# Dipeptidyl Peptidase IV/CD26 in T Cell Activation, Cytokine Secretion and Immunoglobulin Production

HUA FAN, SHULING YAN, SABINE STEHLING, DIDIER MARGUET[#], DETLEF SCHUPPAN[+], WERNER REUTTER

*Institut für Molekularbiologie und Biochemie, UKBF, Freie Universität Berlin, Arnimallee 22, D-14195 Berlin, Germany; [#]Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Marseille, France; [+]Klinik für Innere Medizin I, Abt. Hepatologie, Universität Erlangen-Nürnberg, Ulmenweg 18, D-91054 Erlangen, Germany*

## 1. INTRODUCTION

Dipeptidyl peptidase IV (DPPIV/CD26; EC. 3.4.14.5), a widely distributed multifunctional type II plasma membrane glycoprotein, is involved in different biological processes. It is a serine protease associated with uptake and transmembrane transport of proline-containing peptides as well as with processing of physiological active peptides[1]. As an exopeptidase it cleaves *N*-terminal dipeptides after proline or alanine residues. Furthermore, an endopeptidase activity of DPPIV has also been reported[2].

Due to its interaction with proteins of the extracellular matrix (ECM), such as collagen and fibronectin, DPPIV/CD26 can also be considered as a cell adhesion molecule[3]. The interaction of DPPIV/CD26 with proteins of the ECM has been determined by several binding assays[4-6]. Although the binding properties of DPPIV/CD26 to proteins of the ECM have been well characterized, the significance of this interaction for its biological function, especially for the immune regulation is still unknown.

It has been shown that DPPIV/CD26 plays a crucial role in T cell activation and immune regulation[7-10]. The expression level of DPPIV/CD26 is tightly regulated during the development of T lymphocytes and its density on the plasma membrane is markedly enhanced after lymphocyte activation.

*Dipeptidyl Aminopeptidases un Health and Disease*, Edited by Hildebrandt et al.
Kluwer Academic/Plenum Publishers, New York, 2003

Thus this protein is regarded as an activation marker for T, B and NK cells[11]. Some immunoregulative hormones and chemokines closely related to the immune function have been shown to be substrates of DPPIV/CD26. Among these substrates are substance P, neuropeptide Y, endomorphin-2, GLP-1, RANTES (regulated on activation normal T-cell expressed and secreted), eotaxin, MDC (monocyte-derived chemokine) and SDF-1α and SDF-1ß (stromal derived factor)[10,12-14]. Also of great importance is that the T cell surface molecule DPPIV/CD26 serves as a co-stimulator in the antigen-stimulated activation of T lymphocytes[15,16] and mediates signaling by direct interaction with CD45[17,18]. On human T cells, DPPIV/CD26, as a receptor of adenosine deaminase (ADA), may play an important role for the regulation of the immune response[19].

Synthetic inhibitors of the enzymatic activity of DPPIV have been shown to suppress certain immune reactions *in vitro* and *in vivo*[20,21]. However, in the immune system DPPIV/CD26 is regarded not only as an enzyme, but also as a co-stimulator for T cell activation, a receptor for ADA and a receptor for collagen. So it can be postulated that DPPIV/CD26, depending on the physiological environment and/or pathological conditions, could function in different ways. In the present work we investigated the role of the diverse functions of DPPIV/CD26 in T cell activation. We have found that collagen inhibits the co-stimulating effect of DPPIV/CD26, and hence can modulate the activation of T cells. The enzymatic activity of this molecule is not for its co-stimulating effect on T cell activation. In consideration of the multi-functions of DPPIV/CD26, our experiments with DPPIV/CD26 knockout mice could provide useful information about the physiological role of this plasma membrane glycoprotein in the immune response, as well as additional insights into the underlying molecular mechanisms of signal transduction processes.

# 2. RESULTS AND DISCUSSION

## 2.1 Rat DPPIV/CD26 exhibits a co-stimulatory effect on human T cell activation

Rat DPPIV/CD26 exhibits a high (85%) homology to human DPPIV/CD26. Hence studies on the mechanisms of DPPIV/CD26 in T cell activation were performed with Jurkat stable transfectants containing wild type rat DPPIV/CD26 (rCD26/Jurkat). Cells were stimulated with specific antibodies adsorbed on the surface of wells of microtiter plates (solid-phase immobilization). As an examination of cell activation, the production of the

cytokine IL-2 was measured[22]. After stimulation with anti-CD3 mAb, rCD26/Jurkat transfectants showed a two-fold higher secretion of IL-2 in comparison with Jurkat cells lacking DPPIV/CD26 (Fig. 1). When the cells were co-stimulated with anti-CD3 mAb and anti-rat-DPPIV/CD26 polyclonal antibodies, a four-fold raise in IL-2 secretion was measured in rCD26/Jurkat as compared to the controls (Fig. 1). This suggests that rat-DPPIV/CD26 could also act as co-stimulator and thus mediate the antigen-stimulated activation of human T cells. Since the human ADA does not bind to rat-DPPIV/CD26[23] we can conclude from our results that the interaction of DPPIV/CD26 with ADA is not necessary for the DPPIV/CD26-mediated T cell co-stimulation, which is in accordance with the suggestion of Dong et al.[19].

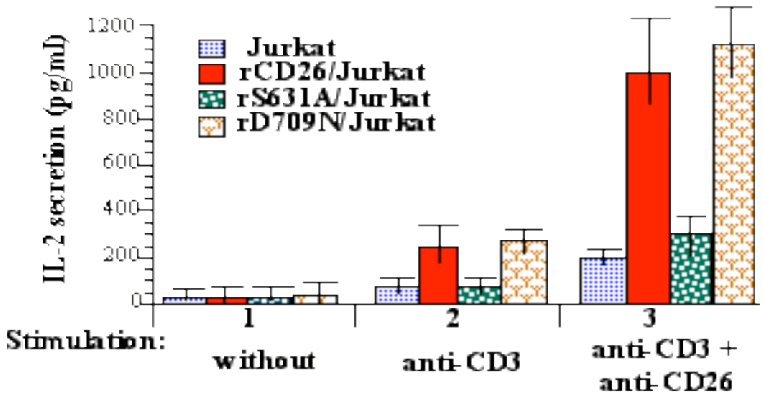


*Figure 1.* IL-2 secretion by Jurkat cells and Jurkat transfectants after stimulation. Jurkat cells and jurkat stable transfectants were stimulated with solid-phase immobilized anti-human CD3 mAb (5 $\mu$g/ml), or with co-immobilized anti-human CD3 mAb (5 $\mu$g/ml) and anti-rat DPPIV/CD26 polyclonal antibodies (6 $\mu$g/ml). After 24 hours, the concentration of IL-2 in cell culture supernatants was assayed by ELISA.

## 2.2 The enzymatic activity of DPPIV is not required for the DPPIV/CD26 co-stimulatory effect on T cell activation

The role of the enzymatic activity of DPPIV/CD26 in its co-stimulatory effect on T cell activation represents a controversial issue in the area of DPPIV/CD26[15,24]. In the present work two enzymatic inactive mutants (S631A and D709N) were tested for their co-stimulatory effect on IL-2 secretion of Jurkat cells, in order to clarify this debatable point. As expected these mutants did not exhibit any enzymatic activity since $Ser^{631}$ and $Asp^{709}$ are components of the amino acid triad of the catalytic site of DPPIV/CD26, but their expression on the Jurkat cell surface was comparable to that of wild type DPPIV/CD26. We observed, in agreement with Tanaka et al. that the

S631A mutation markedly inhibited IL-2 secretion (Fig. 1)[15]. But unexpectedly the D709N mutation did not affect the signaling pathway leading to raised secretion of IL-2, despite that this mutant did not exhibit any enzymatic activity (Fig. 1). Based on the results obtained with the mutant S631A, Tanaka et al., have postulated that the enzymatic activity of DPPIV/CD26 is required for its co-stimulatory effect on IL-2 secretion during T cell activation. Steeg et al. found no reduction in IL-2 secretion of DPPIV/CD26 Jurkat transfectants incubated with inhibitors of the enzymatic activity of this protein[24]. In the present work with the D709N mutant we demonstrate that the enzymatic activity of DPPIV/CD26 is not required for its co-stimulatory effect on IL-2 secretion of T cells. We postulate that the inability of the S631A mutant to mediate the signal transduction pathway of IL-2 secretion may be due to the requirement of the serine residue for signal transduction, or to alterations in the protein functional conformation, rather than to its lack of enzymatic activity (Fan et al., in preparation).

## 2.3 Collagen inhibits the CD26 co-stimulatory effect on T cell activation

The adhesive interactions of cells with the extracellular matrix play a central role in the functions of the immune system, which regulate the migration of lymphocytes and the interactions of activated cells during the immune response. It has been determined that native DPPIV/CD26 not only binds to collagens but also that its binding capacity depends markedly on the type of collagen[6]. We then asked whether the interaction of DPPIV with specific types of collagen differentially influences the DPPIV/CD26 co-stimulatory capacity for T cell activation. For this purpose IL-2 secretion of rCD26/Jurkat cells was measured after incubation with different types of collagen during stimulation with anti-CD3 mAb. We observed that the secretion of IL-2 was inhibited by collagen type I, type XIV, type II, type VI and type III in different levels. While collagen type I showed a stronger inhibitory capacity, collagen type IV had no significant influence on the IL-2 secretion (Fig. 2). These results are consistent with those of *in vitro* binding tests[6] in which collagen type I showed stronger affinity, while collagen type IV bound poorly to DPPIV/CD26. The $\alpha$1(I) chain showed the strongest inhibitory capacity, more than 95%, whereas the $\alpha$2(I) chain caused an inhibition of 40%, only (Fig.2). This is also in accordance with the results of the *in vitro* binding assay, in which a strong binding of the $\alpha$1(I) chain and a significantly weaker affinity of the $\alpha$2(I) chain to DPPIV/CD26 were demonstrated[6].

The monoclonal anti-DDIV/CD26 antibody mAb 13.4 also completely inhibited the IL-2 secretion (Fig. 2). It has been reported that the binding of DPPIV/CD26 to collagen was abolished by this antibody[6]. We could show here that both mAb 13.4 and collagen type I could inhibit the DPPIV/CD26

mediated T cell activation. This suggests that the binding domain(s) of DPPIV/CD26 for collagen and the mAb 13.4 is (are) essential components of the signal transduction pathway of IL-2 secretion in T cells. We show that collagen can modulate the T cell activation via its interaction with DPPIV/CD26. Further experiments are necessary to clarify whether this interaction has additional significance for adhesion, migration and signal transduction of T cells and other leukocytes.

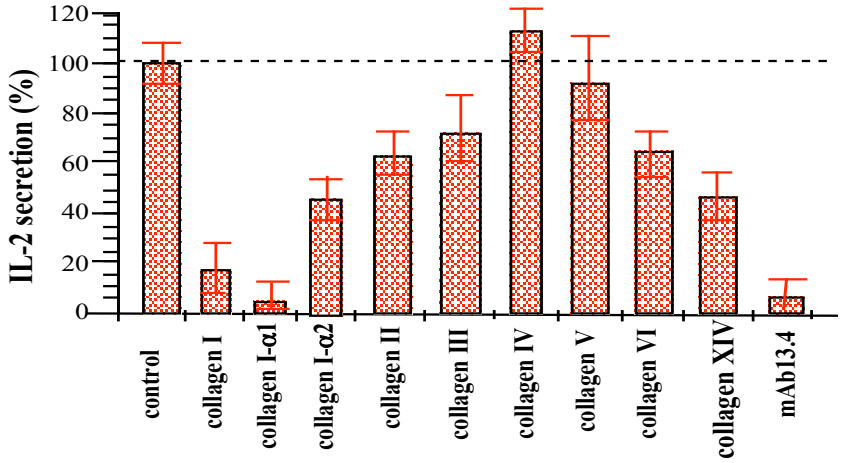


*Figure 2.* Influence of different collagen types on the IL-2 secretion of rCD26/Jurkat transfectants induced by anti-human CD3 mAb. The rCD26/Jurkat transfectants were stimulated with solid-phase immobilized anti-human CD3 mAb (5 μg/ml) alone (control) or in the presence of different types of co-immobilized collagens (1 μg/ml) for 24 hours. The IL-2 concentrations in cell culture supernatants were assayed by ELISA.

## 2.4 Influence of DPPIV/CD26 on T cell development and differentiation

Investigations of the last few years as well as the present work have provided evidence for the involvement of DPPIV/CD26 in processes like T cell activation and cell adhesion to the extracellular matrix[6,8-10,18]. However, the physiological function of this multifunctional molecule in the intact animal has not yet been clarified. Therefore, we studied the role of DPPIV/CD26 on the activation and differentiation of lymphocytes after stimulation with different mitogens in DPPIV/CD26 gene knockout mice.

DPPIV/CD26 gene knockout mice show an apparently normal phenotype. However, the percentage of $CD4^+$ cells (helper/memory cell population in mice spleen lymphocytes (MSLs) is about 30% lower in DPPIV/$CD26^{-/-}$ mice than in DPPIV/$CD26^{+/+}$ mice (data not shown), suggesting an involvement of DPPIV/CD26 in lymphocyte development and

maturation *in vivo*. MSLs were stimulated *in vitro* for 72 h with the following mitogens: phytohemagglutinin (PHA), pokeweed mitogen (PWM), concanavalin A (ConA) and lipopolysaccharide (LPS). The proliferation rates of DPPIV/CD26$^{-/-}$ MSLs were decreased to 70% and 80% after stimulation with ConA and PWM, respectively, whereas no significant changes after stimulation with PHA or LPS were observed. The secretion of several cytokines in MSLs after stimulation with PWM was also determined. A significant reduction in the secretion of the Th2-type cytokine IL-4 of DPPIV/CD26$^{-/-}$ MSLs, to 20-40% of that of MSLs from wild type mice, was measured. An increase in the production of Th1-type cytokine IFN-γ of DPPIV/CD26$^{-/-}$ MSLs was observed, while no changes of the secretion of IL-2 and IL-6 were detected (Fig. 3) (Yan et al., in preparation).

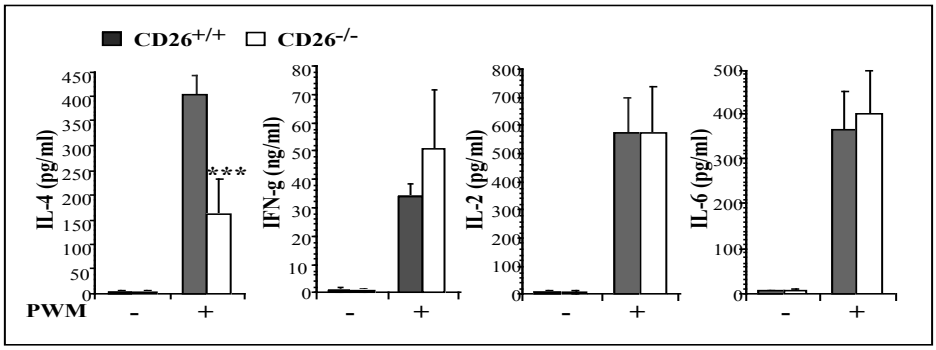


*Figure 3*. Cytokines secretion by mouse sple en lymphocytes after stimulation with PWM. Spleen lymphocytes from CD26$^{-/-}$ and CD26$^{+/+}$ mice were stimulated by PWM (0.5 μg/ml) for 72 h. Cytokine concentrations in culture supernatants were measured by ELISA. The noted values represent the mean ± SD of a minimum of seven mice. ***: P<0.001 as compared to CD26$^{+/+}$ mice.

The lower concentration of IL-4 in the supernatants of CD26$^{-/-}$ MSLs is probably due to the lower percentage of CD4$^{+}$ lymphocytes in the knockout mice (s. above), since CD4$^{+}$ cells are the major source of this cytokine. However, the lower percentage of CD4$^{+}$ cells could not explain the increased IFN-γ production, and it did not result in a decreased production of IL-2 and IL-6 in CD26$^{-/-}$ MSLs. The reduction of IL-4 corresponded with an imperfect differentiation of the Th2 subset[25,26], indicating an involvement of DPPIV/CD26 expression in the regulation of cytokine secretion, as well as in the differentiation of T-lymphocytes.

It has been found that blocking of the enzymatic activity of DPPIV/CD26 with specific inhibitors suppressed the IL-2 and IFN-γ production of human T-cells, and reduced the IL-2 and IL-12 secretion of human peripheral blood mononuclear cells (PBMC) stimulated with PWM[11,27,28]. The apparent contrast between the results of the present work and those of Arndt et al. can be explained on the basis of the different leukocyte populations used. The

activation and differentiation of T cells *in vivo* is influenced by the interaction with other subsets of lymphocytes and antigen presenting cells (APCs), so that the cytokine production from mixed lymphocyte populations, as in our study, may be different to that from purified T cells or PBMC. Moreover, as discussed earlier, DPPIV/CD26 has in the immune system a co-stimulatory effect on lymphocyte activation, which is independent of its enzymatic activity. Therefore, the use of enzymatic inhibitors of DPPIV/CD26 should only interfere with signal transduction events dependent on the proteolytic activity of this plasma membrane glycoprotein. Experiments using DPPIV specific inhibitors can help to elucidate the role of DPPIV/CD26 enzymatic activity in the immune response, whereas studies with DPPIV/CD26 knockout mice provide insights into the physiological role of this multifunctional molecule in the immune system.

## 2.5 The deficiency of DPPIV/CD26 results in reduced immunoglobulin concentrations after stimulation by PWM *in vivo*

To examine whether the differentiation and functions of B-lymphocytes were dependent upon the reduction of IL-4 secretion and ultimately on the expression of DPPIV/CD26, the concentrations of different immunoglobulins in sera were measured by ELISA after stimulation with PWM *in vivo*. As shown in Fig. 4 DPPIV/CD26$^{-/-}$ mice presented significantly reduced IgG concentrations six days after immunization with PWM. The IgE concentrations were also reduced after six days, but the most significant reduction was observed 19 days after immunization with PWM, while no significant differences in the IgM levels between both kinds of mice were detected (Fig 4) (Yan et al., in preparation). These results suggest that the deficiency of DPPIV/CD26 *in vivo* leads to a specific impairment of the production of immunoglobulins. Moreover, this impairment could be related to the lower IL-4 levels of the DPPIV/CD26$^{-/-}$ mice, since this cytokine plays a crucial role in the antibody forming process[25,26]. IL-4 induces activation and differentiation of B cells, as well as acts as a growth factor for T cells, thereby promoting differentiation of Th2 cells and reinforcing the antibody response and plasma cells isotype switching to IgG and IgE production[25,26]. Regarding the importance of DPPIV/CD26 on T cell function, Ohnuma et al. found that soluble DPPIV/CD26 up-regulated the expression of the co-stimulatory molecule CD86 on monocytes, and enhanced the T cell immune response[29]. In the present *in vivo* study, however, we cannot exclude the possibility of a direct effect of DPPIV/CD26 on B cells. Buhling et al. reported that stimulation of isolated

CD20-positive B cells with PWM led to a raise in the proportion of CD26-positive cells, from 5% to 51%, suggesting an involvement of CD26 in B cell activation[30].

In DPPIV/CD26 knockout mice, we have found above all for the first time a disturbed immune response to PWM. Further experiments with stimulation or immunization with other antigens and pathogens *in vivo*, and with purified subsets of lymphocyte *in vitro* are necessary, in order to understand the role of this protein in the pathomechanisms of several diseases with immunological implications.

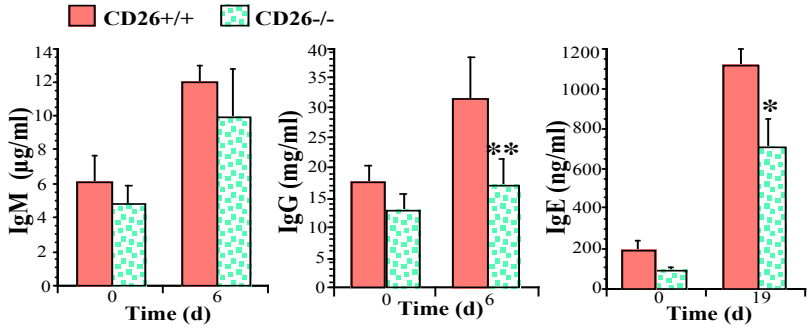


*Figure 4.* Immunoglobulins productions by $CD26^{+/+}$ and $CD26^{-/-}$ mice stimulated with PWM. $CD26^{+/+}$ and $CD26^{-/-}$ mice were injected (i. p.) with 200 µl PWM (40 mg/ml). After indicated days the concentration of immunoglobulins in sera was analyzed by ELISA. The noted values represent the mean ± SD of a minimum of five mice. *: $P<0.05$, **: $P<0.01$ as compared to $CD26^{+/+}$ mice.

# 3. CONCLUSION

Collagen type I inhibits the co-stimulatory activity of DPPIV/CD26, and hence modulates the T cell activation. The enzymatic activity of DPPIV is not necessary for the co-stimulatory activity of this molecule in T cell activation. DPPIV/CD26 plays an important role in the development, maturation, activation and differentiation of T cells as well as on their functions in the immune system. Deficiency of DPPIV/CD26 results in an impaired development and maturation of CD4 lymphocytes and a disturbed response to PWM stimulation. The IL-4 secretion was decreased, and hence the IgG production was reduced and isotype switching to IgE was affected.

# ACKNOWLEDGEMENTS

This work was supported by a grant from the *Deutsche Forschungsgemeinschaft* Bonn (Sonderforschungsbereich 366 and

Graduiertenkolleg 276), the *Sonnenfeld-Stiftung* and the *Fonds der Chemischen Industrie*, Frankfurt/Main.

# REFERENCES

1. Trugnan G., 1995, Control of dipeptidyl peptidase IV/CD26 cell surface espression in intestinal cells. In *Dipeptidyl Peptidase IV (CD26) in Metabolism and the Immune Response* (B. Fleischer, eds), R. G. Landes Company, Texas, pp. 79-98.
2. Bermpohl F., Löster K., Reutter W. & Baum O., 1998, Rat dipeptidyl peptidase IV (DPP IV) exhibits endopeptidase activity with specificity for denatured fibrillar collagens. *FEBS Lett*, **428:** 152-6.
3. Reutter W., Baum O., Löster K., Fan H., Bork J.P., Bernt K., Hanski C. & Tauber R., 1995, Functional aspects of the three extracellular domains of dipeptidyl peptidase IV: Characterization of glycosylation events, of the collagen-binding site and endopeptidase activity. In *Dipeptidyl Peptidase IV (CD26) in Metabolism and the Immune Response* (B. Fleischer, eds), R. G. Landes Company, Texas, pp. 55-79.
4. Hanski C., Huhle T. & Reutter W., 1985, Involvement of plasma membrane dipeptidyl peptidase IV in fibronectin-mediated adhesion of cells on collagen. *Biol Chem Hoppe Seyler*, **366:** 1169-76.
5. Hanski C., Huhle T., Gossrau R. & Reutter W., 1988, Direct evidence for the binding of rat liver DPP IV to collagen in vitro. *Exp Cell Res*, **178:** 64-72.
6. Löster K., Zeilinger K., Schuppan D. & Reutter W., 1995, The cysteine-rich region of dipeptidyl peptidase IV (CD 26) is the collagen-binding site. *Biochem Biophys Res Commun*, **217:** 341-8.
7. Fleischer B., 1994, CD26: a surface protease involved in T-cell activation. *Immunol Today*, **15:** 180-4.
8. Morimoto C. & Schlossman S.F., 1998, The structure and function of CD26 in the T-cell immune response. *Immunol Rev*, **161:** 55-70.
9. Franco R., Valenzuela A., Lluis C. & Blanco J., 1998, Enzymatic and extraenzymatic role of ecto-adenosine deaminase in lymphocytes. *Immunol Rev*, **161:** 27-42.
10. De Meester I., Korom S., Van Damme J. & Scharpe S., 1999, CD26, let it cut or cut it down. *Immunol Today*, **20:** 367-75.
11. Ansorge S., Koahne T., Lendeckel U., Reinhold D., Neubert K., Steinbrecher A. & Brocke S., 2001, Dipeptidyl peptidase IV/CD26 and aminopeptidase N/CD13 in regulation of the immune responce. In *Cell-surface aminopeptidase: Basic and clinical aspects* (S. Mizutani, eds), Elsevier, Amsterdam, pp. 85-94.
12. Hildebrandt M., Reutter W., Arck P., Rose M. & Klapp B.F., 2000, A guardian angel: the involvement of dipeptidyl peptidase IV in psychoneuroendocrine function, nutrition and immune defence. *Clin Sci (Lond)*, **99:** 93-104.
13. Marguet D., Baggio L., Kobayashi T., Bernard A.M., Pierres M., Nielsen P.F., Ribel U., Watanabe T., Drucker D.J. & Wagtmann N., 2000, Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26. *Proc Natl Acad Sci U S A*, **97:** 6874-9.
14. Proost P., De Meester I., Schols D., Struyf S., Lambeir A.M., Wuyts A., Opdenakker G., De Clercq E., Scharpe S. & Van Damme J., 1998, Amino-terminal truncation of chemokines by CD26/dipeptidyl-peptidase IV. Conversion of RANTES into a potent inhibitor of monocyte chemotaxis and HIV-1-infection. *J Biol Chem*, **273:** 7222-7.

15. Tanaka T., Kameoka J., Yaron A., Schlossman S.F. & Morimoto C., 1993, The costimulatory activity of the CD26 antigen requires dipeptidyl peptidase IV enzymatic activity. *Proc Natl Acad Sci U S A*, **90:** 4586-90.
16. Dang N.H., Torimoto Y., Deusch K., Schlossman S.F. & Morimoto C., 1990, Comitogenic effect of solid-phase immobilized anti-1F7 on human CD4 T cell activation via CD3 and CD2 pathways. *J Immunol*, **144:** 4092-100.
17. Ishii T., Ohnuma K., Murakami A., Takasawa N., Kobayashi S., Dang N.H., Schlossman S.F. & Morimoto C., 2001, CD26-mediated signaling for T cell activation occurs in lipid rafts through its association with CD45RO. *Proc Natl Acad Sci U S A*, **98:** 12138-43.
18. von Bonin A., Huhn J. & Fleischer B., 1998, Dipeptidyl-peptidase IV/CD26 on T cells: analysis of an alternative T-cell activation pathway. *Immunol Rev*, 161: 43-53.
19. Dong R.P., Tachibana K., Hegen M., Munakata Y., Cho D., Schlossman S.F. & Morimoto C., 1997, Determination of adenosine deaminase binding domain on CD26 and its immunoregulatory effect on T cell activation. *J Immunol*, **159:** 6070-6.
20. Augustyns K., Bal G., Thonus G., Belyaev A., Zhang X.M., Bollaert W., Lambeir A.M., Durinx C., Goossens F. & Haemers A., 1999, The unique properties of dipeptidyl-peptidase IV (DPP IV / CD26) and the therapeutic potential of DPP IV inhibitors. *Curr Med Chem*, **6:** 311-27.
21. Kubota T., Flentke G.R., Bachovchin W.W. & Stollar B.D., 1992, Involvement of dipeptidyl peptidase IV in an in vivo immune response. *Clin Exp Immunol*, **89:** 192-7.
22. Tanaka T., Camerini D., Seed B., Torimoto Y., Dang N.H., Kameoka J., Dahlberg H.N., Schlossman S.F. & Morimoto C., 1992, Cloning and functional expression of the T cell activation antigen CD26. *J Immunol*, **149:** 481-6.
23. Dinjens W.N., ten Kate J., Wijnen J.T., van der Linden E.P., Beek C.J., Lenders M.H., Khan P.M. & Bosman F.T., 1989, Distribution of adenosine deaminase-complexing protein in murine tissues. *J Biol Chem*, **264:** 19215-20.
24. Steeg C., Hartwig U. & Fleischer B., 1995, Unchanged signaling capacity of mutant CD26/dipeptidylpeptidase IV molecules devoid of enzymatic activity. *Cell Immunol*, **164:** 311-5.
25. Abbas A.K., Murphy K.M. & Sher A., 1996, Functional diversity of helper T lymphocytes. *Nature*, **383:** 787-93.
26. Sallusto F., Lanzavecchia A. & Mackay C.R., 1998, Chemokines and chemokine receptors in T-cell priming and Th1/Th2-mediated responses. *Immunol Today*, **19:** 568-74.
27. Arndt M., Reinhold D., Lendeckel U., Spiess A., Faust J., Neubert K. & Ansorge S., 2000, Specific inhibitors of dipeptidyl peptidase IV suppress mRNA expression of DP IV/CD26 and cytokines. *Adv Exp Med Biol*, **477:** 139-43.
28. Arndt M., Lendeckel U., Spiess A., Faust J., Neubert K., Reinhold D. & Ansorge S., 2000, Dipeptidyl peptidase IV (DP IV/CD26) mRNA expression in PWM-stimulated T-cells is suppressed by specific DP IV inhibition, an effect mediated by TGF-beta(1). *Biochem Biophys Res Commun*, **274:** 410-4.
29. Ohnuma K., Munakata Y., Ishii T., Iwata S., Kobayashi S., Hosono O., Kawasaki H., Dang N.H. & Morimoto C., 2001, Soluble CD26/dipeptidyl peptidase IV induces T cell proliferation through CD86 up-regulation on APCs. *J Immunol*, **167:** 6745-55.
30. Buhling F., Junker U., Reinhold D., Neubert K., Jager L. & Ansorge S., 1995, Functional role of CD26 on human B lymphocytes. *Immunol Lett*, **45:** 47-51.