drug treatments that use anti-diabetic DPP4 inhibitors and anti-hypertensive ACE inhibitors in combination, causing potentiated NPY-induced hypertension and vasoconstriction. A suspected increase of vasocontrictive NPY_{1-36} after treatment with anti-diabetic DPP4 inhibitor may be compensated by the C-terminal inactivation of NPY mediated by ACE, but fails if ACE is also blocked [80]. This hypothesis has been substantiated by physiological animal studies, using spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY), respectively [183]. Intriguingly, when treating SHR and WKY rats with either the pan-DPP inhibitor P32/98 alone or in combination with captopril, only SHR developed hypertension after combined therapy. This suggests a genetic background involving nephropathic hypertension similar to the human metabolic syndrome [184]. However, Y_1-R antagonists ablated the hypertensive effects of combined treatment with DPP4 and ACE inhibitors, supporting the involvement of either NPY or PYY [183]. Similar findings have been observed with substance P and ACE inhibitors [178]. This is of pharmacological significance, as hypertension is a frequent co-morbidity with diabetes. In recent reports, the development of hypertension was associated with the combined application of the anti-diabetic compound sitagliptin and anti-hypertensive drug enalapril in patients suffering from metabolic syndrome [185,186]. Because the anti-diabetic effects of DPP4 inhibition is only required upon glucose challenge, the development of short-acting and highly specific DPP4 inhibitors might minimize side effects due to off-target inhibition.

Conclusion

The introduction of DPP4 inhibitors into clinics aimed to enhance the endogenous insulin secretion in diabetes mellitus type 2 via elevated levels of glucagon, such as GLP-1 and GIP. At present, the majority of findings for non-diabetes effects mediated by DPP4 inhibitor treatment in patients are indicative of largely beneficial secondary effects. Nevertheless, the application of these new compounds might represent a double-edged sword: apart from

the metabolic benefit, the associated immunological effects of long-term DPP4 inhibition on regulatory processes such as T cell maturation and activation are not understood fully at this stage. Several Phase III trials of new DPP4 inhibitors are currently ongoing. These trials, along with postmarketing surveillance data, will hopefully increase our knowledge about the long-term efficacy and safety of DPP4 inhibitor therapy. The scope of these studies should be focused not only on the current questions of incretin action in the cardiovascular system, pancreatitis and cancer, but also on (long-term) immunological parameters such as infections, T cell development and immune homeostasis.

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Disclosure

The authors declare no disclosures.

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