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Review

CD26/dipeptidylpeptidase IV—chemokine interactions: double-edged regulation of inflammation and tumor biology

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ABSTRACT

Post-translational modification of chemokines is an essential regulatory mechanism to enhance or dampen the inflammatory response. CD26/dipeptidylpeptidase IV, ubiquitously expressed in tissues and blood, removes NH_2-terminal dipeptides from proteins with a penultimate Pro or Ala. A large number of human chemokines, including CXCL2, CXCL6, CXCL9, CXCL10, CXCL11, CXCL12, CCL3L1, CCL4, CCL5, CCL11, CCL14, and CCL22, are cleaved by CD26; however, the efficiency is clearly influenced by the amino acids surrounding the cleavage site and although not yet proven, potentially affected by the chemokine concentration and interactions with third molecules. NH_2-terminal cleavage of chemokines by CD26 has prominent effects on their receptor binding, signaling, and hence, in vitro and in vivo biologic activities. However, rather than having a similar result, the outcome of NH_2-terminal truncation is highly diverse. Either no difference in activity or drastic alterations in receptor recognition/specificity and hence, chemotactic activity are observed. Analogously, chemokine-dependent inhibition of HIV infection is enhanced (for CCL3L1 and CCL5) or decreased (for CXCL12) by CD26 cleavage. The occurrence of CD26-processed chemokine isoforms in plasma underscores the importance of the in vitro-observed CD26 cleavages. Through modulation of chemokine activity, CD26 regulates leukocyte/tumor cell migration and progenitor cell release from the bone marrow, as shown by use of mice treated with CD26 inhibitors or CD26 knockout mice. As chemokine processing by CD26 has a significant impact on physiologic and pathologic processes, application of CD26 inhibitors to affect chemokine function is currently explored, e.g., as add-on therapy in viral infection and cancer. *J. Leukoc. Biol.* 99: 955–969; 2016.

Abbreviations: ACKR = atypical chemokine receptor, BCG = *Bacillus* Calmette-Guérin, DPP = dipeptidylpeptidase, GAG = glycosaminoglycan, GCP = granulocyte chemotactic protein, GIP = gastric inhibitory peptide, GLP-1 = glucagon-like peptide-1, GPCR = G protein-coupled receptor, GRO = growth-regulated oncogene, HCC-1 = hemofiltrate CC chemokine, HCV = hepatitis C virus, HPC = hematopoietic progenitor cell,

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Introduction

Inflammation assists in defense against microbial infection and in healing of injury and is often associated with tumor growth. Strict regulation of the inflammatory response is essential for normal tissue function. Inappropriate down-regulation of the inflammatory response may lead to permanent tissue damage, chronic inflammation, and autoimmune diseases. Furthermore, progenitor cell retention in the bone marrow and leukocyte homing to lymphoid organs during physiologic or pathologic processes are strictly regulated [1, 2]. Chemokines have been identified as crucial players in the regulation of leukocyte homing and leukocyte extravasation during inflammatory processes [2]. The biologic activity of chemokines depends on their interaction with 7-transmembrane-spanning GPCRs and ACKRs [3–6]. In addition, chemokine availability in vivo also depends on the interaction of chemokines with specific matrix and cell-associated GAGs [7–9]. Regulation of chemokine activity occurs at multiple levels, including gene duplication, classic transcriptional and translational regulation of ligand and receptor expression, alternative splicing, and enzyme-mediated post-translational modification [10]. Up- and down-regulation of chemokine activity is crucial during the initiation and resolution of an inflammatory response, respectively, and during the migration of progenitor or mature leukocytes between lymphoid organs or between lymphoid organs and peripheral tissues [2]. Alteration of chemokine receptor expression is a crucial mechanism in maturation and differentiation of lymphocytes and dendritic cells and consequently, in their homing to particular organs or tumors [1, 11]. At an initial stage, inflammation can be enforced by a positive regulatory mechanism via an enhanced chemokine production and synergism between chemokines to augment the local leukocyte influx rapidly. For instance, CXCL8/IL-8 can synergize with CC chemokines in leukocyte recruitment to enhance the inflammatory response [12, 13]. Post-translational chemokine modification is more frequently, but not exclusively, observed on inflammatory chemokines [2, 14–16].

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Post-translational modifications, such as NH_2- and COOH-terminal proteolytic processing (e.g., by MMPs or the aminopeptidase CD13 and the DPP IV/CD26) and *N*- or *O*-linked glycosylation, have been detected on natural chemokines and have different consequences for their biologic activity, going from no effect to decreased or increased activity and altered receptor specificity [2, 14, 17]. For some chemokines, e.g., CXCL8, proteolytic NH_2-terminal cleavage results in enhanced biologic activity [18–21]. CXCL7/neutrophil-activating protein-2 even requires NH_2-terminal truncation to become chemotactically active [18, 22–24]. Moreover, proteolytic cleavage differently affects the binding and signaling capacity of some chemokines (e.g., CCL5/RANTES) through their various cognate receptors [25–29]. Furthermore, a receptor antagonist can be generated by proteolytic cleavage. CCL8/MCP-2(6–76), for instance, which is the result of proteolytic processing of CCL8(1–76), is devoid of chemotactic activity and acts as a monocyte chemotaxis antagonist, diminishing inflammation [17]. Glycosylation has been described for the CC chemokines CCL2/MCP-1, CCL5, CCL11/eotaxin, and CCL14/HCC-1, as well as for XCL1 and CX3CL1 [15]. In addition to truncation and glycosylation, citrullination, i.e., deimination of arginine to citrulline, has been identified as a natural chemokine modification. Citrullinated CXCL8 and CXCL10/IP-10 were isolated from cell conditioned medium [30, 31]. The enzymes responsible for the conversion of peptidylarginine to peptidylcitrulline are peptidylarginine deiminases and play an important role in autoimmune diseases, such as rheumatoid arthritis and multiple sclerosis [32]. Whether chemokine citrullination also affects disease outcome or disease progression in such patients needs further investigation.

Similar to these endogenous regulatory mechanisms, microorganisms infiltrating the human body can modulate the activity of the chemokine network through the production of chemokine-binding molecules, chemokine and chemokine receptor analogs, or chemokine-modifying enzymes, which may serve as mechanisms to escape an efficient immune response [4, 14].

In summary, processing often changes the interactions of chemokines with their receptors and hence, influences their biologic activities positively or negatively, depending on the substrate or protease involved. This review focuses on the current knowledge of post-translational modifications by the serine protease CD26 and the biologic consequences of its action on chemokines to understand better the role of the chemokine–CD26 connection in physiologic and pathologic processes.

CD26: SPECIFICITY, EXPRESSION, AND INHIBITORS

Binding and/or cleavage of target proteins

The cell-surface marker CD26 or DPP IV (EC 3.4.14.5) belongs to the peptidase family S9 (http://merops.sanger.ac.uk/cgi-bin/famsum?family=s9), which is the family of the prolyl oligopeptidases that also contains fibroblast activation protein α, DPP II/DPP 7, DPP 8, and DPP 9 [33, 34]. CD26 was first described in 1966 as enzymatic activity hydrolyzing glycyl-prolyl-β-naphthylamide from rat liver [35]. It is a serine type peptidase that removes dipeptides from the NH_2-terminal end of peptide chains if the penultimate residue is proline or alanine, but under certain conditions, other amino acids may be accepted [36–38].

Natural substrates of CD26 include neuropeptides, peptide hormones, vasoactive peptides, chemokines, and a few growth factors and cytokines [39–41]. Remarkably, many substrates activate GPCRs, although their structural characteristics are quite diverse [42]. The best studied function of CD26 is regulation of the incretin hormones that control the glucose metabolism, but hematopoiesis, immunity, and cancer biology are also influenced by CD26. Apart from its catalytic activity, CD26 interacts with several proteins, for instance, adenosine deaminase, the tyrosine phosphatase CD45, fibronectin, collagen, the chemokine receptor CXCR4, and the HIV gp120 protein [36]. In addition to indirectly facilitating entry and the cytopathic effect of X4 HIV strains [43], which is most probably mediated via CD26-cleaved CXCL12/SDF-1 with reduced receptor (CXCR4)-binding capacity, CD26 is a major entry factor for the Middle East Respiratory Syndrome coronavirus [44]. The role of CD26 within the immune system is a combination of its exopeptidase activity and its interactions with different molecules. This enables CD26 to regulate HPC/HSC homing, engraftment, and growth; to serve as a costimulatory molecule influencing T cell activity [45, 46]; and to modulate leukocyte chemotaxis and mobilization [47, 48].

CD26 expression

Cell-bound CD26 is rather ubiquitously expressed on blood cells, fibroblasts, and mesothelial, epithelial, and endothelial cells and can be detected in placenta, kidney, intestine, prostate, gall bladder, pancreas, and liver [37, 38, 45, 46, 49]. Furthermore, modulated expression of CD26 on malignant hematologic or solid tumor cells has been reported and may be of help in cytologic diagnosis [50]. As an adhesion molecule binding the extracellular matrix proteins collagen and fibronectin, CD26 might promote adhesion, migration, and metastasis of tumor cells [51]. Absence of CD26 is associated with tumor development in certain cancers (e.g., expression of CD26 is decreased during ovarian carcinoma and melanoma progression), whereas its presence is also correlated with aggressive clinical behavior in other tumor types, including T cell malignancies [52]. The multifunctional nature of CD26 can explain these seemingly contrasting effects.

Apart from its expression in solid tissues—either normal or malignant—CD26 has been demonstrated on several leukocytes, e.g., dendritic cells, activated B cells, NK cells, T cells, and human $CD34^+$ progenitor cells [38, 53, 54]. CD26 was characterized originally as a T cell subset marker, present on 10–60% of resting T cells [55]. Systematically lower CD26 levels were detected on $CD8^+$ compared with $CD4^+$ cells [55]. Expression of this membrane antigen is strongly up-regulated after activation [56] and therefore, is a suitable marker for activated T cells, which when expressing high levels of CD26, constitute a subpopulation of $CD45RO^+$ memory T cells and produce IL-2 in response to

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HSC = hematopoietic stem cell, I-TAC = IFN-inducible T cell α chemoattracant, IP-10 = IFN-γ-inducible protein 10, MDC = macrophage-derived chemokine, Mig = monokine induced by γ-IFN, MMP = matrix metalloproteinase, MS = mass spectrometry, SDF-1 = stromal cell-derived factor 1, SELDI-TOF-MS = surface-enhanced laser desorption/ionization time-of-flight mass spectrometry, TFPI = tissue factor pathway inhibitor

mitogenic or alloantigenic stimulation [38]. This unique population of $CD4^+$ cells is the only one that responds to recall antigens, induces synthesis of IgG in B cells, and activates MHC-restricted cytotoxic T cells [38]. Furthermore, CD26 may function in an alternative pathway of T cell activation [46]. Up-regulation of CD26 expression on HPCs by G-CSF and GM-CSF may affect chemokine activity (vide infra) [54]. However, as these growth factors are substrates for CD26, this may also provide a negative-feedback loop for the activity of these CSFs [39].

In addition to the membrane-bound protease, a soluble form of CD26 exists, which is enzymatically fully active and occurs at high levels in seminal fluid; lower amounts are detected in plasma, urine, and cerebrospinal fluid [38, 49]. Soluble CD26 activity acts as a biomarker for several pathologic conditions. For instance, the level of CD26 enzymatic activity in plasma is an independent prognostic factor for survival of colorectal cancer patients [57]. In addition, plasma CD26 activity is a predictor of the onset of insulin resistance and metabolic syndrome in apparently healthy Chinese [58]. Recently, CD26 has been suggested to be an adipokine, potentially linking obesity to insulin resistance and the metabolic syndrome [59]. For a long time, the enzyme releasing CD26 from cellular surfaces remained unknown. However, Röhrborn et al. [60] recently demonstrated that several MMPs can cleave CD26, setting free the extracellular catalytic domain. MMP9 is involved in CD26 shedding from adipocytes, and MMP1, MMP2, and MMP14 release CD26 from human vascular smooth muscle cells.

We can conclude that CD26 and its substrates highlighted in this review, namely chemokines, are often expressed under similar conditions (e.g., inflammation, hematopoiesis, and malignant transformation). Indeed, large numbers of $CD26^+$ T cells have been detected in peripheral blood and/or inflamed tissues of patients with multiple sclerosis, Graves' disease, tuberculoid leprosy, and rheumatoid arthritis [45, 61]. In rheumatoid arthritis, CD26 expression is increased on T cells infiltrating the synovial cavity [62], together with enhanced levels of several chemokines [63]. Inflammatory stimuli, up-regulating the expression of CD26, are also well-known inducers of chemokine transcription. For instance, in differentiated adipocytes, TNF-α increases the release of CD26 [59] and the expression of 34 chemokines, including the CD26 substrates CCL5, CXCL2/GRO-β, CXCL11/I-TAC, and CXCL12 [64]. In fibroblasts, IFN-γ alone or in the presence of TNF-α and/or IL-1β induces coexpression of CD26 and its substrate chemokine CXCL10 [65]. Furthermore, mesothelial cells, lining the abdominal cavity, express basal levels of CD26, and CD26 expression/activity is up-regulated by factors present in malignant ascites [66], which contain many inflammatory and constitutive chemokines that are substrates of CD26.

CD26 inhibitors

Currently, 5 CD26 inhibitors are approved by the U.S. Food and Drug Administration or the European Medicines Agency: sitagliptin, saxagliptin, linagliptin, alogliptin, and vildagliptin [67]. Based on their structure, the inhibitors of CD26 can be broadly divided into 2 classes: CD26 substrate-like inhibitors (sita-, saxa-, and vildagliptin) and nonpeptidomimetics (alo- and linagliptin) [68]. Several other anti-CD26 drugs are tested in advanced clinical trials.

CD26 inhibitors are currently applied to inhibit the most prominent studied function of CD26, namely, its role in glucose homeostasis through proteolytic inactivation of the insulinotropic hormones (incretins), glucose-dependent insulinotropic polypeptide (GIP), and GLP-1. These peptides are released in response to food in the intestinal lumen and potentiate the production and release of insulin and glucose clearance. Cleavage by CD26 completely inactivates the incretins. Following evidence in animal models of Type 2 diabetes for the therapeutic benefit of CD26 inhibitors stabilizing the incretins in vivo, CD26 inhibitors and CD26-resistant GLP-1 analogs are now approved therapeutics for diabetic patients. By prolonging the incretin effect, these therapeutics improve glucose tolerance. In addition, CD26 inhibitors (e.g., sitagliptin) improve pancreatic islet cell function in patients with Type 2 diabetes, as prolonged activation of the β-cell GIPR induces proliferation and reduces apoptosis [69]. Currently, sitagliptin is also considered as add-on therapy for treating patients with autoimmune (Type 1) diabetes, as inhibition of CD26 and its T cell-activating properties may preserve or increase β-cell mass [70]. Treatment with drugs from the incretin family is as efficient as the other known oral antidiabetic drugs, and it is safer than sulfonylurea treatment when comparing the incidence of hypoglycemic events [71]. The safety profile of CD26 inhibitors is, in fact, very good, as only few serious adverse events are reported, and their number was comparable with the placebo group. In contrast to CD26 inhibitors, GLP-1 analogs have an effect on body weight (decreased food intake) and are contraindicated in patients with moderate and severe renal failure. In addition, in diabetic patients who also suffer from coronary heart disease, it was claimed initially that treatment with sitagliptin improved their heart function and coronary artery perfusion. However, recently, completed, larger-scale studies failed to demonstrate that CD26 inhibitors lowered the incidence of major adverse cardiovascular events over several years of use in individuals with Type 2 diabetes or cardiovascular disease [67]. The fact that CD26 inhibitors might act cardioprotective is not surprising because of the large spectrum of peptides (including several cardioprotective peptides) metabolized by CD26, indicating that CD26 inhibitors are multitarget drugs having multiple effects. The effect of CD26 inhibitors has also been evaluated in other pathologies in which CD26 is considered important. For instance, the CD26 inhibitor sitagliptin had a protective effect in a rat model of induced colon carcinogenesis, reducing the number of precancerous lesions in the sitagliptin-treated animals [72]. Analogously, sitagliptin treatment was shown to reduce melanoma growth in mice as a result of delayed chemokine processing (vide infra) [73]. The latter study explores the possibility of therapeutic intervention blocking chemokine processing by CD26. Indeed, chemokines are very good substrates (rapidly cleaved) by CD26, as is discussed below.

FACTORS AFFECTING THE CHEMOKINE CLEAVAGE EFFICIENCY OF CD26

CD26 cleaves the first 2 aa from peptides/proteins with penultimate proline or alanine residues. Several cytokines and chemokines are characterized by the presence of such an NH_2-terminal XP motif. Although most cytokines were reportedly not

cleaved by CD26 [74], recently, IL-3 and hematopoietic growth factors, such as erythropoietin, G-CSF, and GM-CSF, were identified to be sensitive to CD26 truncation [39]. However, a number of chemokines were discovered previously as the first CD26 substrates with immune functions (**Fig. 1**). The difference in susceptibility to CD26 cleavage between cytokines and chemokines is probably partially based on the difference in size of these proteins; cytokines are, in general, larger proteins compared with chemokines. In addition, chemokines have an NH_2 terminus, which is flexible in structure and therefore more easily fits in the active site of proteases, including CD26. In the late 1990s, CCL5, CXCL10, and CCL11 were the first chemokines described as CD26 substrates [26, 27]. Over the years, CXCL6/GCP-2, CXCL12α/SDF-1α and CXCL12β/SDF-1β, CCL22/MDC, CCL3L1/LD78β, CXCL9/Mig, and CXCL11 followed [27, 77, 78, 81, 82, 85, 95, 98]. More recently, CXCL2 and post-translationally processed and activated CCL14(9–74) were discovered as additional chemokines sensitive to CD26 cleavage [75, 94]. Contrasting data have been published regarding the susceptibility of CCL4/MIP-1β to cleavage by CD26 [85, 88]. The dissimilar susceptibility published might be a result of the different chemokine concentrations used for incubation. Oligomerization, at higher concentrations [85], might limit CD26 cleavage and therefore, mask potential cleavage of CCL4 by CD26. Further in-depth investigation is required to confirm this hypothesis.

The presence of an NH_2-terminal XP or XA dipeptide is necessary, however not sufficient for truncation of chemokines by CD26, as some chemokines characterized by such a motif, i.e., CCL2, CCL8, and CCL7/MCP-3, proved resistant to cleavage [27]. Resistance of the MCPs is a result of the presence of a pyroglutamic acid at the NH_2 terminus, which is the result of post-translational conversion of the glutamine residue and renders these chemokines fully active [99]. Recombinant CCL8

Chemokines with putative CD26 cleavage site		Consequences of indicated proteolytic cleavage		Cellular source of cleaved isoforms
Chemokine	NH_2-terminal amino acid sequence	Receptor recognition	Biological activity (in vitro)	
CXCL2/GRO-β	A **P** L A T E L R C Q C	N.D.	N.D.	
	[75]			
CXCL6/GCP-2	G **P** V S A V L T E L R C T C	N.D.	neutrophil activity unchanged [27]	MG-63 osteosarcoma cells [76]
	[27]			
CXCL9/Mig	T **P** V V R K G R C S C	decreased (CXCR3)	lymphocyte chemotaxis decreased [77]	
	[77]		angiostatic activity unchanged [77]	
CXCL10/IP-10	V **P** L S R T V R C T C	decreased (CXCR3)	lymphocyte chemotaxis decreased [77]	MG-63 osteosarcoma cells [76], fibroblasts [65]
	[77]		angiostatic activity unchanged [77]	
	L S R T V R C T C	N.D.	N.D.	fibroblasts [65]
CXCL11/I-TAC	F **P** M F K R G R C L C	decreased (CXCR3)	lymphocyte chemotaxis decreased [77, 78]	cultured keratinocytes [79]
	[77, 78]		angiostatic activity unchanged [77]	
	M F K R G R C L C	decreased (CXCR3)	lymphocyte chemotaxis decreased [80]	fibroblasts, PBMCs [80]
	[80]		angiostatic activity decreased [80]	
CXCL12/SDF-1	K **P** V S L S Y R C P C	decreased (CXCR4)	lymphocyte chemotaxis decreased [81, 82, 83]	
	[81,83]		hematopoietic progenitor cell chemotaxis decreased [84]	
CCL2/MCP-1	*pQ* **P** D A I N A P V T C C			
	N.C. [27]			
CCL3L1/LD78β	A **P** L A A D T P T A C C	increased (CCR1)	monocyte chemotaxis increased [85, 86]	monocytes [85]
	[85]	decreased (CCR3)	eosinophil chemotaxis decreased [86]	
		increased (CCR5)	lymphocyte chemotaxis increased [85, 86]	
CCL4/MIP-1β	A **P** M G S D P P T A C C	increased (CCR1)	CCR1 signaling increased [87]	PB lymphocytes [88]
	[88]	increased (CCR2)	CCR2 signaling increased [87]	
		unchanged (CCR5)	CCR5 signaling unchanged [87]	
CCL5/RANTES	S **P** Y S S D T T P C C	decreased (CCR1)	monocyte chemotaxis decreased [25-27, 29]	fibroblasts [27], PB leukocytes [89], sarcoma cells [28]
	[27]	decreased (CCR3)	eosinophil chemotaxis decreased [29]	
		increased (CCR5)	lymphocyte chemotaxis increased [25, 26]	
CCL7/MCP-3	*pQ* **P** V G U B T S T T C C			
	N.C. [27]			
CCL8/MCP-2	*pQ* **P** D S V S I P I T C C			
	N.C. [27]			
CCL11/eotaxin	G **P** A S V P T T C C	decreased (CCR3)	eosinophil chemotaxis decreased [90]	dermal fibroblasts [91, 92]
	[90]			
CCL14/HCC-1	T K T E S S S R G **P** Y H P S E C C			
	G **P** Y H P S E C C	decreased (CCR1)	CCR1 signaling decreased [93]	
	[94]	decreased (CCR3)	CCR3 internalization decreased [94]	
		decreased (CCR5)	CCR5 signaling decreased [93]	
	Y H P S E C C	decreased (CCR1)	CCR1 signaling decreased [93]	human blood filtrate [93]
	[93]	N.D. (CCR3)	CCR3 internalization decreased [94]	
		decreased (CCR5)	CCR5 signaling decreased [93]	
CCL22/MDC	G **P** Y G A N M E D S V C C	decreased (CCR4)	lymphocyte chemotaxis decreased [95, 96]	transformed $CD8^+$T cells [97]
	[95]		monocyte chemotaxis unchanged [95, 96]	

Figure 1. Overview of the chemokines with putative CD26 cleavage sites. The NH_2-terminal sequences of the chemokines are shown in 1-letter code, and *pQ* points to an NH_2-terminal pyroglutamic acid. Cysteine residues are underlined to mark the CXC/CC motif. Observed cleavages by CD26 and CD13 are indicated with full or dotted vertical lines, respectively. When a chemokine is a substrate for CD26 and/or CD13, the consequences of cleavage on receptor binding and biologic activity are depicted, and potential cellular sources for these cleaved isoforms are exemplified. Reference numbers are indicated in brackets. N.D., Not determined; N.C., not cleaved; PB, peripheral blood; *pQ*, pyroglutamic acid.

with an NH_2-terminal Gln, instead of the pyroglutamic acid present in natural CCL8, was indeed cleaved by CD26 [26, 99]. These data show that in addition to the penultimate proline, the surrounding residues and the accessibility of the NH_2 terminus are important for CD26 substrate recognition.

Although all chemokines are cleaved behind a Pro-2 residue, located in the rather unstructured and flexible NH_2-terminal region, huge kinetic differences can be observed [98]. A comparison of the half-life of these chemokines in the presence of CD26 and their kcat/Km values reveals clear substrate selectivity: CXCL12 > CCL22 > CXCL11 > CXCL10 > CXCL9 > CCL11 > CCL5 > CCL3L1. These data confirm that the amino acids surrounding the scissile bond influence substrate selectivity [49]. Furthermore, dimerization of chemokines may impede accessibility of the NH_2-terminal region for CD26 and may explain the lower kinetics of CCL5, CCL3L1, and CCL4, which under the described experimental conditions, may form aggregates [100]. Therefore, the kinetics may be underestimated compared with kinetics in natural conditions. This hypothesis is also supported by the fact that for these chemokines, isoforms missing the NH_2-terminal dipeptide have been isolated from natural sources (vide infra).

Interestingly, cleavage of CCL22 by CD26 is not restricted to the removal of the NH_2-terminal GP dipeptide [95]. On the contrary, after removal of the first 2 residues, CD26 can cleave a second time, more specifically, the peptide bond between Gly-4 and Ala-5. However, cleavage of the Gly-4–Ala-5 peptide bond occurs definitely much slower than the Pro-2–Tyr-3 peptide bond, which results in the accumulation of CCL22(3–69) before further degradation to CCL22(5–69). Cleavage by CD26 behind a penultimate Gly residue is highly uncommon and probably strongly dependent on the nature of the surrounding amino acids.

CD26-mediated cleavage of chemokines can be negatively regulated by the presence of GAGs. Binding to GAGs, important in the formation of a chemokine gradient in the extracellular matrix and the presentation of chemokines on the endothelium, was described to protect CXCL12 from processing by CD26 [101]. Such protection probably depends on the expression level and structure type of the GAGs expressed and might be location specific. These findings, however, should not be generalized, as CXCL12 processing by MMP2 is not inhibited by GAG binding [102].

NH_2-terminal cleavage by CD26 may turn a chemokine susceptible to cleavage by aminopeptidases. Indeed, proline residues near the NH_2 terminus serve as a structural protection of the NH_2-terminal peptide bonds against proteolytic degradation by aminopeptidases [103]. The removal of these proline residues may render proteins susceptible to aminopeptidases and thus, more prone to degradation during an inflammatory response. For example, intact CXCL10, CXCL11, and CCL14(9–74) are resistant to cleavage by aminopeptidase N/CD13 as a result of the presence of a proline residue on the penultimate position. Upon cleavage by CD26 and the removal of this proline residue, these chemokines become efficient substrates for CD13 (unpublished results in **Fig. 2A**) [80, 93].

To study chemokine production, numerous cell cultures (fibroblasts, leukocytes, tumor cell lines, etc.) have been stimulated with TLR ligands and/or cytokines, mimicking an inflammatory reaction, and the produced chemokines have been analyzed. Purification and biochemical characterization by MS revealed the production of significant amounts of chemokine isoforms that might result from CD26 cleavage, as listed in Fig. 1 (examples are given; this list is not meant to be exhaustive) [27, 29, 65, 76, 79, 80, 87, 89, 91, 92, 97]. Purification of such truncated chemokine isoforms from conditioned media of a broad array of cultured cells underscores the importance of the in vitro-observed interactions between CD26 and chemokines.

CONSEQUENCES OF CD26-MEDIATED PROCESSING ON THE RECEPTOR-BINDING AFFINITY AND CHEMOTACTIC ACTIVITY OF CHEMOKINES

Cleavage of chemokines by CD26 clearly influences the activity of the chemokine. However, rather than being characterized by 1 general consequence, the effects of NH_2-terminal truncation are highly diverse. Either no difference in activity or drastic alterations in receptor recognition and specificity and hence, biologic activity is observed (**Figs.** 1 and **3A**).

For most chemokines, CD26 cleavage leads to reduced chemokine activity and occasionally, to the generation of a receptor antagonist. Proteolytic cleavage of CXCL12α by CD26 results in reduced binding affinity for CXCR4 and the loss of its calcium-dependent signaling and chemotactic properties for peripheral blood lymphocytes [81–83, 104]. These data may explain why $CD26^+$ peripheral blood lymphocytes are less efficiently attracted by CXCL12α than $CD26^-$ peripheral blood lymphocytes [82]. Analogously, CD26 inhibition on T cells has been reported to prolong protein kinase B and ERK-2 signaling via CXCR4 upon stimulation with CXCL12 [105]. In parallel, CD26-truncated CXCL12 also loses its chemotactic effect on CXCR4-expressing HPC/HSCs and thus, its capacity to function as a retention signal in the bone marrow [84, 106, 107]. Moreover, truncated CXCL12 blocks the response of HPCs to intact CXCL12. Accordingly, chemotaxis of HPCs to CXCL12 is enhanced when pretreating HPCs with a CD26 inhibitor or when using $CD26^{-/-}$ mouse bone marrow cells [48, 84]. In addition, removal of the NH_2-terminal Lys-Pro dipeptide clearly lowers the heparin-binding affinity of CXCL12α [108]. Analogously, CD26-mediated truncation of CCL11 results in a 30-fold reduced chemotactic potency on eosinophils [90]. However, CCR3 binding is only 6-fold diminished. As a result, CCL11(3–74) is able to desensitize and partially antagonize CCL11-induced chemotaxis [90]. Both NH_2-terminally truncated isoforms of CCL22 generated by CD26 show reduced lymphocyte chemotaxis, which is explained by reduced CCR4 binding and signaling. In contrast, monocyte binding and chemotaxis remain unaffected, indicating the possible involvement of another receptor, apart from CCR4, in these processes [95, 96]. Biologic inactivation, as a result of CD26 cleavage, is observed for the CXCR3 ligands CXCL9, CXCL10, and CXCL11 [77, 78]. Truncated CXCL9, CXCL10, and CXCL11 are unable to induce chemotaxis of CXCR3A-transfected cells at concentrations 30-fold higher than the minimal effective concentration of their intact counterparts. CXCL10 and CXCL11 retain weak

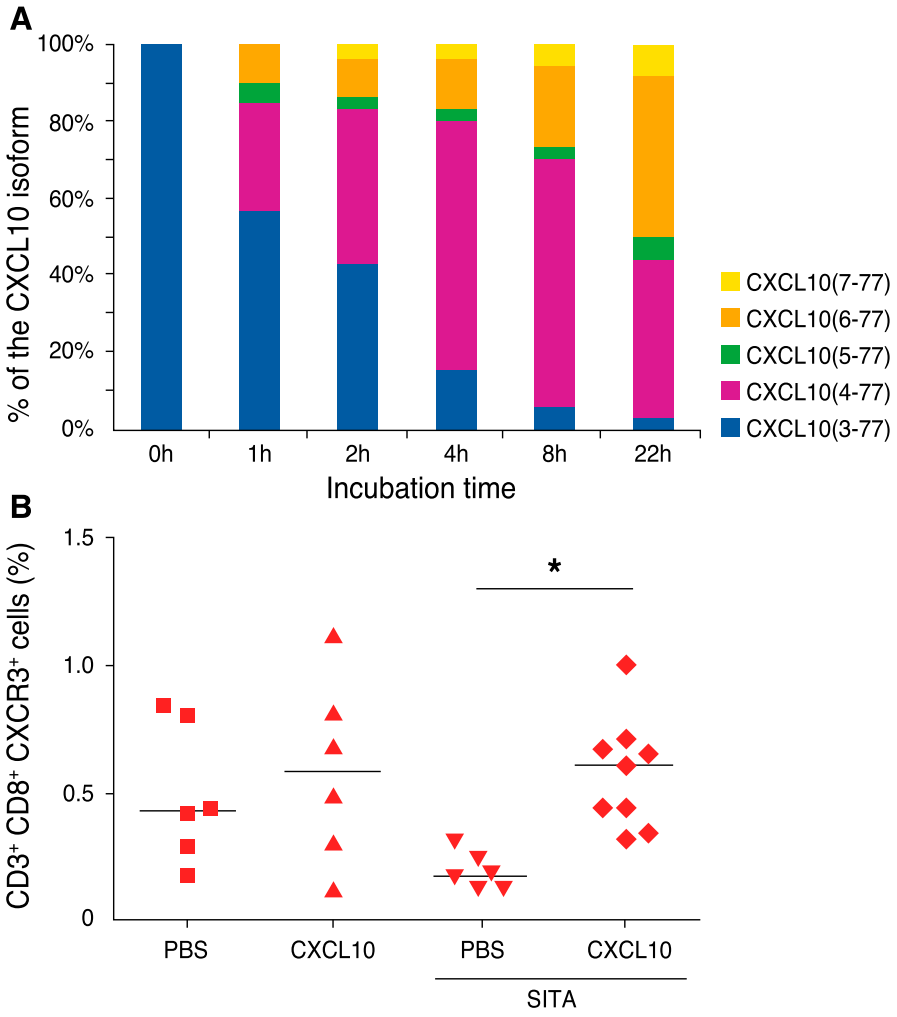

Figure 2. Biochemical and biologic consequences of CD26 cleavage for CXCL10. (A) CD13 efficiently cleaves CD26-truncated CXCL10. Recombinant CXCL10(3–77), the isoform produced upon CD26-mediated cleavage of intact CXCL10, was incubated with porcine CD13 (enzyme:substrate ratio: 1:25) in PBS. The percentages of the different CXCL10 isoforms, present in the incubation mixture after the indicated time periods, are shown. (B) CXCL10-dependent migration of lymphocytes in vivo depends on CD26 inhibition. Sitagliptin-treated (SITA; via drinking water; 10 mg/day, 3 d of treatment) or control Naval Medical Research Institute (NMRI) mice were intraperitoneally injected with 10 μg CXCL10 or vehicle. The peritoneal cavity was washed, and the migrated cells were identified and quantified by flow cytometry. The horizontal lines mark the median number of cells. To detect statistically significant differences, the Mann-Whitney *U* test was carried out ($*P < 0.05$).

CXCR3-binding properties and partially antagonize chemotaxis induced by their intact counterparts. Surprisingly, CXCL9- and CXCL10-mediated angiostatic activity remains unchanged after processing by CD26 [77]. Nevertheless, upon further truncation by CD13, the angiostatic activity of CXCL11 is impaired as well [80]. CCL14, a CC chemokine that is constitutively expressed by a variety of tissue cells and abundantly present in plasma, depends on proteolytic processing for its activity. Processing into CCL14(9–74) by plasmin or urokinase plasminogen activator is necessary for CCL14 to become an agonist for CCR1, CCR3, and CCR5 (with a specific activity comparable to that of CCL5 and CCL3/LD78α) [109, 110]. However, further cleavage by CD26 (possibly in combination with CD13) generates an inactive variant [93, 94]. Therefore, a subtle equilibrium between these enzymes determines the fate of CCL14.

For some chemokines, the effect of truncation depends on the receptors involved. CCL5 is the first identified CD26-processed chemokine that has been studied in detail [25–27, 29]. The activity of CCL5(3–68) is down-regulated regarding signaling assays and chemotaxis tests on monocytes and eosinophils. CCL5(3–68) even acts as an antagonist in in vitro monocyte chemotaxis assays. In contrast, its lymphocyte chemotactic activity remains unaffected [25, 27, 29, 89]. These apparently contradictory results for CCL5(3–68) are explained by a loss of CCR1 (expressed on monocytes)- and CCR3-binding and -signaling potency but an even moderately increased interaction of CCL5(3–68) with CCR5 functionally expressed on lymphocytes [28, 29]. Thus, CD26 appears to modulate CCL5 to a T cell-specific chemoattractant. Changed receptor selectivity is also observed for CCL3L1, the nonallelic variant of CCL3. The latter only differs from CCL3 in 3 aa, among which, the Pro-2 residue making CCL3L1, in contrast to CCL3, a substrate for CD26 [85]. Cleavage by CD26 enhances the binding and signaling of CCL3L1 to CCR1 (strongly) and CCR5 (moderately) [85, 86]. In accordance, CCL3L1(3–70) acts as an extremely potent monocyte (CCR1) and lymphocyte (CCR5) chemoattractant. Truncation has the opposite effect on binding and signaling via CCR3, the third receptor recognized by CCL3L1 [86]. As a result of impaired binding and signaling of CCL3L1(3–70) to CCR3, eosinophils with undetectable expression of CCR1 only migrate in response to intact and not to truncated CCL3L1. If eosinophils show high expression of CCR1, which is the case for ~20% of the donors [111], however, the reduced signaling of truncated CCL3L1 through CCR3 is over-ruled by the enhanced response

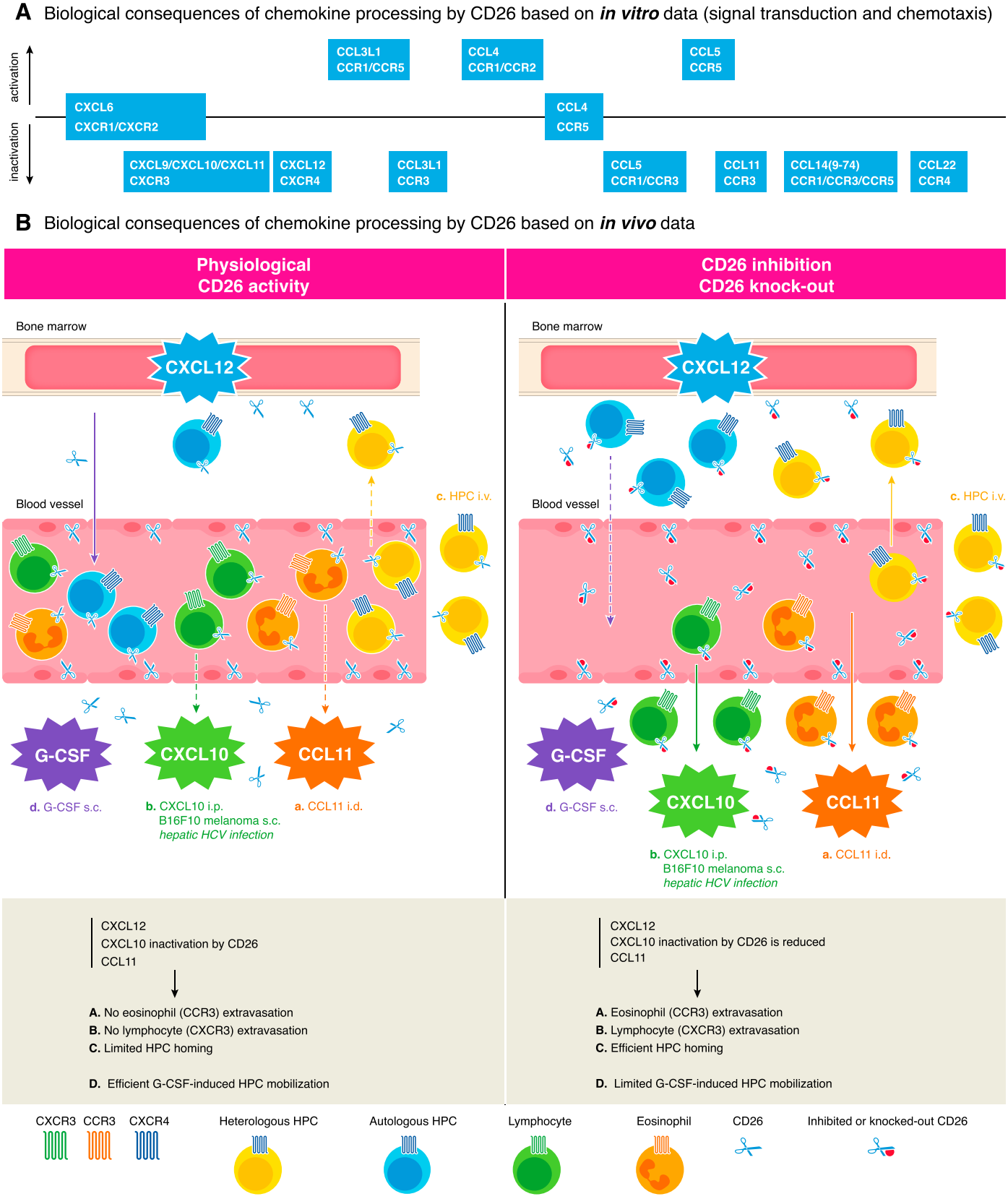

Figure 3. Overview of the consequences of CD26-mediated cleavage on chemokine activity, as evidenced in vitro and in vivo. (A) The consequences of CD26-mediated cleavage of various chemokines on their biologic activity based on in vitro data (capacity to induce signal transduction and

(*continued on next page*)

of CCR1 to CCL3L1, making CCL3L1 and truncated CCL3L1 equal stimulators of $CCR1^+$ eosinophil chemotaxis. The closely related chemokine CCL4 retains its CCR5-dependent activity upon CD26 cleavage [87]. Moreover and in contrast to full-length CCL4, it becomes active on CCR1 and CCR2 [87].

Unlike all other chemokines that are substrates for CD26, the biologic activity of the neutrophil chemoattractant CXCL6 remains unaffected upon CD26-mediated cleavage [27].

As the majority of the chemoattracted leukocytes express CD26, CD26 can provide a positive- or negative-feedback loop. For example, activated T lymphocytes, attracted by the CXCR3 ligands CXCL9, CXCL10, and CXCL11, express CD26 [38]. With the use of a specific CD26 inhibitor, Ludwig et al. [78] demonstrated that cleavage of CXCL11 by PHA- and IL-2-treated T cells was a result of CD26. Therefore, the infiltration of these cells may result in the cleavage of these chemokines and the generation of inactive and even antagonistic variants, preventing further infiltration of activated T cells.

Considering the effect of CD26 cleavage on the different leukocyte subtypes, $CCR1^-$ neutrophil [86] migration can be concluded to be unaffected by the presence of CD26. Indeed, the activity of the only neutrophil chemoattractant being cleaved by CD26, i.e., CXCL6, remains unaffected. In contrast, eosinophil migration mediated through CCR3 will be reduced in the presence of high concentrations of CD26, as the activity of the 3 eosinophil-attracting chemokines CCL5, CCL3L1, and CCL11 is strongly decreased upon CD26-mediated cleavage. However, in case eosinophils show high expression of CCR1, the reduced signaling of truncated CCL3L1, CCL5, or CCL11 through CCR3 might be counteracted by the response of CCR1 to truncated CCL3L1. The effect of CD26 on the migration of monocytes and lymphocytes will depend on the monocyte/lymphocyte subtype (the specific chemokine receptor spectrum expressed) and the chemokine involved. CCR5-mediated migration of Th1 lymphocytes will be increased (CCL5, CCL4, CCL3L1), whereas CXCR3-dependent migration of these cells will be decreased (CXCL9, CXCL10, and CXCL11). Th2 cells, expressing CCR3 and CCR4, might exhibit reduced migration to CCL11 and CCL22. Thus, chemokine processing definitely impacts the receptor-dependent inflammatory properties of chemokines, but the consequences will depend on the chemokines and chemokine receptors involved.

Besides binding their cognate GPCRs, chemokines have been shown to interact with atypical, non-G protein-coupled 7-transmembrane receptors that fine tune chemokine availability through binding, internalization, or degradation [112]. Among these, ACKR2/D6, predominantly expressed by lymphatic endothelial cells, binds most inflammatory CC chemokines. Interestingly, CD26-mediated cleavage influences binding and degradation of such chemokines by ACKR2. The less-potent isoforms of CCL22, i.e., CCL22(3–69) and CCL22(5–69) no longer bind to ACKR2 [113]. Furthermore, CD26-mediated processing of the potent inflammatory isoform CCL14(9–74) into the less-active CCL14(11–74) isoform reduces the rate of degradation by ACKR2, although the binding affinity remains rather unaffected [114]. A proline residue at position 2 was suggested to be required for rapid chemokine degradation by ACKR2 [114]. As ACKR2 selectively recognizes and degrades the active chemokine isoforms, ACKR2 and CD26 might cooperate in the down-regulation of the biologic activity of these chemokines.

CONSEQUENCES OF CD26-MEDIATED PROCESSING ON THE ANTI-HIV ACTIVITY OF CHEMOKINES

Besides being involved in the generation of an adequate immune response upon viral, bacterial, or parasite infection, chemokine receptors are also used by pathogens, such as HIV, to infect host cells successfully. A major breakthrough in HIV research was made when chemokines were identified as potent inhibitors of HIV-1 infection [115]. A number of chemokine receptors have been shown to function as coreceptors for specific HIV strains [97, 116–120]. Through competition for binding to these receptors with the viral glycoprotein gp120, chemokines can inhibit HIV infection. CXCR4 and CCR5 are the main coreceptors for syncytium-inducing T-tropic (or X4-tropic) and nonsyncytium-inducing M-tropic (or R5-tropic) HIV strains, respectively. As CD26 cleavage may alter the receptor specificity or affinity of multiple chemokines, consequences for the antiviral activity of the processed chemokines were anticipated.

CXCL12 impedes CXCR4-mediated HIV-1 infection of T lymphocytes by binding to CXCR4 and inducing endocytosis of this viral coreceptor [81–83, 104]. The inhibitory effect of CXCL12 on infection by T-tropic HIV-1 strains is strongly

chemotaxis) are summarized, taking into account the specific chemokine receptor involved. The horizontal line depicts the basal activity of the intact chemokine (via the receptor indicated). Chemokines positioned above the horizontal line turn into more active isoforms (via the receptor indicated) upon cleavage, whereas for those chemokines under the horizontal line, the activity (on the receptor indicated) is reduced upon cleavage. (B) Based on studies that use mice treated with CD26 inhibitors or CD26 knockout mice, an important role for CD26 (depicted as scissors) in the regulation of CCL11-, CXCL10-, and CXCL12-mediated leukocyte trafficking has been elucidated. Only when CD26 is inhibited or knocked out (right), intradermal (i.d.) or intraperitoneal (i.p.) injection of CCL11 (a) or CXCL10 (b) results in the directional extravasation and migration (solid arrows) of $CCR3^+$ eosinophils and $CXCR3^+$-activated T cells, respectively. In the case of physiologic CD26 concentrations (left), CCL11 and CXCL10 are cleaved and inactivated rapidly, and no directional extravasation and migration can be detected (dashed arrows). Analogously, the antitumoral [B16F10 melanoma (b)] influx of activated T cells is strongly limited, as a result of CD26-dependent abrogation of the CXCL10 guidance gradient. In addition, the efficacy of the human immune response to HCV infection is highly dependent on the degree of CD26 activity in the blood. Higher CD26 activity has been associated with lower concentrations of active CXCL10 and a lower probability to resolve infection spontaneously or to respond to therapy, suggested to be a result of less efficient CXCL10-mediated lymphocyte trafficking to the liver (italics indicate that evidence is based on association with CD26 activity instead of direct CD26 inhibition). Transplanted HPCs (c) show increased homing efficiency and engraftment capability when CD26 on these cells is inhibited or knocked out (right, solid arrow). (i.v., intravenous) In contrast, mobilization of HPCs from the bone marrow to the blood upon subcutaneous (s.c.) G-CSF injection (d) is strongly reduced when CD26 is inhibited or knocked out (right, dashed arrow). The effect of CD26 on homing and mobilization of HPCs has, at least partially, been explained by CD26-dependent inactivation of CXCL12, the chemokine that is responsible for HPC retention in and recruitment to the bone marrow.

reduced upon removal of the NH_2-terminal dipeptide, which is in accordance with the decreased CXCR4 binding affinity of this isoform [81, 104]. Although CXCL12 concentrations in normal serum and serum from AIDS patients are technically sufficient to block HIV-1 infection, CXCL12 seems to fail to prevent HIV-1 infection and spread in vivo, seen the susceptibility of humans to HIV infection. Natural CXCL12, circulating in blood, was shown to be rapidly modified by CD26, resulting in a functionally inactive molecule, which might explain these apparently contradictory observations [121–124].

The major coreceptor for M-tropic HIV-1 strains is CCR5, a receptor that binds multiple CC chemokines, including CCL3L1 and CCL5. In contrast to truncated CXCL12, most CCR5 ligands gain receptor affinity upon truncation by CD26. CCL5(3–68) becomes even a CCR5-specific ligand, explaining its enhanced anti-HIV-1 activity [27, 28]. Interestingly, HIV-1 experiments, with cell lines that express different levels of CD26 on their membrane, have shown an inverse correlation between the IC_{50} values of intact CCL5 and the expression of CD26 on the cell line to be infected [28]. These data suggest that intact CCL5 has very poor antiviral activity but may be rapidly converted into the more potent antiviral isoform CCL5(3–68) upon truncation by soluble or membrane-bound CD26 [28]. In addition, intact CCL4 behaves as a rather specific CCR5 agonist. However, processing of CCL4 by CD26 generates a CCR1 and CCR2 ligand with retained CCR5 interaction and anti-HIV-1 activity [87]. A third CD26-susceptible CCR5 ligand is CCL3L1. Although it only differs in 3 aa from its nonallelic variant CCL3, CCL3L1 is a much more potent inhibitor of infection with R5-tropic HIV strains [125, 126]. The importance of CCL3L1 in the protection against HIV infection is illustrated by the association of low copy numbers of the *CCL3L1* gene with an increased risk of acquiring HIV infection and progressing rapidly to AIDS [127, 128]. CD26-processed CCL3L1(3–70) has even more antiviral activity, yielding a most potent inhibitor for infection with HIV-1 [86].

In addition to CXCR4 and CCR5, several other chemokine receptors, e.g., CCR2 and CCR3, have been identified as coreceptors for specific HIV strains, and the antiviral activity of their chemokine ligands is also variably affected by CD26 processing. NH_2-terminal truncation with CD26 does not change the antiviral activity of CCL11 against an HIV-2 strain [90]. Although CCL22(1–69) and CCL22(3–69) neither signal through CCR5 and CXCR4 nor desensitize these receptors for a CCL5- or CXCL12-induced calcium response, both isoforms partially protect PHA-stimulated PBMCs from HIV infection [96]. As binding to CCR4, the cognate receptor for CCL22, is lost upon truncation, these results suggest that CCL22 is a ligand for an alternative receptor, which is involved in HIV cell entry [96]. In conclusion, host-dependent post-translational modification of chemokines by soluble or membrane-bound CD26 influences the susceptibility of cells to HIV infection.

IMPACT OF CD26 ACTIVITY ON PHYSIOLOGIC AND PATHOLOGIC INFLAMMATORY PROCESSES

For years, evidence for the actual interaction between CD26 and chemokines was merely based on the localization of CD26, including the expression on the cell membrane of cells involved in inflammation and in plasma, the primary specificity and cleavage kinetics of chemokines by CD26, and the purification of truncated chemokine isoforms from conditioned media of a broad array of cultured cells.

Recent in vivo studies underscore the high biologic impact and relevance of CD26 chemokine interactions. First of all, chemokine cleavage, generating isoforms lacking the NH_2-terminal dipeptides, does occur in vivo. Injection (intravenous or subcutaneous) of CXCL12 and analysis of the plasma by SELDI-TOF-MS, 5–30 min postadministration, reveal the presence of NH_2- and COOH-terminally processed CXCL12 [122]. After 5 min, no less than 80% of the intravenously injected CXCL12 is converted to CXCL12(3–67). Analogously, fast NH_2-terminal truncation of CCL5 is observed upon intraperitoneal injection of CCL5(^{44}AANA^{47}; a mutant isoform with reduced GAG-binding affinity) and subsequent SELDI-TOF-MS-based analysis of the CCL5 present in serum [129]. The natural occurrence of such CD26-processed chemokine isoforms in plasma underscores the importance of the observed CD26 cleavages. CD26-cleaved CXCL12 has been identified in human, murine, and rhesus monkey blood plasma [123, 124, 130]. Furthermore, plasma levels of CXCL12(3–67) in mice or rhesus monkeys are reduced significantly upon treatment with the CD26 inhibitor MK-0626 [123]. Analogously, endogenous CXCL12 in plasma of $CD26^{-/-}$ mice is NH_2-terminally intact, whereas plasma of wild-type mice contains significant amounts of CD26-truncated CXCL12 [130]. In addition, the levels of endogenous, intact CXCL10, induced by intravenous injection of CpG, are also increased significantly when mice are treated with sitagliptin, an orally active CD26 inhibitor (vide supra) [73]. All of these data provide in vivo evidence for CD26-dependent processing of chemokines.

Evaluation of the biologic activity of chemokines in mice, in which CD26 activity is inhibited (by sitagliptin) or absent ($CD26^{-/-}$ rodents), further extends our knowledge on the biologic consequences of CD26 processing (Fig. 3B). Mobilization of eosinophils into the blood upon intravenous administration of CCL11 is enhanced significantly in $CD26^{-/-}$ rats. Moreover, also intradermal injection of CCL11 in CD26-deficient rats results in increased eosinophil recruitment into the skin [131]. Analogously, murine CXCL10 is only able to induce significant extravasation of $CD8^+CXCR3^+$ cells upon intraperitoneal injection, when mice are fed with sitagliptin chow or when $CD26^{-/-}$ mice are used [73]. In addition, our data show that administration of sitagliptin via drinking water also restores the lymphocyte-attracting activity of human CXCL10 upon intraperitoneal injection in mice (unpublished results in Fig. 2B).

Through the modification of chemokines, CD26 activity was shown to influence disease progression and therapeutical efficacy. CXCL10(3–77) was found in plasma of HCV patients and correlates with the development of chronic HCV infection and pegylated IFN-α_2/ribavirin treatment failure. The measurement of the concentration of CXCL10 in the plasma of chronic HCV patients using ELISAs specifically discriminating between CXCL10(1–77) and CXCL10(3–77) showed that the dominant form of circulating CXCL10 in these patients is the truncated, antagonistic isoform CXCL10(3–77) [132]. Furthermore, the concentrations of antagonistic CXCL10(3–77) and CD26 activity are significantly higher in patients who do not respond to therapy

with pegylated IFN-α_2/ribavirin. CD26-mediated cleavage of CXCL10 is suggested to result in perturbed lymphocyte trafficking to the liver in chronic HCV patients [133]. Later on, follow-up of patients with acute viral HCV infection showed that those patients who develop chronic infection have higher plasma concentrations of truncated CXCL10 compared with those patients who spontaneously resolve infection. This suggests that a disturbed CXCL10 gradient inhibits the development of an efficacious innate and adaptive host immune response to HCV and favors the evolution of viral persistence [134]. Likewise, following BCG therapy for treatment of nonmuscle invasive bladder carcinoma, CD26 cleavage of CXCL10 might limit the migration of CXCR3-expressing NK cells and T cells and thereby, counteract the antitumor response. Elevated CXCL10 levels are detected in the urine upon treatment [135], but differential CXCL10 measurements showed that a substantial part of the CXCL10 lacked the NH_2-terminal dipeptide. HCV patients and patients with bladder carcinoma treated with BCG might benefit from the use of CD26 inhibitors, as these may enhance the efficacy of therapy. Barreira da Silva and colleagues [73] nicely showed how delayed tumor (B16F10 melanoma and CT26 colon carcinoma) growth in $CD26^{-/-}$ mice can be explained by enhanced $CXCR3^+$ lymphocyte infiltration into the tumor tissue as a result of decreased proteolytic inactivation of CXCL10. Moreover, the combination of immunotherapy (intratumoral injection of CpG, adoptive cell transfer, and checkpoint blockade) with sitagliptin treatment clearly enhances tumor rejection and the response to current therapeutical approaches. However, the beneficial role of CD26 in Sézary syndrome illustrates that the opposite can also be true [136]. The accumulation of $CXCR4^+$ T lymphoma cells in the skin is associated with impaired CD26 expression, which is suggested to result in reduced inactivation of CXCL12 (abundantly expressed in the skin) and accelerated CXCL12-mediated homing to the skin. As a result of the broad spectrum of substrates cleaved by CD26, in addition to its immune-related functions as a costimulatory and adhesion molecule (vide supra) [36], the antitumoral effect of CD26 inhibition cannot be generalized. Indeed, CD26 expression in different tumor types has been associated with good as well as poor prognoses, depending on the mechanism (and CD26 substrates involved) underlying tumor development and progression [51].

A role for CD26 in the regulation of HPC trafficking has been ascribed to its effects on the CXCL12–CXCR4 axis, which is considered to be responsible for retaining HPCs in the bone marrow and for efficient bone marrow engraftment. CD26 has been shown to be involved in G-CSF-induced mobilization. It is expressed by a subpopulation of human cord blood $CD34^+$ cells and negatively regulates CXCL12-induced chemotaxis of these cells [54]. Expression of CD26 on $CD34^+CD38^-$ cells is enhanced upon treatment with G-CSF, resulting in a decreased chemotactic response to CXCL12 [54]. When CD26 is inhibited (diprotin A, a first-generation CD26 inhibitor) or absent ($CD26^{-/-}$ mice) during treatment with G-CSF, mobilization of HPC in mice is reduced dramatically, providing evidence for an in vivo role for CD26 in G-CSF-induced mobilization of HPCs [47, 137]. Therefore, it is hypothesized that G-CSF, by up-regulating CD26 expression on $CD34^+$ cells, induces the cleavage of CXCL12, resulting in inactivation of CXCL12 and a loss of its function as a retention signal for HPC. Alternative proteases, such as neutrophil elastase and cathepsin G, have also been suggested to influence HPC mobilization through degradation of CXCL12 and might cooperate with CD26 [138, 139]. The constitutive HSC mobilization seen in patients with chronic myeloproliferative neoplasms has been, at least partially, ascribed to the predominance of CXCL12 truncation products [CXCL12 (3,4,5,6–67)] within the bone marrow plasma compared with the bone marrow plasma of healthy controls, which contains significantly more intact CXCL12 [140]. Further research showed that inhibition (diprotin A) or loss of endogenous CD26 activity on donor HPCs results in increased homing efficiency and engraftment capability (a CXCR4-dependent process [141]) in lethally irradiated congenic mice [48]. Interestingly, glypican-3, which is coexpressed with CD26 on HSC/HPCs, blocks CD26 activity and serves as an endogenous regulator of CD26, important for HSC maintenance in the bone marrow [142, 143]. Glypican-$3^{-/-}$ HSC/HPCs exhibit a decreased potential to migrate to CXCL12 and to home to and reside in the bone marrow. In addition, endogenous TFPI, an important mediator in the coagulation cascade and a ligand for glypican-3, enhances glypican-3-mediated inhibition of CD26 activity [142]. Thus far, glypican-3 and TFPI are the first endogenous CD26 inhibitors with clinical relevance described.

With the use of sitagliptin, the effect of CD26 inhibition on the HPC engraftment after cord-blood transplantation in patients with hematologic malignancies was evaluated in a clinical trial pilot study [144]. Although the dosing schedule of sitagliptin needs improvement [145], this pilot study reveals that CD26 is a promising target for improving the engraftment capacity, allowing for the use of HPCs from sources containing only limited cell numbers, such as cord blood. Effects of CD26 inhibition on HPC maintenance and trafficking are believed to be linked to inhibition of CXCL12 inactivation; however, the hematopoietic growth factors GM-CSF, G-CSF, IL-3, and erythropoietin have recently been shown to be negatively regulated by CD26 truncation as well [39]. Furthermore, plenty of proteins involved in hematopoiesis with a putative CD26 truncation site await investigation and might also be involved [40, 41].

CONCLUDING REMARKS

In summary, post-translational modification by CD26 is a general regulatory mechanism of chemokine activity. Modification by CD26 affects the biologic activity of many chemokines. However, rather than being characterized by 1 common effect, the consequences of NH_2-terminal chemokine truncation are highly diverse. Either no difference or drastic alterations in chemotactic activity can be observed, the latter being mediated by changes in chemokine receptor recognition, specificity, and signaling. Besides chemokine receptors, chemokines tend to bind to and be regulated by many other molecules, such as GAGs and microbial proteins. The effect of CD26 cleavage on the affinity of a chemokine for these molecules needs further exploration. Furthermore, binding to such chemokine-binding molecules might affect the chemokine's susceptibility to CD26 cleavage and

therefore, might constitute an additional dimension of regulation of chemokine activity.

The finding that truncated isoforms are present in vivo and that intravenous/intraperitoneal injection of certain chemokines in mice leads to rapid NH_2-terminal truncation underscores the importance of cleavage by CD26 in vivo. Furthermore, its potential regulatory function in many physiologic and pathologic processes has been illustrated using rodents treated with CD26 inhibitors or CD26 knockout rodents. Leukocyte, HPC, or tumor cell migration is up- or down-regulated in the absence of CD26, depending on the chemokines involved in the process. Although in vivo data are limited, the first in human evidence supporting an important role for CD26-mediated regulation of chemokine-dependent cell trafficking has been published recently. Increases in CD26 activity and in concentrations of CD26-inactivated CXCL10 have been associated with reduced responsiveness to therapy in HCV patients as a result of an impaired CXCL10 gradient and hence, reduced lymphocyte trafficking. In addition, provisional data showed that treatment with sitagliptin, a CD26 inhibitor approved for treatment of patients with Type II diabetes, resulted in increased HPC homing to the bone marrow upon transplantation in patients with hematologic malignancies. Therefore, the control of CD26 activity might offer a potential add-on therapeutic strategy to modulate chemokine activity, thereby enhancing or decreasing immune responses in inflammation and tumorigenesis.

Particular care should be taken when chemokines are quantitatively (e.g., by ELISA) detected in biologic samples, as no information on their in vivo processing and functionality is provided. Differentiation among different post-translationally modified chemokine isoforms should be considered when measuring chemokines to determine their bioavailability (when used as therapeutics) or their use as diagnostic molecules or to elucidate disease mechanisms. However, assays that allow discrimination among the different post-translationally modified chemokine forms are almost nonexisting. Therefore, the development of such specific assays is a crucial step in our understanding of the role of the individual enzymes and members of the chemokine network in pathology.

AUTHORSHIP

A.M., M.G., P.P., and S.S. wrote and corrected the manuscript. J.V.D. supervised research and corrected the manuscript.

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DISCLOSURES

The authors declare no conflicts of interest.

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