

# The dipeptidylpeptidase-IV inhibitors sitagliptin, vildagliptin and saxagliptin do not impair innate and adaptive immune responses

Inhibitors of dipeptidylpeptidase IV (DPP-IV) represent a novel class of frequently used anti-diabetic drugs. In addition to its function in metabolic regulation, DPP-IV also plays a role in the immune system. Whether the DPP-IV inhibitors sitagliptin, vildagliptin or saxagliptin impair immune responses is, however, currently unknown. Here, we investigated the effect of these agents on both innate and adaptive immunity. We found that the DPP-IV inhibitors did not affect the innate immune response induced by Toll-like receptor (TLR) ligands, as cytokine secretion and induction of co-stimulatory molecules by human blood mononuclear cells was not impaired. Furthermore, proliferation of T cells and suppressive function of regulatory T cells was preserved. Mice treated with vildagliptin showed normal cytokine production, immune cell activation and lymphocyte trafficking upon TLR activation. Thus, crucial immunological parameters remain unaffected upon treatment with DPP-IV inhibitors, a fact that is reassuring with respect to safety of these drugs.

**Keywords:** DPP-IV inhibitor, drug mechanism, pharmacology, type 2 diabetes

## Introduction

The dipeptidylpeptidase-IV (DPP-IV) inhibitors sitagliptin, vildagliptin and saxagliptin are novel drugs for the treatment of type 2 diabetes with several advantages, as oral administration, a neutral effect on body weight and a low risk of hypoglycaemia [1]. DPP-IV inhibitors prevent the inactivation of glucagon-like peptide 1 and thus act against hyperglycaemia [2]. Several reports suggest that DPP-IV also plays a role in the immune system. DPP-IV is expressed by immune cells, in particular by T and B lymphocytes [3,4]. DPP-IV-expressing T cells proliferate more strongly in response to antigen-dependent stimulation than DPP-IV-negative T cells [5] and interaction of DPP-IV with adenosine receptors on dendritic cells can trigger a stronger release of IFN- $\gamma$  [6]. Soluble DPP-IV enhances T cell proliferation via up-regulation of CD86 and interaction with caveolin-1 [7]. Furthermore, the enzymatic function of DPP-IV is immunologically relevant as it inactivates chemokines by cleavage [8]. Thus, as DPP-IV is involved in the regulation of the immune system it is essential to investigate potential immunological effects of the novel DPP-IV-inhibiting anti-diabetes drugs.

## Methods

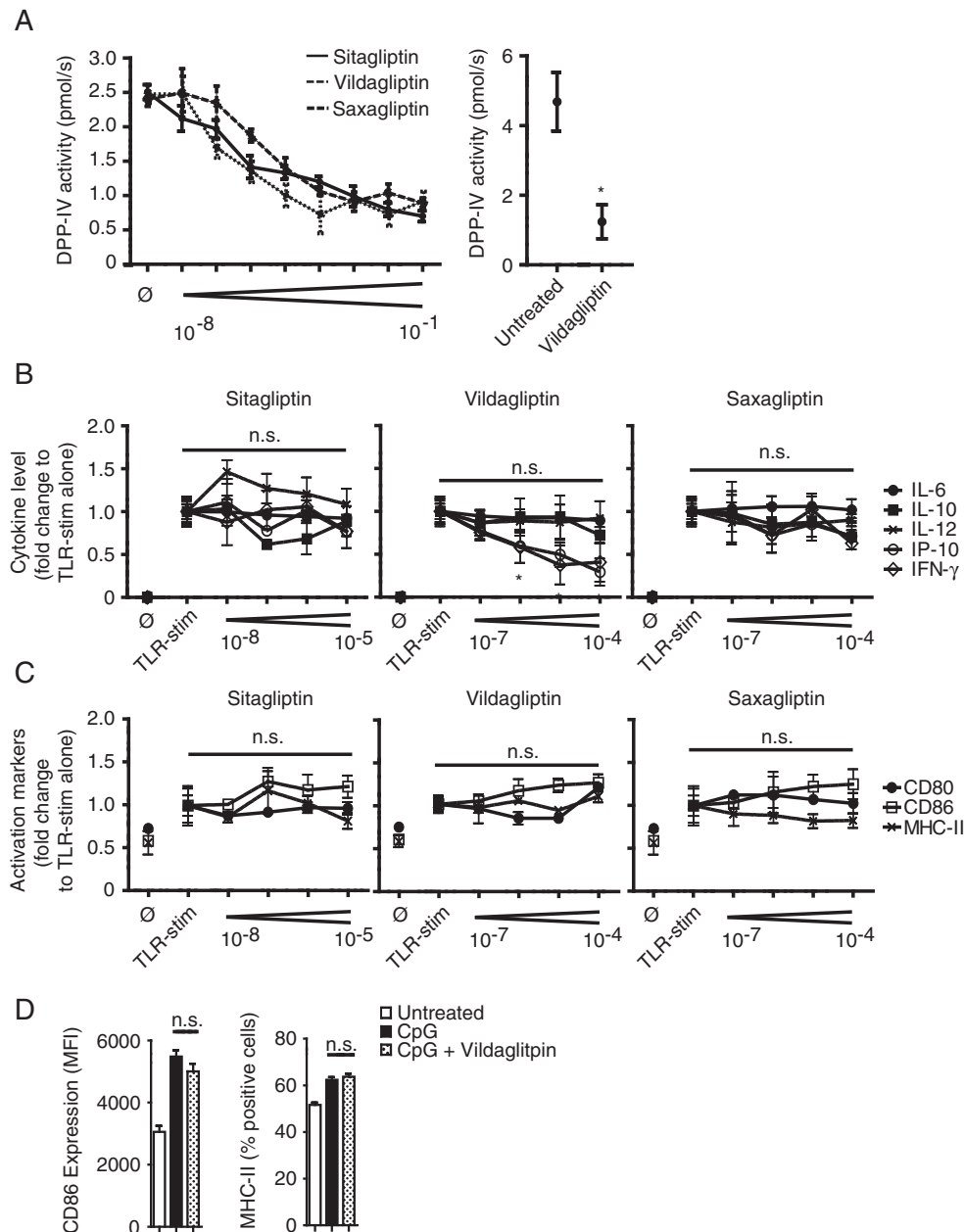
Peripheral blood mononuclear cells (PBMC) were obtained from healthy volunteers who had given informed consent.

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Animal studies were approved by the local regulatory agency. For *in vitro* use tablets were pestled and a 150 mM solution of the active component was prepared. Enzymatic activity of DPP-IV was determined by measuring the light absorbance of the synthetic DPP-IV substrate Z-Gly-Pro-4-nitroanilide (Sigma-Aldrich, Munich, Germany). For flow cytometry cells were stained with the respective antibodies and analysed by an FACSCanto II (BD Biosciences, Heidelberg, Germany). Cytokine concentrations were determined using enzyme-linked immunosorbent assay (ELISA) Kits (BD Biosciences). T cell proliferation was measured by BrdU incorporation with a chemoluminescence assay (Roche Diagnostics, Mannheim, Germany) or by flow cytometry. CD4+CD25<sup>neg</sup> and CD4+CD25+ cells were gained by magnetic cell sorting (Miltenyi Biotec, Bergisch Gladbach, Germany). For lymphocyte isolation organs were disrupted, digested using Collagenase and DNase (Sigma-Aldrich) and passed through a cell strainer. *In vitro* assays were done in triplicates and repeated threefold, *in vivo* experiments were done twice, all with similar results. Data followed Gaussian distribution and were analysed as appropriate by unpaired Student's *t*-test or by ANOVA test.

## Results

We first examined the production of inflammatory cytokines upon Toll-like receptor (TLR) stimulation and DPP-IV inhibition. DPP-IV inhibitors were solubilized from the tablet (see Methods) and efficient DPP-IV inhibition was confirmed both *in vitro* and *in vivo* (Figure 1A). The 10<sup>5</sup>-fold dilution of the inhibitor corresponds to the situation in patients, where peak plasma levels of 1  $\mu$ M are usual. First, human PBMC

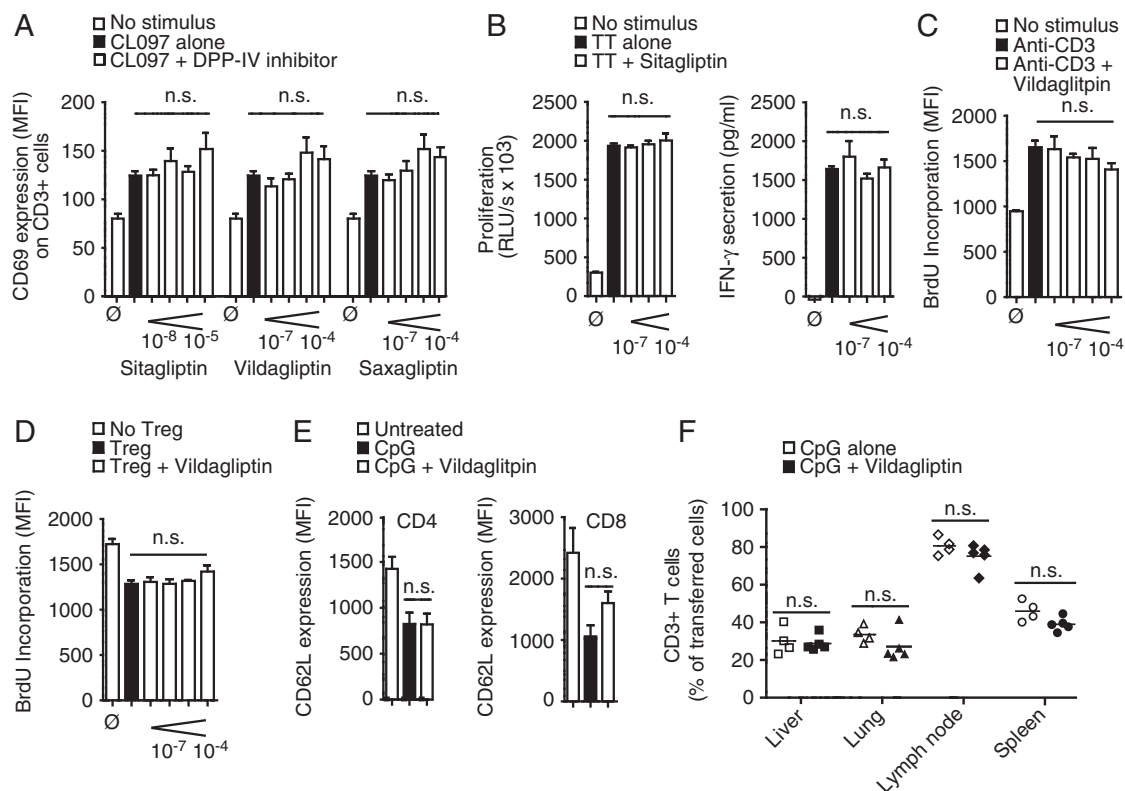


**Figure 1.** Impact of dipeptidylpeptidase-IV (DPP-IV) inhibitors on cytokines and activation markers. (A) Peripheral blood mononuclear cells (PBMC) were treated with the indicated dilutions of a 150 mM stock of the inhibitor and DPP-IV activity was measured using the Gly-Pro-pNA assay (left). DPP-IV activity on splenocytes was measured *ex vivo* after oral treatment of mice ( $n = 4$ ) with 15  $\mu$ g of vildagliptin every 12 h over 2 days (right). (B and C) Human PBMC were treated with increasing concentrations of the DPP-IV inhibitor and stimulated with 1  $\mu$ g/ml R848 (or 0.2  $\mu$ g/ml CL097 for CD86) for 24 h (TLR-stim), then cytokine levels (pg/ml) in the supernatants and expression of activation markers [mean fluorescence intensity (MFI)] were determined. Values are normalized to TLR stimulation alone. (D) Mice ( $n = 5$  per group) received vildagliptin as in (A) and 100  $\mu$ g of CpG (*s.c.*). After 24 h expression of CD86 and MHC-II on splenocytes was determined. Error bars indicate s.e.m. Significance was calculated in comparison to untreated (A) or only TLR-stimulated (B–D) samples (\* $p < 0.05$ , n.s. = not significant).

were treated with the DPP-IV inhibitors and stimulated with the TLR7 ligand R848. Levels of IL-6, IL-10, IL-12, IP-10 and IFN- $\gamma$  were not significantly altered in the presence of the DPP-IV inhibitors. As an exception, vildagliptin inhibited IP-10 and IFN- $\gamma$  secretion (Figure 1B), a finding that may be reflected by a less specific inhibition of DDP-IV by vildagliptin [9]. None of the DPP-IV inhibitors impaired the TLR-induced

up-regulation of the co-stimulatory molecules CD80, CD86 and MHC-II (Figure 1C). This was confirmed *in vivo* in mice treated with vildagliptin and the TLR9 ligand CpG (Figure 1D).

To investigate effects of DPP-IV inhibitors on T cell activation, CD69 expression and proliferation of human T cells was analysed. TLR7 stimulation increased CD69 levels on T cells irrespective of DPP-IV inhibition (Figure 2A).



**Figure 2.** Impact of dipeptidylpeptidase-IV (DPP-IV) inhibitors on T cell function. (A) Human peripheral blood mononuclear cells (PBMC) were treated with indicated dilutions of the DPP-IV inhibitor and 0.2  $\mu\text{g/ml}$  of the TLR7 agonist CL097 for 24 h and expression of CD69 on CD3+ cells was determined [mean fluorescence intensity (MFI)]. (B) Human PBMC from individuals vaccinated against Tetanus were incubated with Tetanus toxoid (0.5  $\mu\text{g/ml}$ ) for 17 h and treated with sitagliptin. Proliferation and IFN- $\gamma$  secretion was measured on day 7 by detection of BrdU and enzyme-linked immunosorbent assay (ELISA), respectively [relative light units (RLU)]. (C and D): CD4+CD25<sup>neg</sup> cells ( $7.5 \times 10^4/\text{well}$ ) were incubated with anti-CD3 or CD4+CD25+ cells ( $4 \times 10^4/\text{well}$ ) and vildagliptin. Proliferation (BrdU incorporation, MFI) into CD4+FoxP3<sup>neg</sup> cells was determined by flow cytometry. (E and F) Mice ( $n = 5$  per group) were treated with vildagliptin and CpG as in Figure 1. (E) 24 h later CD62L expression on T cells was determined. (F) 19 h later CFSE+ splenocytes ( $2 \times 10^3/\text{mouse}$ ) were adoptively transferred. 6 h later lymphocytes were prepared from the indicated organs and the proportion of CD3+ cells within the transferred CFSE+ cells was determined. Error bars indicate s.e.m. of  $n = 3$  (A–D) or  $n = 5$  (E and F), significance was calculated in comparison to the conditions without DPP-IV inhibitor (n.s. = not significant).

Furthermore, proliferation and IFN- $\gamma$  secretion of T cells remained unimpaired upon both antigen-specific (Tetanus toxoid) or unspecific (anti-CD3) stimulation (Figure 2B and C). Further, regulatory T cells suppressed the proliferation of effector T cells irrespective of DPP-IV inhibition (Figure 2D). In addition, mice treated with CpG and vildagliptin showed no difference in T cell activation, reflected by an unimpaired suppression of the marker CD62L (Figure 2E). As chemokines are substrates for cleavage and inactivation through DPP-IV [8], we investigated the migration of lymphocytes upon DPP-IV inhibition *in vivo*. Mice were treated with vildagliptin and CpG, followed by an adoptive transfer of lymphocytes. Lymphocyte migration and distribution into several organs was, however, not altered upon inhibition of DPP-IV (Figure 2F).

## Conclusion

Stimulation of TLRs is essential for the induction of an immune response and the clearance of pathogens by the host [10]. An inhibition of the involved pathways could lead to increased susceptibility to infections [11]. Although

infectious complications are not typically associated with DPP-IV inhibitors [12] it is so far still unknown whether a higher rate of overall infections occurs on long term. We show here that sitagliptin, vildagliptin and saxagliptin do not influence the development of an immune response in terms of cytokine secretion, co-stimulation, T cell proliferation and migration. While these findings are observed in human cells *in vitro* and in mice *in vivo*, they are reassuring with respect to the drug safety in patients. In conclusion, DPP-IV inhibitors do not impair key parameters of the innate and adaptive immune response.

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## Conflict of Interest

Experiments were performed by D. A., S. K., S. H. and M. R., data were analysed and discussed by all authors, the manuscript was written by D. A., S. K. and S. E., the work was designed and supervised by D. A. and S. E. The authors have no competing interest.

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