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# Non-substrate peptides influencing dipeptidyl peptidase IV/CD26 activity and immune cell function

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## 1. ABSTRACT

Investigations using inhibitors of dipeptidyl peptidase IV (DP IV) activities and DP IV-/- mice indicated an immunoregulatory role of DP IV that could not be compensated by DP IV-like enzymes. The HIV-1 Tat protein was identified as the first natural inhibitor of DP IV and as immunosuppressor. This review summarizes our investigations on the identification of the amino acid motif of Tat responsible for DP IV inhibition and of endogenous DP IV-inhibitory ligands that suppress immune cell activation. Examinations on numerous peptides carrying the N-terminal Xaa-Xaa-Pro motif of Tat revealed that tryptophan at position two strongly enhanced DP IV inhibition and immunosuppression. Here, we present evidence that the thromboxane A2 receptor exposing N-terminal Met-Trp-Pro at the cell surface could be a potential endogenous, inhibitory DP IV ligand. Moreover, our data suggest that the major envelope proteins p37k of the orhtopoxviruses *variola* virus and *vaccinia* virus, as well as the B2L antigen of the parapoxvirus *orf,* that also carry N-terminal Met-Trp-Pro, could mediate immunosuppressive effects. Further examinations are in progress to identify new physiologic, inhibitory DP IV ligands and to enlighten the mechanism underlying the DP IV-mediated effects.

## 2. INTRODUCTION

Dipeptidyl peptidase IV (DP IV, EC 3.4.14.5) is a transmembrane type II glycoprotein exposing the catalytic site in a large extracellular domain. It releases N-terminal dipeptides from peptides with proline in the penultimate position (1). With less efficiency it accepts substrates carrying alanine, hydroxyproline, serine, glycine, valine and leucine instead of proline in the penultimate position. The occurrence of proline in the third position of the peptide, however, prevents cleavage. DP IV exists in a membrane-bound and a soluble, secreted form and has access to extracellularly localized substrates.

DP IV is identical with the surface marker CD26, a T cell activation marker. The DP IV expression levels and the enzymatic activity of DP IV on T cells increase upon T cell activation indicating an important role of DP IV in the immune response. In the immune system, DP IV is also expressed on the surface of B cells and NK cells. DP IV/CD26 on leukocyte surfaces functions as a receptor or ligand for various immunological effectors. DP IV/CD26 has been characterized as a co-stimulatory molecule, a collagen receptor, an adenosine deaminase (ADA) binding protein and a ligand for CD45RO (2-4). Recently, the interaction of CD26 with caveolin-1 on antigen-presenting

cells was reported to upregulate CD86 on antigen-presenting cells, a ligand for the T cell-expressed costimulatory molecule CD28 (5, 6).

DP IV exopeptidase activity has been shown to regulate the biological function of several peptides and oligopeptides, including hormones, growth factors, neuropeptides and chemokines. Some chemokines, e.g. CCL5 (RANTES) and CXCL12 (SDF-1alpha), alter the receptor affinities and the chemoattracting properties after N-terminal truncation by dipeptidyl peptidase (7-9). Inhibition of DP IV activity with synthetic inhibitors like Lys[Z($NO_2$)]-thiazolidide, Lys[Z($NO_2$)]-pyrrolidide was shown to suppress proliferation and cytokine release of activated T cells in a way involving the immunosuppressive cytokine TGF-beta1 (for review see Reinhold 2002 (10)). However, the physiologic role of the tightly regulated surface expression of DP IV on T cells and its function in the proliferation and differentiation processes has not been elucidated in detail as yet.

In recent years, more proteins with DP IV-like activity or DP IV-like structure were discovered and summarized to a protein family designated as "dipeptidyl peptidase IV activity and/or structure homologues" (DASH) (11). DP IV-like enzymes are differentially distributed in the cell compartments. Among them are the ectopeptidases fibroblast-activating protein-alpha (FAP-alpha) and attractin (DPPT-L, mouse *Mahogany*, rat *Zitter*) and the intracellularly localized peptidases DPPII, DPP8 and DPP9 disclosing the fact that DP IV is not a unique enzyme with DP IV-like activity. FAP-alpha is not expressed on cells of the immune system. Attractin was described to be expressed by T cells and monocytes (1, 12). However, the protease activity of attractin is still subject of controversial discussions (13).

The existence of proteins with DP IV-like structure and activity indicates that the functions all attributed in the past to DP IV could be at least in part be mediated by DP IV-related proteins. However, the immunoregulatory role of DP IV was confirmed in studies on experimental autoimmune encephalomyelitis (EAE) in DP IV-/- mice compared to wild type mice. DP IV-/- mice showed a markedly increased severity coupled with an earlier onset of disease compared to wild type mice (14). Altogether, these data clearly demonstrated that DP IV plays an important role in the regulation of the immune response.

Due to their blocking effects on T cell activation and proliferation, inhibitors of DP IV-like activities were assumed to be useful for immunosuppressive therapy. It was demonstrated that inhibitors of DP IV activities abrogate acute allograft rejection in a rat cardiac transplantation model (15, 16) and suppress collagen- and alkyldiamine-induced arthritis, which represent accepted animal models for rheumatoid arthritis (17, 18). In multiple sclerosis (MS), a devastating autoimmune disease of the central nervous system, triggered mainly by auto-reactive myelin-specific T cells (19, 20), we observed that myelin-specific T cell clones of MS patients express increased DP IV activity as compared to T cells of healthy controls (21). Furthermore, we could show that targeting DP IV activities with synthetic inhibitors suppresses EAE in mice. In this MS animal model, injections of Lys[Z($NO_2$)]-pyrrolidide reduced disease severity in a preventive as well as in a therapeutic manner indicating that inhibition of DP IV activities represents a novel therapeutic approach for the treatment of this autoimmune disorder (21, 22). The beneficial effects of inhibitors to DP IV activities in combination with the more severe progression of the disease in mice lacking DP IV could be explained by an active immunosuppressive function of DP IV that is not compensated by DP IV-related proteins: Interaction of DP IV with endogenous inhibitory ligands could mediate persistent reduction of immune system responsiveness. This review summarizes our examinations on effects of non-substrate peptides on DP IV activity and immune cell function and the identification of potential endogenous inhibitory DP IV ligands with putative immunoregulatory role.

## 3. HIV-1 TAT – FIRST NATURAL DP IV INHIBITOR WITH IMMUNOSUPPRESSIVE EFFECT

If DP IV inhibitor-mediated modulation of T cell activity is a physiological relevant pathway for the control of the immune response *in vivo*, naturally occurring molecules with an inhibitory function on DP IV and immunomodulatory effects should exist.

Of special interest was the finding that the Tat protein of the human immunodeficiency virus-1 (HIV-1) binds with high affinity to CD26 and inhibits the cleavage of the synthetic DP IV substrate Gly-Pro-*p*NA (23, 24). This suggests that the immunosuppressive effects of Tat could be mediated at least in part by DP IV. With an alternative DP IV assay, which exploits the sensitivity of capillary electrophoresis and allows the use of N-terminal oligopeptides of putative physiological DP IV substrates, we observed that Tat was as potent as the synthetic DP IV inhibitor Lys[Z($NO_2$)]-thiazolidide (IC50 = 2.7 + 0.3 μM) in the inhibition of DP IV-catalyzed IL-2(1-12) degradation (25). Moreover, Tat(1-86) dose-dependently suppressed proliferation of PWM-stimulated T cells down to 58±5 % $^3$H-thymidine incorporation with 1 μM Tat(1-86) (26). N-terminal modification of Tat with rhodamine prevented inhibition of enzymatic activity of DP IV as well as suppression of DNA synthesis of mitogen-stimulated human T cells suggesting that the immune repression observed in AIDS patients could at least in part be mediated by DP IV/Tat interaction (26). Tat was the first natural inhibitor of DP IV identified indicating the existence of endogenous molecules modulating the immune response *via* inhibition of DP IV activity.

## 4. NON-SUBSTRATE PEPTIDES WITH N-TERMINAL Xaa-Xaa-Pro INHIBIT DP IV

The short N-terminal nonapeptide of HIV-1 Tat, Tat(1-9), also inhibited purified human DP IV and interfered with the proliferation of tetanus-toxoid-stimulated peripheral blood mononuclear cells (PBMC) indicating the importance of

**Table 1.** Influence of Xaa-Xaa-Pro peptides on IL-6(1-12) degradation catalyzed by human kidney DP IV

| Peptide | Amino acid sequence | Inhibition [%] |
|---|---|---|
| Tat(1-9) | MDPVDPNIE | 29±11 |
| Tat(1-21) | MDPVDPNIEPWNHPGSQPKTA | 57±6 |
| GRP(14-27) | MYPRGNHWAVGHLM-$NH_2$ | 66±9 |
| Peptide YY(3-36) | IKPEAPGEGA… | 36±16 |
| ACTH(34-39) | AFPLEF | 34±9 |
| Neuromedin N | KIPYIL | 34±21 |
| Bradykinin | RPPGFSPFR | 34±11 |
| $Lys^0$-Bradykinin | KRPPGFSPFR | 34±7 |
| $Met^0$-IL-2(1-24) | MAPTSSSTKKTQLQL… | 34±11 |
| $Met^0$-IL-2(1-6) | MAPTSSS | 32±7 |
| [$Lys^0Ala^2$]- Bradykinin | KRAPGFSPFR | 22±11 |
| $Met^{-1}Lys^0$- Bradykinin | MKRPPGFSPFR | 21±9 |
| Xenopsin-related peptide II | FHPKRPWIL | 15±12 |

Peptides were added in equimolar concentration to the substrate. Samples were analyzed by capillary electrophoresis as described elsewhere (26). Values are given in mean±SD of two separate experiments each carried out in triplicate.

the N-terminal domain of Tat for the interaction with DP IV and the suppression of immune cell proliferation (26, 27). With a DP IV assay using the N-terminal dodecapeptide of IL-2 as substrate, we observed a competitive inhibition mechanism for Tat(1–9) ($k_i$ = 111±12 μM), suggesting specific binding to or at least near by the active site, although Tat(1–9) is not a putative DP IV substrate because it contains Asp in the penultimate position and Pro in the third position (27). We observed that a number of non-substrate oligopeptides containing the N-terminal Xaa-Xaa-Pro- motif of Tat, including peptide YY(3-36), GRP(14-27) and Met-IL-2(1-6), also inhibited the cleavage of IL-2(1-12) by porcine kidney DP IV (26, 28). Similar results were obtained for the inhibition of IL-6(1-12) degradation catalyzed by human kidney DP IV (Table 1). Since Tat and other peptides containing the N-terminal Xaa-Xaa-Pro sequence of Tat inhibit DP IV we assume the existence of endogenous DP IV inhibitors regulating DP IV activity and cell proliferation.

Interestingly, some cytokines and cytokine precursors contain many prolines near the N-terminus (29). Erythropoietin, IL-6 and TGF-beta1-prepropeptide are Xaa-Xaa-Pro-peptides and G-CSF, IL-lalpha-precursor, IL-1beta-precursor, leukemia inhibitory factor, lymphotoxin-precursor and IL-13 are putative substrates for DP IV that release Xaa-Xaa-Pro-peptides after cleavage of the N-terminal Xaa- Pro- or Xaa-Ala-dipeptide.

## 5. PEPTIDES WITH STRONGLY IMPROVED DP IV INHIBITION

To identify the amino acids important for the inhibitory interaction with DP IV and the immunosuppressive effects we synthesized Tat(1-9)-related peptides carrying amino acid exchanges. A summary of all Tat(1-9)-related peptides examined is given in Figure 1. At position 1 of Tat(1-9), we observed that exchanging methionine by small amino acids like alanine, glycine or proline completely abolishes inhibition of DP IV. Loss of DP IV inhibition was also observed in the case of N-terminal methionine sulfoxide instead of methionine. Moreover, Tat(1-9)-related peptides carrying phenylalanine, leucine or norleucine at the N terminus inhibit DP IV indicating that the N-terminal hydrophobic residue is a prerequisite for a peptide to inhibit DP IV. Amino acid exchanges in the 2nd position of Tat(1-9) revealed that tryptophan at this position (leading to N-terminal $H_2N$-Met-Trp-Pro) strongly enhanced DP IV inhibition as well as the suppressive effects on DNA synthesis of tetanus toxoid-stimulated PBMC (30, 31). In equimolar concentration to the substrate, $Ala^2$-, $Phe^2$-, $Lys^2$–, $Gly^2$-, and $Ser^2$-Tat(1–9) inhibited DP IV-catalyzed IL-2(1-12) cleavage in the region of 70 % compared with 23 % for Tat(1–9). The Tat(1-9) peptide with tryptophan at position 2 inhibited DP IV-catalyzed IL-2(1–12) degradation nearly completely (96%) under the conditions used and represents the best DP IV inhibitor of all Tat(1-9)-related peptides examined. Thus, the N-terminal sequence Xaa-Trp-Pro turned out to be important for DP IV inhibition. Analysis of the solution conformations of Tat(1-9) and $Trp^2$-Tat(1-9) by NMR spectroscopy and molecular modeling demonstrated an overall similar backbone structure of the peptides suggesting that tryptophan itself enters favorable interactions with DP IV that are responsible for enhanced DP IV inhibition by $Trp^2$-Tat(1-9) (30).

The role of proline in position 3 was not examined in detail as yet. $Ile^3$-Tat(1-9) and $Gly^3$-Tat(1-9) were at least as potent as Tat(1-9) (Figure 1). However, the peptide $Trp^2/Ile^3$-Tat(1-9) carrying isoleucine instead of proline at position 3 has strongly reduced DP IV inhibiting capacity, 54±8 %, compared to 96±9 % for $Trp^2$-Tat(1-9). Moreover, we found a series of peptides with proline in position 3 that did not act as DP IV inhibitors (26) indicating that proline itself seems not to be crucial for the inhibitory interaction with DP IV but could somehow be involved in the formation or stabilization of the peptide conformation required for inhibition of DP IV. Exchanges in positions 4 to 6 of the Tat(1-9) peptide also influenced the inhibitory potential and the immunosuppressive effects of the peptide. The peptides $Ile^5$-Tat(1-9) and $Leu^6$-Tat(1-9) nearly completely lost both, inhibition of DP IV-catalyzed IL-2(1-12) hydrolysis and suppression of DNA synthesis of tetanus toxoid-stimulated PBMC (27). Exchange of proline at position 6 by phenylalanine, aspartic acid, alanine and serine increased the inhibitory potential of the emerging peptides up to 50±2 % for $Ala^6$-Tat(1-9) compared to 23±4 % for Tat(1-9) indicating that not only the N-terminal residues next to the cleavage site but also amino acids at position 4 to 6 could build attractive interactions with the DP IV active site (Figure 1).

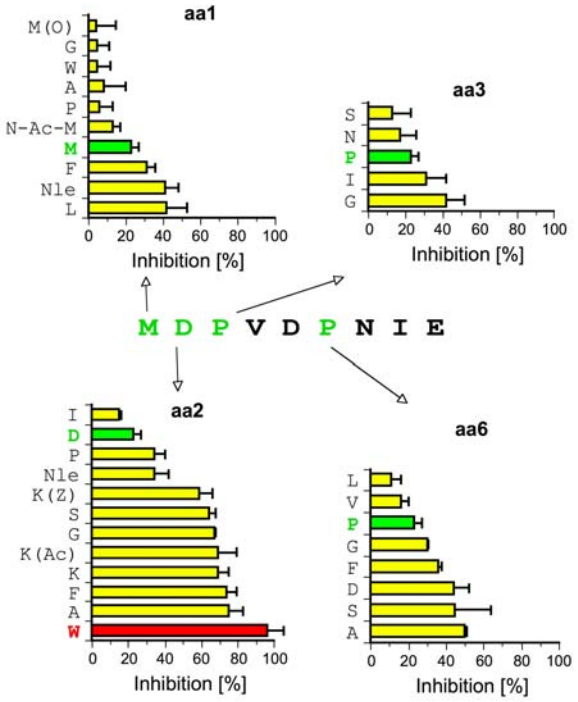


**Figure 1**. Influence of single amino acid exchanges at positions one, two, three and six of Tat(1-9) on the inhibition of DP IV-catalyzed IL-2(1-12) degradation. The original Tat sequence is shown in the center of the figure. Amino acids are given in single letter code. K(Z): benzyloxycarbonyl group at the epsilon-amino group of lysine; K(Ac): benzoyloxycarbonyl group at the epsilon-amino group of lysine. Effector peptides and substrate were used in equimolar concentrations (400 µM). Error bars indicate standard deviation of two different experiments each being carried out in triplicate (refs (34, 42) and unpublished results).

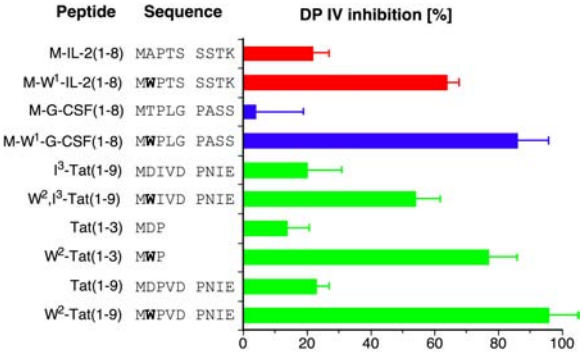


**Figure 2**. Strongly enhanced inhibition of DP IV-catalyzed hydrolysis of IL-2(1-12) by different peptides carrying Trp at the 2nd position. Amino acids are given in single letter code. Error bars indicate standard deviation of three to four different experiments each being carried out in triplicate.

## 6. IMPORTANCE OF TRP AT POSITION 2 FOR THE INHIBITION OF DP IV

The insertion of Trp at position 2 into peptides with weak inhibitory potential generally generated peptides with strongly enhanced inhibition of DP IV-catalyzed IL-2(1-12) cleavage clearly demonstrating the central role of $Trp^2$ for the inhibition of DP IV (Figure 2). To analyze the highly attractive interaction of $Trp^2$ with the DP IV active site in more detail we synthesized a series of Tat-peptides carrying other aromatic amino acids at position 2. Most of the Tat(1-9) derivatives carrying phenylalanine or tyrosine or unusual aromatic amino acids like 4-nitrophenylalanine, beta-(4-pyridyl)-alanine, beta-(3-biphenyl)-alanine, beta-(1-naphthyl)-alanine, beta-(2-naphthyl)-alanine at the 2nd position exhibit strong inhibition of DP IV activity comparable with $Trp^2$-Tat(1-9) suggesting that the aromatic ring is favored to exhibit attractive interactions with DP IV (32).

## 7. STRUCTURE FUNCTION RELATIONSHIPS OF TAT(1-9)-DERIVED PEPTIDES

Recently, co-crystals from the $[DP\ IV{\bullet}ADA]_2$ tetramer with $Trp^2$-Tat(1-9) and the DP IV homodimer with Tat(1-9) were analyzed (33). The N-terminal 4 residues of $Trp^2$-Tat(1-9) are clearly defined in the electron density and form intermolecular interactions with the DP IV active site. The main chains of $Asp^5$-$Pro^6$ are also well defined in the electron density, but only poor density was observed for the side chains because they do not form contacts to DP IV. Remarkably, the crystal structure of the N-terminal 6 residues of $Trp^2$-Tat(1-9) is similar to that determined by us by NMR studies (27, 30). From Tat(1-9) in $[DP\ IV{\bullet}Tat(1-9)]_2$ co-crystals only the N-terminal 3 amino acids form intermolecular interactions explaining the lower inhibition of DP IV by this peptide in comparison to $Trp^2$-Tat(1-9). Neither $Trp^2$-Tat(1-9) nor Tat(1-9) could be attacked by the active site $Ser^{630}$ since both peptides adopt other conformations at the active site than substrate peptides (33).

Altogether, the crystal structures can in the most cases explain our data from enzyme kinetics, NMR and docking studies of Tat(1-9) peptides with single amino acid exchanges. Strong inhibition exerted peptides carrying hydrophobic N-terminal amino acids. Accordingly, the crystal structure of co-crystals of DP IV with Tat(1-9) or $Trp^2$-Tat(1-9) indicated that hydrophobic N-terminal amino acids (P2) favor binding of their side chains to the S1 binding pocket of the enzyme as it was found for the binding of both inhibitory peptides to the active site (33). The enhancement of the DP IV inhibition by insertion of Trp in position 2 of Tat(1-9) instead of Asp is mirrored in the crystal structure by the fact that Trp nicely fits into the S2 pocket of DP IV. Moreover, the crystal structure of the DP IV/$Trp^2$-Tat(1-9) complex showed intermolecular interactions of residues 3 to 6 with the DP IV active site explaining the fact that the inhibitory potential of $Trp^2$-Tat(1-9) was clearly higher than that of the tripeptide Met-Trp-Pro (Figure 2). These findings indicate that optimizations at positions 3 to 6 of the peptide should lead to peptides exerting further enhanced inhibition of DP IV.

## 8. N-TERMINAL PEPTIDES OF VIRAL ENVELOPE PROTEINS INHIBIT DP IV

The N-terminal structure $H_2N$-Met-Trp-Pro is

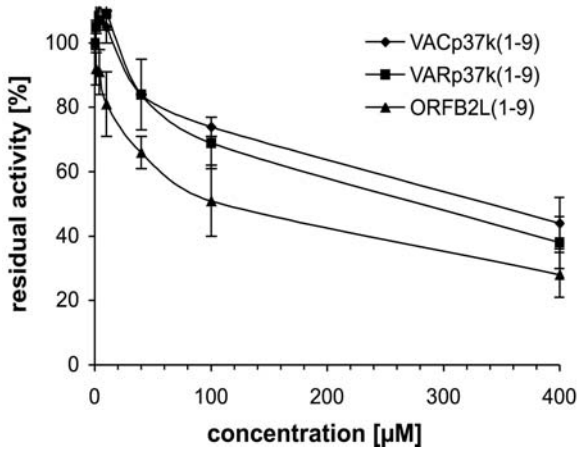


**Figure 3.** Inhibition of DP IV-catalyzed IL-2(1-12) degradation by the N-terminal nonapeptides of the major envelope proteins p37k of the *vaccinia* virus strain IHD-J, VACp37k(1-9), and the *variola* virus strain India-1967, VARp37k(1-9) and the putative virion envelope antigen B2L of the *orf* virus strain NZ-2, ORFB2L(1-9) in the concentrations indicated in the figure. Error bars indicate standard deviation of three to four different experiments each being carried out in triplicate.

present in the 37 kDa major envelope proteins (p37k) of many pathogenic *vaccinia* and *variola* virus strains and in the putative virion envelope antigen B2L of the *orf* virus. *Orf* is a parapoxvirus causing *orf* disease primarily in sheep and goats. Humans can get this disorder through direct contact with infected animals. It causes a purulent locally-appearing papule and generally no systemic symptoms. In human *orf* is usually a benign self-limiting illness. However, *vaccinia* and *variola* viruses belong to the orthopoxviruses causing smallpox, a severe infectious disease. The infection with *variola* major leads to death in 10 to 90 % depending on the virus strain. *Vaccinia* virus is the exciter of cowpox. It causes only mild disease in human and was used in the past to immunize against smallpox. To examine if these viruses could suppress the immune reaction by inhibition of T cell-expressed DP IV activity and subsequent suppression of T cell proliferation we prepared the N-terminal nonapeptides of the major envelope proteins p37k of the *vaccinia* virus strain IHD-J, VACp37k(1-9), and the *variola* virus strain India-1967, VARp37k(1-9) and the putative virion envelope antigen B2L of the *orf* virus strain NZ-2, ORFB2L(1-9), that is homologous to p37k. DP IV-catalyzed IL-2(1-12) degradation was dose-dependently inhibited by each of the viral peptides (Figure 3). In equimolar concentration to the substrate IL-2(1-12) ORFB2L(1-9) inhibited 72±7 %, VARp37k(1-9) 62±8 % and VACp37k(1-9) 56±8 % of the DP IV activity. Using Ala-Pro-*p*NA in a continuous photometric DP IV assay, we estimated for ORFB2L(1-9) a $K_i$ of 13.2±4.4 µM and for VARp37k(1-9) a $K_i$ of 29.3±2.1 µM. For both peptides Dixon plots and slope from Dixon versus reciprocal substrate concentration secondary plots demonstrate a competitive inhibition mechanism indicating binding of the inhibitory peptides directly in the active site or at least near by the active site. If the viral envelope proteins also influence the proliferation of activated T cells remains to be examined.

## 9. DOES THE THROMBOXANE A2 RECEPTOR FUNCTION AS AN ENDOGENOUS DP IV INHIBITOR?

Based on the N-terminal $H_2N$-Met-Trp-Pro sequence, we identified the thromboxane A2 receptor (TXA2-R) as a first potential endogenous inhibitor of DP IV (34). TXA2-R is a G-protein-coupled receptor exposing the N-terminal $H_2N$-Met-Trp-Pro sequence at the cell surface. In the immune system TXA2-R is expressed on monocytic cells. Interaction of T cell-expressed DP IV with TXA2-R during antigen presentation could be a mechanism for the limitation of T cell activation.

The N-terminal peptide TXA2-R(1-9) strongly inhibited DP IV. The $K_i$ value of 5.06 µM for TXA2-R(1–9), determined using Ala-Pro-*p*NA and soluble human DP IV, is in the same range as the $K_i$ value of 2.12. µM for $Trp^2$-Tat(1–9). Moreover, the reduction of DNA synthesis in tetanus toxoid-stimulated PBMC by TXA2-R(1–9) is as strong as by $Trp^2$-Tat(1–9), which stands in striking correlation with the effects of both peptides on DP IV activity. This suggests the contribution of DP IV in the immunosuppressive effect of both peptides.

Accordingly, immune responses to foreign antigens are enhanced in TXA2-R deficient mice indicating, that the TXA2 receptor is involved in negative regulation of the immune cell activation (35). If the entire, cell surface-expressed TXA2-R really inhibits DP IV and represents a prototype of an endogenous DP IV inhibitor that influences T cell activation or if such a large protein has structural constraints interfering with the binding to the DP IV active site remains to be examined. A recent report on docking studies of the trypsin inhibitor aprotinin, which is hydrolyzed by DP IV, to the DP IV active site demonstrated that the side cleft could be widened slightly for substrate entering (36). Moreover, we demonstrated N-terminal truncation of the 116 aa long procalcitonin, a marker for systemic bacterial infections, by DP IV (37). However, the N termini of many larger oligopeptides, like the cytokine IL-2, seem to be protected against proteolytic cleavage probably by intramolecular constraints (38). Thus, the access of inhibitory ligands to the active site should not only depend on the size of the ligand but also on the conformation of its N-terminal region and on the kind of interaction it undergoes during entering the active site. Altogether, these findings indicate that in principle not only peptides but also oligopeptides and even proteins have to be taken into account as potential inhibitory ligands for DP IV.

## 10. SUMMARY AND PERSPECTIVE

Increased DP IV activity and CD26 expression upon T cell stimulation suggest an important role of this enzyme in T cell activation. Some years ago, HIV-1 Tat was identified as the first natural DP IV inhibitor. By modifying the N-terminal amino group of Tat we could

demonstrate the importance of the N-terminal region for the inhibition of DP IV and for the suppression of DNA synthesis in stimulated T cells by Tat. The inhibition of DP IV by the N-terminal nonapeptide of Tat and other peptides carrying the N-terminal Xaa-Xaa-Pro motif of Tat confirmed the importance of this N-terminal region for the observed effects. Amino acid exchanges in the N-terminal Tat region revealed that peptides with Trp at position 2 strongly increased DP IV inhibition and suppression of T cell proliferation. With TXA2-R we identified the first endogenous potential DP IV inhibitor carrying N-terminal Met-Trp-Pro. TXA2-R(1-9) strongly inhibited DP IV and suppressed the proliferation of tetanus toxoid-stimulated PBMC.

In contrast to previous studies using dipeptide analog DP IV inhibitors, we induced suppression of immune cell proliferation with DP IV-inhibiting nonapeptides. Dipeptide analogs could enter cells via the small peptide carriers, PEPT1 and PEPT2, that both mediate active transport of di- and tripeptides and a broad range of small peptide-like peptidomimetics (39). Longer peptides such as tetrapeptides or pentapeptides are not recognized by these transporters. PEPT1 is expressed primarily in the intestine, whereas PEPT2 is expressed in a variety of nonintestinal tissues including the brain. The PEPT2 transcript was identified in human PBMC (S. Wrenger, unpublished results). Thus, many of the inhibitors of DP IV activities should enter the cells and apart from inhibition of cell surface DP IV also inhibit intracellular DPPs. Recently, DPP8/9 specific inhibitors were found to suppress T cell activation suggesting that the effects of the less specific inhibitors of DP IV activities are at least in part mediated by intracellular DPPs (40). DPP II, DPP8 and DPP9 were identified in human leukocytes using several selective DPP inhibitors (41). However, if DP IV-inhibiting nonapeptides enter the cells and if nonapeptides target apart from cell surface DP IV DP IV-like intracellular enzymes remains to be elucidated.

In concern with the tight control of DP IV activity during T cell activation and the increased severity of EAE in DP IV-/- mice our results suggest a function of DP IV in the regulation of the immune response. For the identification of further potential endogenous DP IV inhibitors more information about the sequence and the inhibiting conformation is needed.

## 11. ACKNOWLEDGEMENTS

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**Abbreviations:** ADA: adenosine deaminase, CCL: CC chemokin ligand, CD: cluster of differentiation, CSF: colony stimulating factor, CXCL: CXC chemokin ligand, DP IV: dipeptidyl peptidase IV, DPP: dipeptidyl peptidase, EAE: experimental autoimmune encephalomyelitis, GRP: gastrin-releasing peptide, HIV: human immunodeficiency virus, IL: interleukin, MS: multiple sclerosis, NMR: nuclear magnetic resonance, p37k: 37 kDa major envelope proteins (p37k), PBMC: peripheral blood mononuclear cells, *p*NA: *para*-nitroanilide, PWM: pokeweed mitogen, TGF: transforming growth factor, VAC: *vaccinia* virus, VAR: *variola* virus, Z: benzyloxycarbonyl

**Key Words:** Dipeptidyl Peptidase IV, T Cell Activation, Human Immunodeficiency virus-1 Tat Protein, Thromboxane A2 Receptor, Orthopox Virus, *Variola* Virus, Major Envelope Protein, Putative Virion Envelope Antigen B2L, Review

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