

The Role of Dipeptidyl Peptidase 4 as a Therapeutic Target and Serum Biomarker in Inflammatory Bowel Disease: A Systematic Review

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Background: The roles dipeptidyl peptidase 4 (DPP4), aminopeptidase N (APN), and their substrates in autoimmune diseases are being increasingly recognized. However, their significance in inflammatory bowel diseases (IBD) is not entirely understood. This systematic review aims to discuss the pathophysiological processes related to these ectopeptidases while comparing findings from preclinical and clinical settings.

Methods: This review was conducted according to the PRISMA guidelines. We performed a literature search in PubMed, SCOPUS, and Web of Science to identify all reports from inception until February 2020. The search included validated animal models of intestinal inflammation and studies in IBD patients. Quality assessment was performed using SYRCLE's risk of bias tool and CASP qualitative and cohort checklists.

Results: From the 45 included studies, 36 were performed in animal models and 12 in humans (3 reports included both). Overall, the methodological quality of preclinical studies was acceptable. In animal models, DPP4 and APN inhibition significantly improved intestinal inflammation. Glucagon-like peptide (GLP)-1 and GLP-2 analogs and GLP-2-release-inducing drugs also showed significant benefits in recovery from inflammatory damage. A nonsignificant trend toward disease remission with the GLP-2 analog teduglutide was observed in the sole interventional human study. All human studies reported an inverse correlation between soluble DPP4/CD26 levels and disease severity, in accordance with the proposal of DPP4 as a biomarker for IBD.

Conclusions: The use of DPP4 inhibitors and analogs of its substrates has clear benefits in the treatment of experimentally induced intestinal inflammation. Further research is warranted to validate their potential diagnostic and therapeutic applications in IBD patients.

Key Words: pathogenesis, inflammation, translational, biomarker, ectopeptidase

INTRODUCTION

Inflammatory bowel diseases (IBDs) are a group of chronic relapsing autoimmune disorders, comprising Crohn's disease (CD),¹ ulcerative colitis (UC),² and an intermediate spectrum of unclassifiable conditions designated as indeterminate colitis.³ Inflammatory bowel disease present many extraintestinal manifestations and may pertain to a cluster of autoimmune diseases affecting the same patient.⁴ Left untreated, these conditions are highly debilitating and potentially life-threatening and represent a high economic burden.^{1, 2, 4} In recent decades, IBD has been the target of intensive

research, with considerable progress in the therapeutic management of the disease and related pathologies. However, most available treatment strategies have a significant amount of nonresponders and a wide range of adverse effects.^{1, 2} Thus, the development of new and optimized therapeutic weapons is now supported by research on the underlying pathophysiological mechanisms of IBD.

Dipeptidyl peptidase 4 (DPP4), homologous to cell-surface marker CD26 (cluster of differentiation 26), is a near-ubiquitous membrane protease that cleaves N-terminal dipeptides from many endogenous and exogenous peptides.⁵

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This enzyme is well-known for its physiological action in the incretin axis as the main cause for the rapid inactivation of the incretins glucagon-like peptide (GLP)-1 and gastric inhibitory polypeptide (GIP).⁵ The GLP-1 analogs and DPP4 inhibitors (DPP4i), which act by increasing the half-life of endogenous GLP-1, are potent insulinotropic drugs used in the therapy of diabetes mellitus.^{6,7}

In the last 2 decades, interest in DPP4, aside from its utility in glycemic control, has grown. In fact, plasma levels of this protein have been inversely correlated with disease severity in IBD and other autoimmune diseases (ADs), making it a potentially new biomarker and a valid therapeutic target for these conditions.⁸

The 766-amino acid (aa) CD26/DPP4 is a membrane protein, with many distinct physiological roles (Fig. 1). It contains

an independent C-terminal catalytic region, a cysteine-rich region, a glycosylation-rich region, a flexible stalk, a transmembrane domain, and a short cytosolic tail. It presents specific binding sites to fibronectin (and other extracellular matrix components) and extracellular adenosine deaminase (ADA).⁹ After dimerization, CD26/DPP4 is able to activate intracellular signaling pathways as a type 2 membrane receptor.⁵ The mechanism underlying its cleavage and shedding to plasma in its soluble form, sCD26/DPP4 (aa 39–766), is still unclear.¹⁰ Either through direct cell-signaling or by cleaving immune mediators, it interferes in several immunoregulatory processes.⁹ By binding to caveolin-1 on the surface of antigen-presenting dendritic cells (DCs), DPP4/CD26 stimulates the expression of costimulatory CD86, through NF-κB signaling, thereby promoting T-cell activation.¹¹ Cosignaling with CD45, it enhances T-cell expression

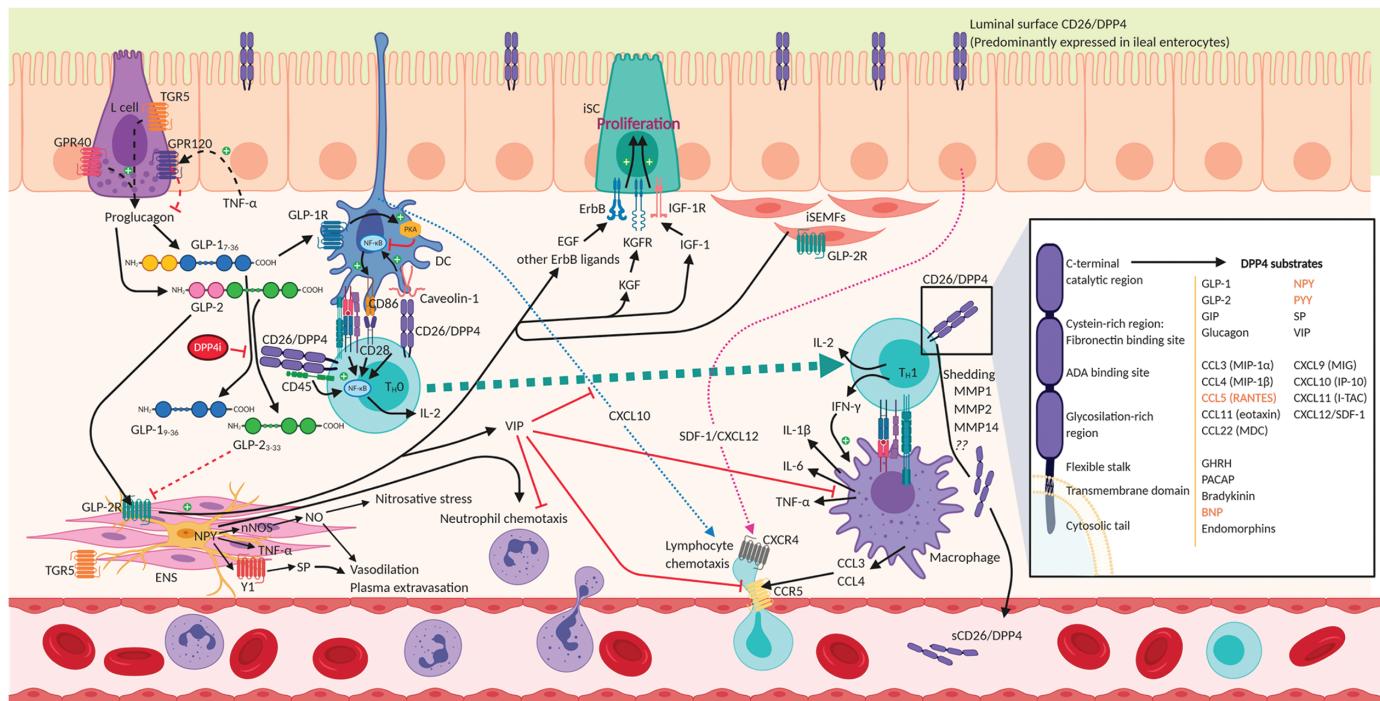


FIGURE 1. Proposed mechanistic view of CD26/DPP4-centered interactions in intestinal inflammation—Th1-polarizing perspective. Membrane CD26/DPP4, coupled to CD45 in naïve T helper cells, directly stimulates NF-κB-dependent T-cell activation and differentiation into a Th1 phenotype. Additionally, it proteolytically cleaves N-terminal dipeptides from a variety of substrates, including GLP-1, GLP-2, VIP, and NPY. GLP-1 is a negative regulator of NF-κB and costimulatory DC signals. GLP-2, acting via GLP-2R, is an intestinotrophic peptide that stimulates the production and release of intestinal growth factors (EGF, KGFR, IGF-1), which stimulate iSC proliferation and differentiation, effectively counteracting mucosal inflammatory lesions. GLP-2_{3–33} acts as partial agonist/antagonist at GLP-2R. VIP is a negative regulator of neutrophil and lymphocyte chemotaxis and inhibits macrophage activation. NPY is an ENS-derived pro-inflammatory peptide. CD26/DPP4 is shed to plasma through a mechanism not yet completely understood. Abbreviations: BNP, brain natriuretic peptide; CCL/CXCL, Chemokines; CCR/CXCR, Chemokine receptors; CD, cluster of differentiation; DC, dendritic cell; ENS, enteric nervous system; EGF, epidermal growth factor; GHRH, growth hormone-releasing hormone; GIP, gastric inhibitory polypeptide; GLP-1/2-1R/-2R, glucagon-like peptide 1/2-receptor/2-receptor; GPR40, G-protein coupled receptor 40 (FFA1, free fatty acid receptor 1); GPR120, G-protein coupled receptor 120 (FFA4, free fatty acid receptor 1); IGF-1/1R, insulin-like growth factor 1/1-receptor; IL, interleukin; IFN-γ, interferon-gamma; iNOS, neuronal nitric oxide synthase; NO, nitric oxide; NPY, neuropeptide Y; PACAP, pituitary adenylate cyclase-activating polypeptide; PYY, peptide YY; sCD26/DPP4, soluble CD26/DPP4; SDF-1/CXCL12, stromal cell-derived factor 1; SP, substance P; TGR5, G-protein coupled bile acid receptor 1 (GPBAR1); Th, T helper cell; TNF-α, tumor necrosis factor alpha; VIP, vasoactive intestinal peptide; Y1, NPY receptor type 1. Substrates in orange have altered receptor subtype specificity after cleavage by DPP4. Substrates in black mainly lose their bioactivity.

of interleukin (IL)-2 and ultimately contributes to differentiation into a T helper (Th) 1 phenotype.^{12,13} Adenosine deaminase binding to CD26 colocalizes ADA to the cell surface, allowing local degradation of adenosine (a known inhibitor of T-cell activation) to inosine, hence controlling its extracellular levels at the immunological synapse. Furthermore, ADA binding to CD26 was found to produce a direct costimulatory response in T-cell activation.^{14,15}

Many bioactive or inactive precursor peptides are substrates of DPP4, including peptide hormones (GLP-1, GLP-2), neuropeptides (neuropeptide Y, substance P), and many chemokines and growth factors. Additionally, this enzyme acts as long as the penultimate amino acid is either proline or alanine. It is also capable of cleaving N-terminal X-glycine/serine/valine/leucine, albeit at a slower rate. Cleavage is hindered by the presence of proline in NH₂-Xaa-Xaa-Pro position.⁵ In inflammatory settings, the most relevant substrates of DPP4 are GLP-1, GLP-2, and vasoactive intestinal peptide (VIP).

In addition to its insulinotropic action, GLP-1, acting through GLP-1R, stimulates protein kinase A (PKA) and leads to the inhibition of the T-cell costimulatory CD28/CD86 signal.¹⁶ It is rapidly inactivated by DPP4 into GLP-1_{9-36/7}, with a short half-life of around 1 to 4 minutes.¹⁷ The GLP-2 is a sister molecule of GLP-1 that is co-expressed and coreleased from enteroendocrine L cells, after processing of their common precursor, proglucagon. Through its receptor, GLP-2R, localized in enteric nervous plexuses (myenteric, submucosal)¹⁸ and subepithelial myofibroblasts,¹⁹ stimulates the release of key intestinal growth factors such as KGF, IGF-1, and EGF; this mechanism reverts inflammatory changes in the intestinal epithelium.^{20,21} As with GLP-1, GLP-2 is rapidly inactivated by local DPP4 to GLP-2₃₋₃₃, with a half-life of 7 minutes; however its metabolite has a half-life of around 27 minutes.²² Additionally, GLP-2₃₋₃₃ acts as a competitive inhibitor of GLP-2 at GLP-2R.²² Teduglutide, a DPP4-resistant long half-life GLP-2 analog, is approved for use in short bowel syndrome and is under investigation for its applicability in IBD.²³ In addition, GLP-2 stimulates the release of VIP from enteric neurons.²⁴ Vasoactive intestinal peptide is a 28-amino acid peptide with a short half-life of around 1 minute and a wide range of effects, including neurotransmitter, immunomodulatory, and secretagogue activities.^{25,26} It inhibits TNF- α production by macrophages²⁷ and promotes T_H cell differentiation toward a Th2 phenotype.²⁸

Aminopeptidase N (APN) is an ectopeptidase homologous to CD13.²⁹ It is being studied in the setting of hematological disorders and gained interest as a potential co-effector of DPP4/CD26 in immune regulation.³⁰ Aminopeptidase N is involved in antigen processing and interaction with extracellular matrix proteins.³⁰ Its substrates include several immunoregulatory molecules.³⁰ It cleaves off N-terminal neutral amino acids of oligopeptides but stops if proline is in the penultimate position.³¹ These catalytic specificities, the

subcellular localization similar to CD26, and increased expression in activated T cells point to a potential role of APN/CD13 as a DPP4/CD26-substrate generator and vice versa, acting in tandem as regulators of immune responses. This is further supported by an improved anti-inflammatory response of the dual APN/DPP4 inhibition compared with antagonism of only one of either of these proteins.³² For this reason, we decided to extend the scope of this review to also include reports on the role of APN/CD13 in intestinal inflammation.

Although some reviews on the importance of DPP4 and APN on AIDs and inflammation have been published, the knowledge on their specific role in IBD pathogenesis is limited. This systematic review aims to fill this gap and provide a link between preclinical and clinical data on the role of these ectopeptidases in IBD (as well as other molecules of their related axes), through the use of validated animal models of intestinal inflammation³³ and studies on IBD patients.

METHODS

This review was conducted following the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement (PRISMA 2009).³⁴ Study screening was conducted in 3 electronic databases: PubMed, Web of Science, and SCOPUS, covering all reports published through February 4, 2020. The query used for PubMed was as follows: “DPPR OR DPPIV OR Dipeptidyl peptidase 4 OR Dipeptidyl peptidase IV OR CD26 OR ADCP2 OR Adenosine deaminase complexing protein 2 OR Aminopeptidase N OR Alanyl aminopeptidase OR Alanine aminopeptidase OR CD13 OR AAP OR APN AND inflammatory bowel disease OR crohn’s disease OR ulcerative colitis.” Similar queries were used for the other 2 databases, after syntax adaptation. To ensure the inclusion of all pertinent studies, the reference lists of the included reports were reviewed by 2 independent researchers.

All preclinical reports emphasizing the pathophysiological role of DPP4/CD23 and APN/CD13 in intestinal inflammation models, in addition to clinical reports demonstrating a link between DPP4/APN and IBD, were assessed for inclusion. Reports concerning other molecules pertaining to the physiological axes of these factors were also considered, as long as there was at least an indirect link to DPP4 or APN.

The inclusion criteria were (1) articles studying the association between DPP4/CD26 and IBD in animal models of intestinal inflammation and human patients; (2) articles studying the association between APN/CD13 and IBD in animal models of intestinal inflammation; and (3) articles studying the association between related molecules of the DPP4 axis with known therapeutic potential, specifically GLP-1 and GLP-2 (and molecules that influenced GLP-1 or GLP-2 levels, such as TGR5, G-protein coupled receptor [GPR]-40, and GPR120), and IBD in animal models of intestinal inflammation and human patients. No restrictions on publication language were applied.

The exclusion criteria were (1) review papers, metanalyses, letters, commentaries, guidelines, editorials, meeting abstracts, and case reports; (2) studies with no relation to IBD or related animal models of colitis (intestinal cancer studies, radiation-induced injury, etc.); (3) studies with no pathophysiological association with DPP4 or APN; (4) studies including other substrates of DPP4 and APN (setting a limit to study screening and to avoid an overreaching and unfocused review of all possible substrates and their influence in DPP4-dependent inflammatory pathways); and (5) studies without available abstract.

The risk of bias in individual studies was assessed using quality evaluation tools/scales adapted to study type. For pre-clinical animal studies, SYRCLE's risk of bias (RoB) tool was used.³⁵ For studies in humans, CASP checklists were used for qualitative³⁶ and cohort³⁷ reports. These tools were applied by 2 independent reviewers, and discrepancies were solved by consensus.

RESULTS

Study Selection and Characteristics

Study selection, following the PRISMA 2009 Flow Diagram,³⁴ is outlined in Figure 2. Of the 45 studies selected for review, 36 used animal models of intestinal inflammation,^{38–73} and 12 concerned human IBD patients^{50, 66, 67, 74–82} (3 reports included both^{50, 66, 67}). The characteristics and main results of

animal studies are described in **Supplementary Table 1** and those of human studies in **Table 1**. For animal studies, disease and animal models, with respective variations, are compiled in **Supplementary Table 2**.

Most animal studies consisted of interventional protocols of varying durations that tested the effects of DPP4i, APNi, GLP-1, or GLP-2 analogs (mostly long-acting, DPP4-resistant, or by continuous infusion) and related drugs on the recovery from experimentally induced intestinal lesions.

The most used model of colitis was the dextran sulfate sodium (DSS) model ($n = 25$), followed by 2,4,6-trinitrobenzenesulfonic acid (TNBS, $n = 7$), indomethacin ($n = 5$), HLA-B27 ($n = 2$), CD4+ transfer ($n = 1$), and irinotecan ($n = 1$) models. The most commonly used strains of mice were Balb/c and C57BL/6 mice. Seven reports utilized knock-out mice for CD26/DPP4, and 1 study used Glp2r^{-/-} mice.

Despite wide variations in protocol, we decided to interpret each model's group as a whole, inasmuch as all experiments were able to elicit an inflammatory response to some extent and were therefore deemed internally valid.

With a single exception of an interventional study,⁷⁴ all studies in human IBD patients were observational, focusing on serum activity, levels of DPP4, APN, GLP-2, GLP-2R, and others, in addition to associated clinical and endoscopic findings. None of the included studies in IBD patients used DPP4 inhibitors.

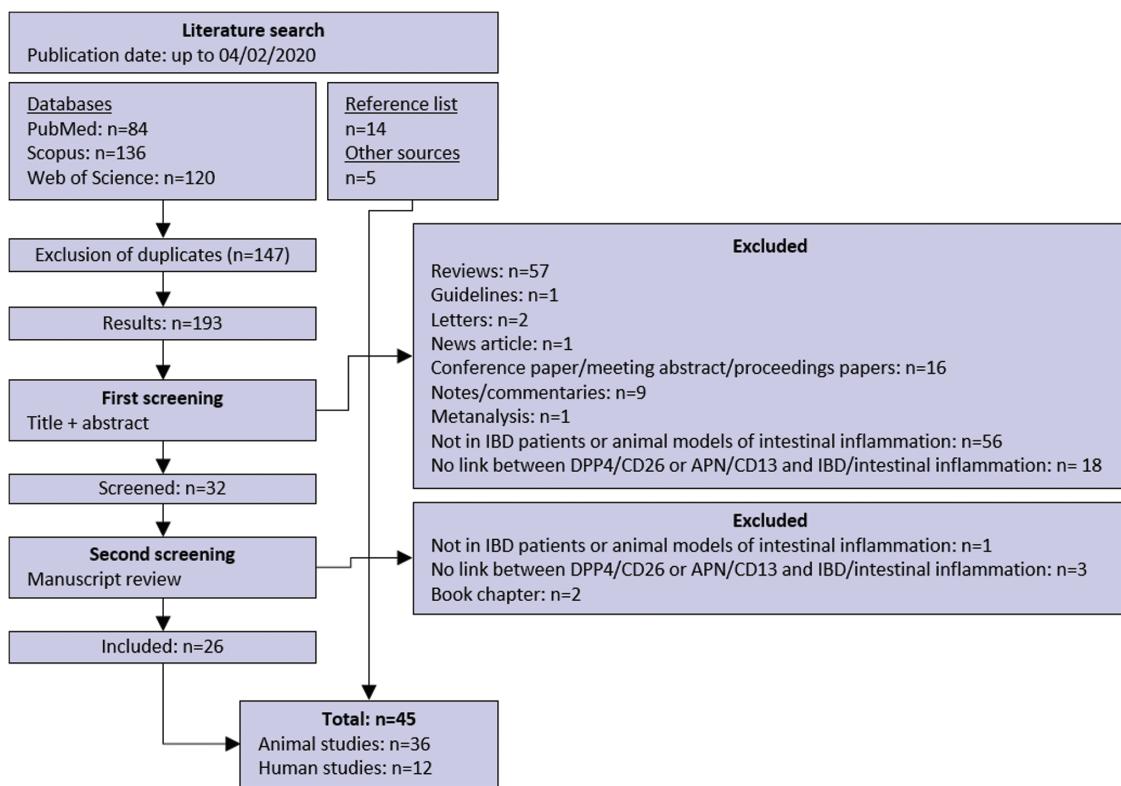


FIGURE 2. Study screening and selection process.

TABLE 1. Characteristics and Main Results of Human Studies

Authors (Year)	Study Origin	Study Type IBD	IBD Assessment	Disease Characteristics	Intervention	Outcomes Assessed	Results
Buchman AL et al (2010)	USA and Canada (multicenter)	Prospective CD, moderate-to-severe (n = 100) (Extension: n = 48)	CDAI Mean disease duration 11.1–13.7 ± 9.5–10.9 mg/kg/d, sc, 24% concomitant therapy with immunomodulators	Teduglutide 0.05/0.10/0.20 mg/kg/d, sc, lid, 8w (Extension: teduglutide 0.10 mg/kg/d, sc, lid, for 12 additional weeks)	Primary: response (100pts CDAI or remission (CDAI ≤ 150) at week 8; Secondary: response and remission at weeks 2 and 4, mean changes in disease severity (CDAI), mean decrease in # of liquid bowel movements, mean decrease in CRP.	No statistically significant differences from placebo. In the 0.2 mg/kg/d group, 40% achieved remission at 8 weeks. No difference was observed in serum CRP at any timepoint.	
El-Jamal N et al (2014)	Not reported	Cross-sectional CD (n = 19) UC (n = 15)	Not reported	Insufficient data	—	GLP-2R expression in human intestine of IBD patients.	Significantly higher expression of GLP-2R mRNA in the colon of healthy and IBD patients, vs in the ileum.
Hildebrandt M et al (2001)	Germany, single center	Cross-sectional CD (n = 63) UC (n = 47) Controls (n = 28)	CDAI Rachmilewitz score (UC)	Mean disease duration—CD: 10.8 ± 8.1, s years UC: 11.9 ± 8.5, s years	sDPP4 activity and lymphocyte CD26/DPP4 expression in IBD patients.	Similar percentages of CD2+/CD26+ and CD2+/CD25+ cells in IBD patients. DPP4 activity was inversely correlated with disease activity, orosomucoid concentrations and CRP.	

TABLE 1. Continued

Authors (Year)	Study Origin	Study Type IBD	IBD Assessment	Disease Character- istics	Intervention	Outcomes Assessed	Results
Magro D et al (2017)	Brazil, single center	Cross-sectional CD (n = 20)	CDAI (active: >150; remission: ≤150) Montreal Classification	Active CD: n = 10 Remitting CD: n = 10 —	—	Correlation between serum levels of LPS and CD26, and serum levels of CRP, interleukins, TNF-α and CDAI.	Significantly higher levels of serum LPS and CRP in active and inactive CD group, vs controls.
Moran GW et al (2012)	Not reported	Cross-sectional CD (n = 26)	CDAI CRP	CDAI = 174.5 ± 14.26— CRP = 20.4 ± 5.4 mg/L	—	DDP4 expression and correlation to underlying intestinal inflammation (CDAI, CRP).	Significantly lower levels of plasma and tissue (terminal ileum) DPP4 in CD patients, vs controls. Negative correlation between CRP and LPS in CD groups.
Pinto-Lopes P et al (2020)	Portugal, multicenter	Prospective CD cohorts UC (n = 94)	CD: HBI (clin.-Active CD: HBI = 7.6— UC: pMS (clinical remissio: ≤1) Montreal Classification	CD: HBI (clin.-Active CD: HBI = 7.6— UC: pMS (clinical remissio: ≤1) Montreal Classification	—	Role of sDPP4 as a biomarker of IBD activity (potential in predicting the need for treatment escalation and monitoring response to biological therapy); correlation between sDPP4 levels with endoscopic activity and clinical activity scores.	Patients with active IBD had significantly higher serum CRP and FC levels and lower sDPP4, vs those in clinical remission. Correlation with FC, serum CRP and DAI. FC was positively correlated with serum CRP and DAI. sDPP4 was inversely correlated with both disease activity scores (HBI and pMS) and endoscopic activity groups (stronger in CD, vs UC).
Salaga M, Mokrowiecka A, Zielińska M et al (2017)	Not reported	Cross-sectional UC (n = 12)	CD (n = 9) UC (n = 12) Controls (n = 8) 13 colon biopsy samples	Montreal Classification	CD: 5-ASA (n = 5) Anti-TNF-α (n = 4) UC: 5-ASA (n = 12) 29 serum samples	Expression of GLP-2 and GLP-2R in the serum and colon of IBD patients.	Significant decrease in the expression of serum GLP-2 in CD patients. Significant decrease in the expression of colon GLP-2R in UC patients.

TABLE 1. Continued

Authors (Year)	Study	IBD	Disease Characteristics	Intervention	Outcomes Assessed	Results
	Origin	Type IBD	Assessment			
Salaga M, Mokrowiecka A, Jacenik D et al (2017)	Not reported	Cross-sectional	Montreal Classification	CD: — 5-ASA (n = 10) Anti-TNF-α (n = 7) UC: — 5-ASA (n = 10)	Characterization of intestinal tissue and serum expression levels of APN and NEP in IBD patients.	Significantly higher expression of APN mRNA in colonic tissue of CD patients (nonsignificant increase in UC patients).
Schmidt PT et al (2005)	Sweden, single center	Cross-sectional	Not reported	Active disease: CD (n = 4) UC (n = 5) Chronic/no inflammation: UC (n = 10) CD: 5-ASA (n = 1) Glucocorticoids (n = 1) Metronidazole (n = 1) UC: 5-ASA (n = 11) Prednisolone (n = 1) Azathioprine (n = 1)	Meal stimulation (430 kcal) secretion of GLP-2 and PYY in IBD patients	No significant increase in the expression of NEP protein in colonic tissue of CD patients. Nonsignificant increase in the expression of NEP protein in colonic tissue of UC patients. No changes in serum levels.
Tsukahara T et al (2015)	Japan, single center	Cross-sectional	Not reported	Previous therapeutic exposure: Anti-TNF-α (n = 4) Immunomodulator (n = 2) Corticosteroids (n = 0)	Expression of GPR40 and GPR120 in the ileal mucosa of CD patients and its correlation with inflammatory parameters.	Intestinal epithelial cells express GPR40, but rarely express GPR120, in the normal ileal mucosa. Both were overexpressed in inflamed ileal mucosa. GPR40 and GPR120 are co-expressed in L cells (significant positive correlation).
		Controls (n = 15)				HBI values significantly correlated with GPR120 expression, but not GPR40. Significantly higher levels of TNF-α. Both GPR120 and GPR40 expression levels significantly correlated with levels of TNF-α, but not those of IL-6 or IL-1β.
						No differences in protein and mRNA expression of proglucagon in CD patients vs controls.

TABLE 1. Continued

Authors (Year)	Study Origin	Study Type IBD	IBD Assessment	Disease Characteristics	Intervention	Outcomes Assessed	Results
Varljen J et al (2005)	Croatia, single center	Cross-sectional CD (n = 38) UC: Truelove and Witts' (TW) classification	CDAI UC: Truelove and Witts' (TW) classification	Insufficient data	—	Relation between sDPP4 activity with clinical and inflammatory parameters in patients with IBD; potential differences were found between the 2 IBD groups.	sDPP4 activity was significantly decreased in both CD and UC patients, vs controls, although no significant differences were found between the 2 IBD groups. DPP4 activity inversely correlates with CDAI score in CD patients, and TW in UC patients.
Xiao Q et al (2000)	Canada, single center	Cross-sectional GLP-2	Not reported	Mean disease duration— <i>GLP-2</i> CD without bowel resection: 4.5 ± 5.1 years CD with bowel resection (n = 30) = 9) UC (n = 21) Healthy controls (n = 14) Immune controls (n = 38)	Mean disease duration— <i>GLP-2</i> CD without bowel resection: 4.5 ± 5.1 years CD with bowel resection: 12.9 ± 12.7 years UC: 4.3 ± 5.9 years <i>DPP4</i> CD without bowel resection: 8 years CD with bowel resection (n = 1) = 5) UC: 1 year	Abnormalities in the levels and/or molecular forms of circulating GLP-2 in IBD patients.	Significant difference in sDPP4 activity between male and female patients with UC. No correlation between sDPP4 activity and routine laboratory parameters in either disease, nor in relation to the location and extension of pathological lesions.

Abbreviations: CD#, cluster of differentiation #; CDAI, Crohn's disease activity index; DAI, disease activity index; HB, Harvey-Bradshaw index; IFN- γ , interferon-gamma; LPS, lipopolysaccharide; pMS, partial Mayo score; Th, T helper cell; TNF- α , tumor necrosis factor alpha; 5-ASA, 5-aminosalicylic acid; mesalazine

Quality Assessment

The results of the methodological quality evaluation are summarized in [Supplementary Tables 3–5](#). Overall, preclinical studies do not report sufficient details to allow for proper risk of bias assessment, with most items being answered as “unclear,” according to SYRCLE’s RoB tool³⁵ ([Supplementary Table 3](#)). Unlike randomized clinical trials (RCTs), experimental studies in animals usually do not implement tools to assess internal validity and risk of bias in their study design. Consequently, the generalizability of their findings is compromised due to poor reporting.³⁵ In the revised studies, the most affected domains were inadequate randomization during allocation of study groups and outcome assessment and lack of blinding/concealment.

For the included reports on human patients, we used 2 separate CASP checklists, according to study design: qualitative³⁶ ([Supplementary Table 4](#)) and cohort³⁷ ([Supplementary Table 5](#)). Human qualitative studies showed a better performance under appraisal when compared with animal studies due to better reporting. Nevertheless, some doubts arise (eg, “Can’t tell”) in the items related to adequate recruitment, representativeness, and significance of the study population. Furthermore, the studies revealed a low ability to extrapolate findings to the general population, most often due to low sample size and high homogeneity (eg, only female subjects, only recruited from tertiary centers, etc.). As expected, cohort studies had better classifications, yet the generalizability of the results was also an important drawback.

DPP4/CD26 and APN/CD13

Animal studies

None of the studies in CD26^{-/-} mice with experimentally induced colitis showed significant differences in histological and clinical scores compared with wild-type strains.^{44, 46–48, 53} Conversely, Iwaya et al⁵⁶ demonstrated a significant yet transient improvement of intestinal inflammation in an early phase of colitis development in DPP4-deficient (F344/Du) rats.

Reports using DPP4 inhibitors showed either partial or significant improvement of clinical and histological scores and reduction of MPO activity and pro-inflammatory cytokine levels.^{42, 43, 51, 52, 55, 61, 65, 67, 71–73}

Bank et al⁴³ studied the effects of combined inhibition of both DPP4 and APN on colitis attenuation. The dual DPP4/APN inhibitor IP12.C6 promoted the expression of TGF-β and FOXP3 compared with separate inhibition and controls. In a similar manner, dual inhibition of APN and neprilysin (NEP, CD10) by sialorphan or a sialorphan analogue also significantly improved colitis, in part through μ- and κ-opioid receptor-dependent mechanisms.^{57, 66}

Human studies

Serum CD26/DPP4 expression and/or activity was found to be significantly lower in IBD patients compared with healthy

controls.^{75–77, 80–82} Patients with active disease showed lower levels than patients in remission, and sCD26/DPP4 levels were negatively correlated with disease severity and classical inflammatory markers, such as C-reactive protein (CRP).^{75, 77, 80, 82} In addition, Hildebrandt et al⁸² reported significant increases in CD25⁺/CD26⁺ and CD2⁺/CD25⁺ peripheral blood lymphocytes in IBD patients vs controls but no differences in the population of CD2⁺/CD26⁺ cells. Moran et al⁷⁶ showed that DPP4 expression was significantly reduced in tissue samples from the terminal ileum of IBD patients. Still, the authors reported significantly higher levels of DPP4 in a Caco-2 cell-based study after exposure to rhTNF-α.⁷⁶ As with similar reports, they showed an inverse correlation between serum DPP4 (sDPP4) levels and CRP; however, such correlation was not found for CDAI.⁷⁶

In a recent multicentric prospective cohort undertaken by our study group,⁷⁷ sDPP4 was found to have a strong inverse correlation with clinical and endoscopic activity. It performed equally well in postoperative CD patients. Optimal cutoff points were defined based on receiver operating characteristics (ROC) curve analysis of sDPP4 and 2 other biomarkers, CRP and fecal calprotectin (FC). These were used to predict clinical activity, endoscopic activity, and treatment escalation in both CD and UC patients. Stratification according to these cutoffs in a Kaplan-Meier curve showed that after 1 year, 62.2% of CD patients and 36.3% of UC patients with DPP4 levels below the cutoff (≤ 1452 ng/mL and ≤ 1472 ng/mL, respectively) had escalated treatment, as opposed to 7.8% of CD and 4.1% of UC patients with DPP4 levels above the cutoff. The use of 3 simultaneous biomarkers proved to have a higher discriminative power. Eighty percent of the CD patients with 3 positive biomarkers escalated treatment after 1 year vs 3.3% of the patients with triple negative biomarkers. Regarding UC, 85.0% of the patients with 3 positive biomarkers escalated treatment after 1 year vs none with 3 negative biomarkers. All 3 biomarkers had a similar ability to distinguish between IBD responders and nonresponders. In a subset of the IBD population with active disease but without CRP elevation, sDPP4 was able to discriminate endoscopic activity better than FC.

Aminopeptidase N mRNA expression was significantly higher in colonic tissue of CD patients (nonsignificant increases in UC patients and in the expression of NEP protein levels in CD patients), but no changes were detected in serum levels.⁶⁶

Glucagon-like Peptides

Animal studies

Thirteen reports studied the effects of GLP-2, either by continuous infusion,^{38, 41} long-acting polymer-coupled (XTEN,³⁹ PEG^{62, 63}) or microsphere-associated⁶⁹ molecules, or degradation-resistant analogs.^{45, 49, 54, 60, 70} These studies reported significant improvements in histological and clinical scores, reduction in pro-inflammatory cytokine levels and MPO activity,

and a significant intestinal growth (intestinotrophic) response. This was assessed by increased crypt cell proliferation and increased survival/reduced apoptotic rates and manifested by decreased intestinal length reduction after experimental induction of inflammation. A study conducted in $\text{Glp}2r^{-/-}$ mice demonstrated a functional Paneth cell defect, with reduced bactericidal activity and reduced expression of intestinotrophic factors.⁵⁹ Furthermore, similar outcomes were obtained by activation of GPR40 (FFAR1, free fatty acid receptor 1⁸³) and TGR5 (GPBAR1, G protein-coupled bile acid receptor 1⁸⁴) in enteroendocrine L cells by specific agonists with increased expression and release of GLP-2.^{58, 64}

A report on GLP-1 nanomedicine revealed a significant decrease of pro-inflammatory cytokine IL-1 β levels and tissue damage, in addition to a partial attenuation of the diarrheal phenotype.⁴⁰

Human studies

In accordance with the results in animal models, a pilot study⁷⁴ of a marketed GLP-2 analog, teduglutide, demonstrated a trend toward an increased response and remission rate in IBD patients, although these differences were not statistically significant. A significant increase in plasma citrulline levels, an indirect marker of intestinal mucosal mass, was also reported.

Other human studies found lower expression of GLP-2R and GLP-2 in an inflammatory setting in IBD patients compared with noninflamed sites and healthy controls.^{50, 67, 78, 81} Schmidt et al⁷⁸ found no differences in tissue or plasma levels of GLP-2 or peptide YY (PYY) after meal-stimulation between IBD patients and healthy controls.

A report on CD patients showed overexpression of both GPR40 and GPR120 (FFAR4, free fatty acid receptor 4)⁸⁵ in inflamed ileal mucosa and a GPR120-dependent inhibition of GPR40-induced GLP-2 expression by L cells, promoted by upregulation of GPR120 by TNF- α .⁷⁹

DISCUSSION

Dipeptidyl peptidase 4/CD26 and its substrates have been recognized as important mediators of inflammation and immunity. However, data on the efficacy of manipulating the incretin axis as a treatment modality in IBD lack consistency.^{49, 70, 74}

Despite the success of DPP4 inhibitors as antidiabetic drugs, the use of DPP4 inhibitors raises special concerns regarding the potential short and long-term adverse effects of inhibiting a molecule with such a broad spectrum of interactions. This is conditioned by the specific cell population expressing this protein; the local availability, half-life, and biactivity of its substrates; reaction rates; and substrate generation by other proteases (such as APN). In this context, different tissues may present different metabolic signatures resulting from DPP4 action, depending on the underlying pathophysiological conditions.

Through its enzymatic activity, DPP4 can inactivate several inflammatory mediators, such as Mig (CXCL9), IP-10 (CXCL10), and I-TAC (CXCL11), greatly reducing their chemotactic activity, although without hindering antiangiogenic activity.⁸⁶ Conversely, cleavage of chemokine LD78 β by CD26/DPP4 significantly enhanced its lymphocyte and monocyte chemotactic properties.⁸⁷ In addition, DPP4-truncated products show different receptor interaction²² and selectivity⁸⁸ compared with their noncleaved precursor peptide. Drugs acting on these pathways can, therefore, have different net effects. Further research is warranted to assess for significant differences between truncated or nontruncated forms, with a comprehensive assessment of their regulatory mechanisms and their relevance to the inflammatory milieu.

In our systematic review, all studies showed at least a moderate therapeutic benefit of DPP4i in animal models of colitis. On the one hand, these results reflect the inhibition of the catalytic activity of DDP4i over bioactive substrates such as GLP-2, GLP-1, and VIP, greatly extending their half-lives. This indirectly inhibits costimulatory signals of T-cell activation and the production of Th1-polarizing cytokines and chemokines while promoting intestinal proliferation and tissue recovery (Fig. 1). On the other hand, a more direct effect of DPP4i action cannot be excluded because $\text{CD}26^{-/-}$ mice did not possess any inherent resistance to colitis development, nor did they display an enhanced rate of repair of the damaged mucosal tissue. The protective effect of DPP4i was only observed in the presence of DPP4/CD26, suggesting that DPP4i may have unknown mechanisms of action besides blocking catalytic activity.^{53, 72, 73}

In line with these findings, all animal studies with GLP-2 and GLP-1 analogs showed a clear benefit (Supplementary Table 1) regarding intestinal proliferation, preservation of tissue architecture, and prevention of weight loss in the setting of induced inflammatory lesions. This is supported by similarly positive findings in reports using drugs that induce the release of endogenous GLP-1 and GLP-2, such as GPR40⁵⁸ and TGR5 agonists.⁶⁴

The extrapolation of these data to humans is challenging. One study⁷⁴ enrolling patients with moderate to severe CD treated with the GLP-2 analog teduglutide failed to achieve significance from placebo, although a trend toward remission was observed (Table 1). However, this report was limited because it was based on a pilot study with a relatively small sample size. Moreover, the study had a high dropout rate, especially due to adverse effects (up to 31%) and uncomfortable posology (daily subcutaneous injections).

Lower expression of GLP-2R was reported in IBD patients vs controls, further significant reduction was reported in inflamed tissue vs healthy samples, and a significant decrease was reported in the expression of serum GLP-2.^{50, 67} One report, however, found no significant differences between IBD patients and controls in tissue content or plasma concentration

of GPL-2 or PYY after meal stimulation.⁷⁸ Whether this is a cause or a consequence of the underlying pathological process remains to be elucidated. Still, the use of GLP-2 analogs and DPP4i in IBD is a double-edged sword. Despite their benefits, these drugs promote GLP-2-dependent intestinal tumor cell proliferation and migratory activity,⁸⁹ which may be of concern in IBD patients who are already at higher risk of developing colorectal carcinoma.^{1,2} Further research is needed to assess their viability and safety as IBD drugs.

Despite their anti-inflammatory effects, DPP4i also block DPP4-mediated degradation of pro-inflammatory chemokines,⁸⁶ possibly contributing to the perpetuation of inflammatory stimuli. As such, one could argue that DPP4 inhibition enhances certain pro-inflammatory pathways and leads to either a new onset or a flare-up of the underlying IBD or other inflammatory conditions. Nevertheless, a recent meta-analysis did not find an increased risk of IBD in patients under DPP4i therapy.⁹⁰ This same mechanism can also be beneficial in specific pathologies. A recent study by Barreira da Silva et al⁹¹ showed that DPP4 inhibition prevented CXCL10 truncation (and inactivation) and enhanced CXCR3-mediated antitumor immunity and trafficking of T cells into the tumor parenchyma. Furthermore, DPP4 is cleaved by a not fully understood mechanism (eg, shedding), and its soluble form, which is excreted to plasma, may have yet uncharacterized endocrinological effects.¹⁰

Aminopeptidase N acts in synergy with DPP4 for the regulation of immune responses by cleaving peptides preprocessed by DPP4 or by generating substrates susceptible to cleavage by DPP4.⁹² Dual inhibition of APN/DPP4 had statistically significant beneficial effects in animal models of colitis and may be of clinical significance as a new direct or adjuvant treatment modality in many pathologies.^{43,93}

In recent studies, human Th17 cells were implicated in the pathogenesis of many autoimmune diseases, including IBD⁹⁴; this represents a step forward from the previous dichotomous Th1/Th2 paradigm. Bengsch et al⁹⁵ demonstrated that CD26⁺⁺ (highly expressing) cells express typical markers of type 17 differentiation, even before stimulation, suggesting that CD26⁺⁺ T cells harbor the Th17 lineage. Moreover, Th1 and Th2 cells were shown to be compatible with an intermediate CD26⁺ phenotype, whereas regulatory CD25⁺CD127⁻FOXP3⁺ IL-10-producing T cells showed an even lower expression. Patients with active IBD were found to have the highest frequency of tissue-infiltrating Th17 cells. A strong increase was observed in inflamed tissue lesions, where 25%–50% of CD26⁺⁺ T cells produced IL-17 upon stimulation, in contrast to peripheral blood, where only about 5% of CD26⁺⁺ T cells produced IL-17. These findings suggest an incomplete differentiation of Th17 cells in peripheral blood due to the lack of sufficient stimulatory signals found in the proinflammatory cytokine-rich environment of lesion sites. Thus, a link has been established between CD26 and Th17, corroborating the preponderant role of CD26 in autoimmunity.

In the dawning of the microbiome age, DPP4 gains even more interest. A recent proof-of-concept study demonstrated a DPP4-like activity of gut microbiota,⁹⁶ extending toward uncharted territories the importance of intestinal microbiome–host interactions in pathological settings.^{59,97}

Dipeptidyl peptidase 4 is also a potential serum biomarker for many diseases, including cancer and IBD, which have become an intense target of investigation in recent years.^{77,98} As evidenced in recent studies, sDPP4 levels and activity are significantly lower in IBD patients vs healthy controls, and sDPP4 correlated negatively with other disease activity markers such as C-reactive protein, orosomucoid and fecal calprotectin, and disease activity scores (Harvey-Bradshaw Index [HBI], partial Mayo Score [pMS], Crohn's Disease Activity Index [CDAI], and Truelove and Witts index [TW]) and endoscopic activity groups.^{75–77,80,82} As reported in our previous study,⁷⁷ an important benefit of the use of sDPP4 as a biomarker is the ability to predict the need for treatment escalation from baseline sDPP4 levels at an early stage of the disease process. This enables the identification of patients who would benefit *a priori* from a more aggressive treatment strategy, avoiding exposure to potentially ineffective drugs and the consequent risk of adverse effects. A biomarker that could direct clinicians to more effective treatment options (skipping the necessary steps of treatment escalation with safety) may, at the end of the road, prove to be more efficient and resource-sparing, and enable a faster control of the underlying disease. This translates to obvious gains for the patient in terms of time, expenses, and quality of life. Higher-powered prospective studies with larger sample sizes are needed to confirm these findings and prove their usefulness in a clinical real-world setting.⁷⁷

This review includes 45 studies and is, to the best of our knowledge, the first attempt to systematize the role of DPP4, APN, and related substrates in IBD. It also provides, for the first time, an overview of the integration of the underlying pathophysiological processes and potential applications in clinical practice. We highlight our translational approach to this subject (from preclinical animal models of disease to studies in IBD patients), which allowed to emphasize the existing knowledge gaps within and between both settings.

Nevertheless, this systematic review is hindered by some limitations. Stemming from the inherent limitations of the included reports, the overall quality of preclinical experimental studies was less than desirable. As illustrated in **Supplementary Table 2**, protocol variations within the “same” model and the considerable variability of animal strains and species do not allow for a reliable comparison between experiments. Underreporting of the protocol execution and the subjective nature of the quality assessment tools also limit the internal validity and the extrapolation of data from animal to human subjects. Most reports with human populations suffered for having a cross-sectional design (only one study was

a prospective cohort⁷⁷) or low sample size (Table 1), and only one⁷⁴ had an interventional approach akin to animal studies.

CONCLUSION

Despite the ubiquitous position and wide spectrum of interactions of these ectopeptidases intertwining with many known—yet poorly understood—pathophysiological processes, their diagnostic and therapeutic benefits may be soon applied to the growing roster of clinical tools for the management of IBD. Still, many concerns regarding their potential for stimulating carcinogenesis and immune dysregulation and their viability as biomarkers still need to be evaluated in depth. Further research is required to achieve the necessary data robustness to introduce these new applications into clinical practice with confidence and safety.

SUPPLEMENTARY DATA

Supplementary data is available at *Inflammatory Bowel Diseases* online.

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