5 Discussion

5.1 Purification and 3D-structure analysis of rat homogeneous dimeric CD26/DPPIV

Although physico-chemical investigations implicate a three-domain structure of the extracellular part, the tertiary structure of CD26/DPPIV has not yet been elucidated before the beginning of our work. For an in-depth structure study, homogeneous dimeric DPPIV/CD26 with enzymatic activity is required.

In the present work, combination of mAb-conjugated affinity column and gel filtration column was used for rapid purification. Enzymatically active DPPIV/CD26 was isolated from CHO/CD26 cell lysate by an affinity chromatography under a moderate elution condition. Homogeneity of dimeric CD26 was achieved by an additional SE-FPLC gel filtration step. Four peaks corresponding to molecular weight 3140 kDa, 650 kDa, 210 kDa and 5 kDa, respectively, were separated. The proteins in the first three peaks showed DPPIV enzymatic activity, which were polymer, hexamer, and dimer, respectively. The dimer showed the highest specific activity. In addition, oligosaccharide side chains of the protein expressed in CHO cells consist of both mature complex oligosaccharides and mannose-rich glycans. We successfully isolated not only the dimeric CD26 but also the dimeric CD26 with different oligosaccharides. Only the fraction containing homogeneous mature *N*-glycan structure of complex form was used for further 3D-structure investigation.

By application of this simple two-step purification protocol, high purity of homogeneous dimer rat CD26/DPPIV with enzyme activity was obtained. Successful resolution of 3D-structure confirms that this protocol is highly effective for purification of CD26. Moreover, following this protocol with slight modifications, soluble form of human dimeric CD26/DPPIV was purified from insect cell transfectants in our laboratory too (Dobers et al., 2002).

The 3D-structure of rat CD26/DPPIV dimer was determined by cryo-TEM and single particle analysis. Two distinct, nearly identical barrel-shaped subunits are twisted against each other. The catalytic triad, Ser 631, Asp 709 and His 741, of this enzyme lies at the bottom of the cavity, which is accessible via two openings. One opening is in the center of the propeller domain. Another is in the side between the hydrolase and propeller domains. Our results (Ludwig et al., 2003) are concordant with recently resolved 3D-structure of human and pig CD26/DPPIV (Engel et al., 2003; Hiramatsu et al., 2003; Rasmussen et al., 2003).

CD26 is a member of the prolyl oligopeptidase (POP, EC 3.4.21.26) gene family. In comparison with POP, the most striking structure of CD26/DPPIV is the second side opening, which is absent in POP, indicating that the functionality of CD26/DPPIV is most probably driven by a different mechanism. POP functions as a monomer, in which the unique opening of about 4 Å is too small to pass the substrate peptide into the catalytic center (Fulop et al., 1998). It is assumed propeller blades act as a gating filter during catalysis permitting a sequential opening and closing for substrate entry (Fulop et al., 1998). Unlike POP, CD26 is expressed as a homodimer. Dimerization makes it possible to form the second side opening. Substrates could reach the catalytic center of DPPIV/CD26, locating at the bottom of cavity in each subunit, by two different routes. By entering from the top, the catalytic triad would be accessible by a direct route leading down to the bottom of the subunit. Alternatively, the substrate might enter through the side opening. In both cases, the products of enzymatic cleavage could be expelled through the complementary opening. Further structure analysis of CD26/DPPIV with a non-hydrolyzing substrate peptide will give a clear explanation on the underlying mechanisms.

Besides its function as an enzyme, CD26 dimerization may be important for its co-stimulatory activity and its interaction with other molecules, such as ADA, CD45, HIV-tat, gp120 and so on. Hence 3D-structure resolution will shed light on its multiple biological functions.

5.2 The role of CD26 in immunoregulation

5.2.1 Influence of CD26 deficiency on the development, maturation, and distribution of lymphocyte subpopulation

Although $CD26^{-/-}$ mice display an apparently normal phenotype, a subpopulation analysis of spleen lymphocyte population (MSLs) reveals that they present a lower percentage of $CD3^+$ T lymphocytes together with a higher percentage of NK cells in their MSLs. Further analysis showed that the diminishing T lymphocytes in MSLs are mainly $CD4^+$ cells (helper/memory population). In the $CD4^+$ lymphocyte population, no significant difference was found on the ratio of memory vs. naive lymphocytes between $CD26^{-/-}$ and $CD26^{+/+}$ mice (data not shown). On the other hand, in peripheral blood lymphocytes (MPBLs) of $CD26^{-/-}$ mice, the percentage of T cells as well as their subpopulations, $CD4^+$ and $CD8^+$ cells, was not significantly changed. In contrast, there was a marked decrease in the proportion of NKT cells in MPBLs. Among three subsets of NKT population, $CD4^+$, $CD4^-$ $CD8^-$ and $CD8^+$ NKT cells, $CD4^+$ NKT cells are the most affected subsets. Notably, although $CD8^+$ NKT cells have been found in the peripheral blood, their development is thymus- and CD1d-independent. While $CD4^+$ NKT cells are thymus- and CD1d-dependent (Brutkiewicz and Sriram, 2002). The significant decrease of $CD4^+$ NKT cells in $CD26^{-/-}$ MPBLs indicates an involvement of CD26 in the development of NKT in thymus. Ruiz et al. have suggested that CD26 is ontogenetically controlled during T cell maturation (Ruiz et al., 1996; Ruiz et al., 1998a; Ruiz et al., 1998b). However, as far as we know, there was no evidence about the influence of CD26 expression on the development and maturation of NK and NKT cells. Here we show that the deficiency of CD26 expression results in an increase of NK cells in MSLs and a decrease of NKT cells in MPBLs, indicating for the first time that CD26 expression is crucial for development, maturation and migration of convential T cells including $CD4^+$ as well as NK and NKT cells.

5.2.2 Involvement of CD26 in the regulation of PWM-stimulated cytokine and immunoglobulin secretion *in vitro* and *in vivo*

$CD4^+$ cells are T helper cells, which, upon activation, produce a number of different cytokines and play a role in the activation and/or proliferation of B cells, cytotoxic T lymphocytes and macrophages. NK cells are important effector cells involved in innate immunity against tumors and a variety of pathogens. They also participate in the induction and regulation of subsequent immune responses via release of various cytokines and chemokines, such as IFN-γ and TNF-α (Biron et al., 1999). NKT cells are a distinct population of mature lymphocytes that co-express NK receptor and T cell antigen receptors. Upon *in vivo* stimulation, NKT cells produce large amounts of both IL-4 and IFN-γ, which might be important for the initiation and regulation of immune responses (Godfrey et al., 2000; Kronenberg and Gapin, 2002). Thus, the change in percentages of $CD4^+$, NK and NKT cells observed in the present work was expected to influence the immune response of $CD26^{-/-}$ mice, which prompted us to examine the response of $CD26^{-/-}$ mice to produce cytokines and immunoglobulins after stimulation *in vitro* and *in vivo*.

5.2.2.1 Influence of CD26-deficiency on cytokine production *in vitro* and *in vivo*

The activation and proliferation of MSLs were determined after stimulation with different mitogens *in vitro*. A reduction in the proliferation rates was detected in $CD26^{-/-}$ MSLs stimulated by ConA or PWM. Cytokine secretion is a hallmark of T cell activation. T helper cells can be divided into at least two subsets, Th1 and Th2, based on different cytokines produced upon stimulation. Th1 cells produce IL-2 and IFN-γ, whereas Th2 cells produce IL-4, IL-5, IL-6, IL-10 and IL-13. Overwhelmed papers indicated the role of CD26 in T cell activation, especially its function as a co-stimulator in activation of T helper cells. Therefore, in order to further investigate the influence of CD26 expression on lymphocyte activation and differentiation, the production of different cytokines of MSLs was analyzed after stimulation *in vitro*. Compared with ConA, PWM was found to be a better stimulator of IL-2 and IL-4 secretion. The IL-2 and IL-4 concentrations exhibited distinct maxima at 72 h after stimulation by PWM, which were much greater than those

following stimulation by ConA. Hence PWM was used as the stimulator during the following experiments. The deficiency of CD26 affected mostly IL-4 secretion. After PWM stimulation, MSLs of $CD26^{-/-}$ mice produced significantly reduced IL-4, while increased IFN-γ (Fig. 44A), which attracted us to try to identify IL-4- and IFN-γ-producing subsets in MSLs. FACS analysis verified that IL-4 was produced mainly by $CD4^+$ lymphocytes. Less IL-4 in the supernatants of $CD26^{-/-}$ MSLs is probably due to the lower percentage of $CD4^+$ cells or to the impaired differentiation of $CD4^+$ cells to Th2 cells producing IL-4. However the IFN-γ-producing cells consisted of not only $CD4^+$ but also $CD4^-$ populations. Although IFN-γ is a typical Th1 type cytokine, it can be produced by Th1 cells as well as by $CD8^+$, NK cells and others. Considering the capacity of NK cells to produce IFN-γ, an increased percentage of NK cells in $CD26^{-/-}$ MSLs potentially contributed to the enhancement of IFN-γ and inhibition of IL-4.

The cytokine concentrations in sera of both strains of mice were measured after immunization by PWM *in vivo* (Fig. 44B). Similar to the results from the *in vitro* test, deficiency of CD26 affected mainly IL-4 secretion; however, unlike the *in vitro* assay, deficiency of CD26 influenced not only Th2 cytokine IL-4 secretion, but also Th1 cytokines, IL-2 and IFN-γ production (a reduced IL-2 and delayed IFN-γ secretion). CD26 may exert its influence on the immune system through two mechanisms. On the one hand, this protein exhibits a co-stimulatory function on T cell activation via its ability to mediate signaling through the direct interaction with the cytoplasmic domain of CD45 (Ishii et al., 2001), which could influence the differentiation of T lymphocytes and hence their cytokine production; on the other hand, some substrates of dipeptidyl peptidase IV, such as RANTES, Eotaxin, MDC, SDF-1α and SDF-1β, have a direct effect on the immune response. The cleaved products of these chemokines trigger Th1- but not Th2-specific chemokine receptors (McCaughan et al., 2000). Deficiency of this enzyme could reduce the Th1 response, and hence reduce the Th1 cytokine (IL-2 and IFN-γ) secreting in sera. Furthermore, $CD26^{-/-}$ mice exhibited significantly lower percentage of $CD4^+$NKT cells in their PBLs. NKT cells are one of the earlier players involved in cytokine production and immune response initiation. After stimulation *in vivo*, NKT cells produce large amount of

both IL-4 and IFN-γ (Godfrey et al., 2000; Brutkiewicz and Sriram, 2002; Kronenberg and Gapin, 2002). Thus, the decreased NKT cells in peripheral blood of knockout mice may produce much less IFN-γ and IL-4 upon stimulation. Our results suggest that CD26 expression is involved in the regulation of cytokine secretion.

To characterize the function of CD26 on T helper cell differentiation, $CD4^+$ cells were isolated from mouse spleen lymphocytes using $CD4^+$ MicroBeads and MiniMACS column. Interestingly, obviously different from results of MSLs, $CD26^{-/-}CD4^+$ cells secreted much more IFN-γ, IL-4, IL-6 and IL-10 than $CD26^{+/+}CD4^+$ cells after stimulation with PWM. Only IL-2 production of $CD26^{-/-}$ $CD4^+$ cells was less than that of $CD26^{+/+}$ $CD4^+$ cells (Fig. 44C). Enhanced IL-4, IL-6, IL-10 and decreased IL-2 secretion indicate a Th2 bias of $CD26^{-/-}$ $CD4^+$ T cell differentiation, but increased IFN-γ production does not support this notion. Dissociation between IFN-γ and IL-2 production could not be explained by the data available. It is obvious that our studies provide evidence that in $CD4^+$ T lymphocytes CD26 deficiency can cause selective impairment of IL-2 but enhancement of IFN-γ gene expression although IL-2 and IFN-γ are often associated.

A. Influence of CD26-deficiency on cytokine secreting of MSLs stimulated by PWM *in vitro*

Th1		**Th2**	
IFN-γ	↑	**IL-4**	↓↓
IL-2	–	**IL-5**	–
		IL-6	–
		IL-10	↑
		IL-13	–

B. Influence of CD26-deficiency on cytokine production in mouse sera upon PWM stimulation *in vivo*

Th1		**Th2**	
IFN-γ	↓	**IL-4**	↓↓
IL-2	↓	**IL-5**	–

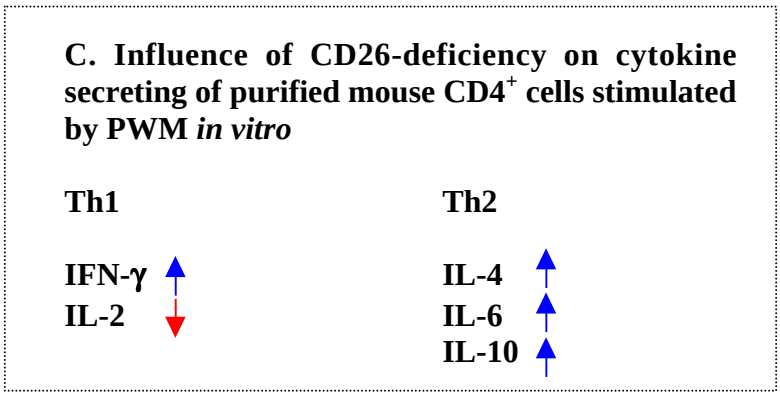

Fig. 44. Influence of CD26 deficiency on cytokine production *in vitro* and *in vivo*. ▬ : No change; ↑ **:** Increase; ↓ **:** Decrease.

The cytokine secretion tendency of isolated $CD26^{-/-}$ $CD4^+$ cells was quite different from that of MSLs. The main difference, compared with their wild type counterpart, is that MSLs of $CD26^{-/-}$ mice produced significantly less IL-4, while $CD26^{-/-}$ $CD4^+$ cells produced more IL-4, IFN-γ and less IL-2 (Fig. 44A and C). As we know, $CD4^+$ lymphocytes are purified T helper cells, whereas MSLs include not only $CD4^+$ lymphocytes, but also $CD8^+$, NK, B lymphocytes, granular cells, monocytes and dendritic cells. Upon stimulation, other cell types are capable of producing cytokines too. Like $CD4^+$ T cells, $CD8^+$ lymphocytes can differentiate into Tc1 and Tc2 cell as well based on cytokine production. However, unlike $CD4^+$ T cells, which can differentiate readily into either Th1 or Th2 cells, naive $CD8^+$ T cells show a strong preference to differentiate into Tc1 cells, whereas Tc2 differentiation requires the presence of substantial amounts of IL-4 during initial stimulation (Fitch et al., 1995; Mosmann et al., 1997a; Mosmann et al., 1997b). It was reported that $CD8^+$ T cells produce relatively more IFN-γ and TNF-α but almost no IL-2 was measured after triggering of CD3 and CD26 in comparison with $CD4^+$ T cells. But the influence of CD26 expression on the function of $CD8^+$ T cells was not extensively studied (De Meester et al., 1995). NK cells are the crucial players in innate immunoresponse and their capability to secrete IFN-γ can influence the subsequent immune response. In addition, the interaction between $CD4^+$ cells and antigen presenting cells may be important for cytokine production. CD26 is possibly involved in this interaction since it was reported

that exogenously added soluble CD26 up-regulates the expression of the co-stimulatory molecule CD86 on antigen presenting cells and results in enhanced tetanus toxoid-stimulated proliferation of human T cells (Ohnuma et al., 2001). Experiment with isolated $CD4^+$ cells deprived the influence of the different percentage of $CD4^+$ and NK lymphocytes and the interaction of $CD4^+$ lymphocytes with antigen presenting cells in MSLs. Besides, the CD4 molecules have a complex regulatory function in T cell activation (Fleischer and Schrezenmeier, 1988). Considering that $CD4^+$ cells were isolated by positive selection, we do not exclude the influence of crosslinking of CD4 antigen by CD4 mAb conjugated to MicroBeads in PWM-mediated cytokine production.

5.2.2.2 Influence of CD26-deficiency on immunoglobulin production *in vivo*

In the present study, a conspicuous decrease of the serum concentrations of IgG (also subclasses IgG1 and IgG2a) and IgE was found in $CD26^{-/-}$ mice immunized with PWM. PWM has been reported to stimulate antibody production and to induce immunoglobulin class switching from IgM to IgG. And PWM-induced immunoglobulin production is highly T cell dependent (Saiki et al., 1980). B cell response to T cell dependent antigen requires cognate interaction between T and B cells as well as interactions between T-cell-derived lymphokines and B cells (Stevens et al., 1988). Recently, a number of studies have demonstrated that Th2 cells are not unique with respect to the ability to induce T cell dependent B cell responses, but both, Th1 and Th2 cells and cytokines are capable of supporting B cell clonal expansion and antibody synthesis (Faquim-Mauro et al., 1999; Smith et al., 2000) (Fig. 45). IL-2 stimulates proliferation of both B and T lymphocytes (Dubois et al., 1999). IL-4 induces the activation and differentiation of B cells, and thus reinforces the antibody response and plasma cell isotype switching to produce IgG1 and IgE, while IgG2a isotype switching is promoted by IFN-γ (Snapper and Paul, 1987). Therefore, the reduced concentrations of these cytokines in MPBLs of $CD26^{-/-}$ mice correspond to the disturbed humoral response of these animals. Our results indicate that the reduced IL-4 and IL-2 production, combined with delayed IFN-γ secretion in sera of $CD26^{-/-}$ mice, contribute to decreased immunoglobulin production. The impaired class switching to IgG1 and IgE in $CD26^{-/-}$ mice is related to the decreased serum IL-4

concentration, whereas the delayed IFN-γ secretion is responsible for the disturbed switching to IgG2a.

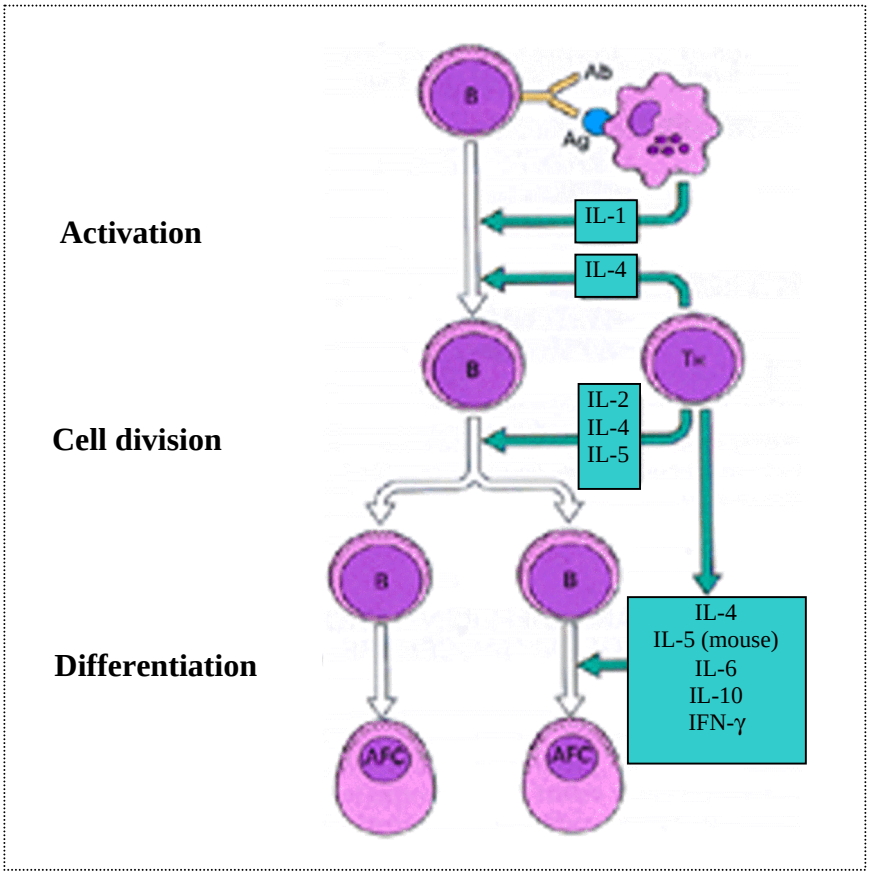

Fig. 45. Cytokines in B-cell activation and differentiation (Roitt et al., 2001)

5.2.3 Impaired immunoglobulin secretion and enhanced lung inflammation in CD26$^{-/-}$ mice upon OVA (Ovalbumin) immunization

PWM-induced antibody production and immunoglobulin class switching is highly T cell dependent. CD26 deficiency results in impaired cytokine and immunoglobulin production upon PWM stimulation, indicating an involvement of CD26 in humoral immunoresponse and in the function of T cell helping B cell to produce antibodies. Thereafter an antigen-mediated humoral immune response was necessary to be performed. Considering the facts that the highest specific enzyme activity of CD26/DPPIV is presented in the lung of C57BL/6 wild type mice (Fig. 9) and that some chemokines and neurotransmitters which

play a crucial role in asthma are demonstrated to be substrates of CD26/DPPIV, such as eotaxin, RANTES (Van Damme et al., 1999), MCP-2 (Oravecz et al., 1997) and MDC (Lambeir et al., 2001), we used a murine model of ovalbumin-induced asthma to examine the involvement of CD26/DPPIV in the humoral immunoresponse and pathogenesis of bronchial asthma.

5.2.3.1 Immunoglobulin production in sera of mice immunized with OVA

Sensitization of mice by intraperitoneal injection of OVA with adjuvant aluminum hydroxide gel provoked the marked increase of immunoglobulins in sera. After OVA immunization, both IgM and IgG (total IgG and subclasses IgG1 and IgG2a) production in $CD26^{-/-}$ mice were significantly lower. But IgE production was not different between these two kinds of mice. Combining these results with those from PWM stimulation, we confirm that CD26 deficiency leads to a decreased production of IgG, the majority antibody in sera, in T cell dependent antigen-mediated immunoglobulin production.

An increased concentration of circulating IgE is often detected in asthma. Crosslinking of IgE bound to mast cells by FceRI triggers degranulation of mast cells and the release of vasoactive mediators (Postma et al., 1995). But the accurate role of IgE in this process is still obscure so far. Bronchial inflammation can be induced in IgE deficient mice as well (Mehlhop et al., 1997). Mast cells express approximately 300 000 high affinity IgE receptors per cell, but aggregation of only 100 receptors is required for detectable responses (Kaliner, 1989). Besides IgE, it was reported that IgG1 isotype effectively sensitises mast cells and can confer hypersensitivity in mice (Kaliner, 1989). In the present experiment, no difference was found on sera IgE production between these two kinds of mice. $CD26^{+/+}$ mice can produce more IgG1 as well as IgG2a isotype, indicating involvement of both IL-4 and IFN-γ in immunoglobulin class switching. However, the lung histology revealed that the challenge by aerosol antigen induced a much more severe eosinophilia and airway inflammation in the lungs of $CD26^{-/-}$ mice. These results suggest that antibody production in sera does not directly associate with the severity of disease in our experiment.

5.2.3.2 Eosinophila and local Th2 cytokines

Accumulation of eosinophilic cells and subsequent activation in bronchial tissues are critical features in the pathogenesis of asthma bronchiale. Eosinophils and their products have been identified in sputum, bronchoalveolar lavage fluid and biopsy material of the airways of patients with asthma (Blease et al., 2000). Infiltration of eosinophils is governed by the up-regulation of adhesion molecules on lung endothelial cells and the production of various cytokines and chemotactic molecules by mast cells, T cells and endothelial cells.

(a) Cytokines

It is well known that Th2 cytokines, IL-4, IL-5 and IL-13, are key mediators of eosinophilic inflammation in the airways. IL-4 and IL-13 evokes transendothelial migration of eosinophils by inducing VCAM-1 expression. IL-5 strongly promotes the maturation, adhesion and activation of eosinophils. In addition, IL-9 and TGF-β are involved too (Renauld, 2001; Herrick and Bottomly, 2003). In the present experiment knock out mice exhibited a higher eosinophilic infiltration into the lungs than wild type mice, which is consistent with the higher mRNA expression and protein concentrations of IL-4, IL-5 and IL-13 measured in lung of knock out mice than wild type mice.

(b) Chemokines

T cell differentiation, cytokine secretion, and recruitment of mononuclear cells and eosinophils to inflammatory sites can be mediated by chemokines. Airway epithelial cells generate a wide variety of cytokines and chemokines, e.g. eotaxin, MCP-1, RANTES and others, which results in an immediate impact on the environment of the airway and surrounding lung tissue (Lukacs et al., 1999; Renauld, 2001). Eotaxin is a specific chemoattractant for eosinophils by interaction with CCR3 expressed on eosinophils shown in different species including the guinea pig, mouse, and human (Broide and Sriramarao, 2001). Eosinophilic chemotaxis involves cooperation between IL-5 and eotaxin as shown in the guinea pig and mouse (Palframan et al., 1998; Foster et al., 2001). IL-5 at this step triggers a rapid mobilization of eosinophils and progenitors from the bone marrow into the blood. Eotaxin recruits the eosinophils from the vasculature to the inflammatory sites in the

lung. But eotaxin is not the only chemokine able to modulate eosinophil accumulation within the lung. The movement of eosinophils during the early stages of asthma depends on RANTES and MIP-1α (Campbell et al., 1998; Campbell et al., 1999). In addition to eosinophils, Th2 cells orchestrate the asthmatic inflammation through the secretion of a series of cytokines. Chemokines involved in recuiment of T cell include RANTES, MIP-1a and MIP-1b, MCP-1, -2, -3 and -4. MDC and I-309 recruit Th2 cells preferentially, which would further exacerbate asthmatic responses and significantly alter the airway environment at specific stages of the disease. IP-10, MIG and ITAC are specifically activated by IFN-γ and enhance Th1-type lymphocyte recruitment and activation (Lukacs et al., 1999). Except for the initiation and maintenance of leukocyte accumulation, CC chemokine members might have the capacity to modulate T cell differentiation towards a Th1 type or Th2 type (Karpus et al., 1997). In particular, MCP-1 can drive undifferentiated T cell populations towards an IL-4-producing Th2-type cell, whereas MIP-1α appears to promote a Th1 type response by enhancing IFN-γ and decreasing IL-4 production *in vitro*.

(c) Neurotransmitters

Substance P (SP) is a widespread neuropeptides, which is produced by eosinophils, monocytes and macrophages, lymphocytes and dendritic cells. Its release from these inflammatory cells might further stimulate and activate these cells in an autocrine or paracrine fashion. SP is chemotactic for human eosinophils and potentiates chemotaxis induced by platelet-activating factor, leukotriene B_4 and IL-5 (Joos and Pauwels, 2000). Simulation of its preferred NK1 receptors induces mast cells degranulation, vasodilatation, plasma protein extravagation, mucus secretion and stimulation of inflammatory and immune cells (Joos, 2001).

(d) CD26/DPPIV-mediated processing of chemokine and neurotransmitters involved in pathogenesis of asthma

The highest specific enzyme activity of CD26/DPPIV was presented in the lung of C57BL/6 wild type mice (Fig. 9). Its expression on alveolar pneumocytes and endothelia has been demonstrated by Shipp (Shipp and Look, 1993). In the human bronchus, besides

its expression on leukocytes, DPPIV is present in serosal submucosal glands strongly and blood vessels weakly. Enzymehistochemistry revealed strong DPPIV activity in submucosal glands, blood vessels and leukocytes (van der Velden et al., 1998). The presence of CD26 on serosal glands and blood vessels indicates that CD26 may play a role in the processing of intravascular and intraluminal peptides. Among chemokines involved in asthma, several members share a conserved Xaa-Pro or Xaa-Ala sequence at their N-termini, which conforms to the substrate specificity of DPPIV. Indeed, DPPIV liberates the terminal Xaa-Pro dipeptides from full length RANTES, eotaxin (Van Damme et al., 1999), IP-10, MCP-2 (Oravecz et al., 1997) and MDC (Lambeir et al., 2001). The chemokines eotaxin, RANTES and MCP-2 stimulate the receptor CCR3 that is expressed on eosinophil and basophil granulocytes as well as on Th2-lymphocytes. Besides chemotaxis, CCR3 mediates degranulation of eosinophils and basophils as well as the synthesis of interleukines in Th2-lymphocytes which further stimulate the production of substances involved in killing of parasites or allergic reactions (Karpus et al., 1997). These chemokines are inactivated or even converted into CCR3 antagonists by the action of DPPIV. Thus these chemokine-induced reactions are stopped. However, IP-10, a chemokine enhancing Th1 cell recruitment, is not inactivated by CD26/DPPIV mediated truncation (Oravecz et al., 1997). The net effect of CD26 expression in the lung is to inactive chemokines enhancing inflammation. Moreover, it is shown recently that CD26/DPPIV may impair the ability of parenchymal cells (including endothelial and epithelial cells) to establish cell-cell interactions necessary to sustain leukocyte migration *in vitro* and tissue infiltration *in vivo* by metabolizing chemoattractants bound to the endothelial/epithelial cell surface (James et al., 2003). Thus, the enhanced inflammation in the lungs of CD26 deficient mice possibly results from the inability to degrade key chemokines important for the recruitment of eosinophils and activated $CD4^+$ Th2 cells.

In addition, CD26 liberates the dipeptides Arg-Pro and Lys-Pro sequentially from the N-terminus of substance P (SP) (Heymann and Mentlein, 1978; Nausch and Heymann, 1985). The fragment substance P-(5-11) has a significantly lower binding activity for the preferred SP receptor, the NK1 receptor. Moreover DPPIV truncated substance P can be further

attacked by aminopeptidase N (APN, EC3.4.11.2, CD13). This sequential sequestration by DPPIV and subsequently by APN is the most important degradation pathway in human plasma, at the vascular endothelium, at fibroblasts and muscle myocytes (Mentlein, 1999). In $CD26^{-/-}$ mice, the degradation of substance P is limited and leads to its accumulation in the lung. Accumulated substance P could mediate eosinophila and severe inflammation by NK1 receptor in $CD26^{-/-}$ mice. This assumption is supported by the findings that loss of DPPIV activity in chronic rhinosinusitis contributes to the neurogenic inflammation induced by substance P in the nasal mucosa; pretreatment with recombinant DPPIV significantly improves the symptoms of patients (Grouzmann et al., 2002).

Taken together, in wild type mice, CD26 enhances the IgM and IgG production induced by OVA immunization, but does not change IgE concentration. CD26 deficiency leads to severe lung inflammation induced by inhaled antigen in $CD26^{-/-}$ mice. Based on experiments performed by us and others, it is strongly suggests that CD26 in the mice lung possibly functions in regulation the local inflammation by cutting some inflammatory mediators, such as eotaxin, RANTES, MCP-2 and substance P which recruit eosinophils and Th2 cells and promote Th2 cell differentiation. As a result, the inability to inactive these key inflammation mediators in $CD26^{-/-}$ mice leads to enhanced Th2 cytokine secretion and severe eosinophilia and inflammation in the lung following aerosol antigen challenge.

5.2.4 Influence of DPPIV activity on lymphocyte proliferation and cytokine production

There are a lot of references showing the influence of DPPIV activity on T-cell activation. But the results are controversial depending on different experimental conditions. One approach is to study the activation of transfectants of CD26 gene. Upon CD26-mediated co-stimulation, IL-2 production is higher in Jurkat cells expressing wild type CD26 than in those expressing enzymatically inactive CD26 mutants (Morimoto and Schlossman, 1998). However, the enzymatic activity of CD26 is reported not to be required for its stimulatory and co-stimulatory activity using a mouse TCR^{+} T-cell hybridoma (von Bonin et al., 1998).

Another approach is the application of specific inhibitors. Reduction of the enzyme activity by specific inhibitors suppresses T-cell proliferation *in vitro* (Reinhold et al., 1993) and decreases antibody production in mice immunized with bovine serum albumin (Kubota et al., 1992). By use of different competitive inhibitors, a significant suppression of IL-2, IL-10, IL-12 and IFN-γ production and an increase of TGF-β are demonstrated in PWM-stimulated murine PBMC and purified T cells (Reinhold et al., 1997a; Reinhold et al., 1997b).

In our experiment, Ile-Pro-thiazolidide, a specific inhibitor of DPPIV activity, inhibited the proliferation of $CD26^{+/+}$ MSLs and the exogenous CD26 obviously enhanced the proliferation of $CD26^{-/-}$ MSLs induced by PWM, indicating an involement of DPPIV activity in immunoregulation. On the other hand, a very weak inhibition was also observed in $CD26^{-/-}$ MSLs stimulated with PWM combined with Ile-Pro-thiazolidide. At the concentration of 1 mM, this inhibitor inhibits survival (without PWM stimulation) of $CD26^{+/+}$ and $CD26^{-/-}$ MSLs by 20% and 10%, respectively, which is possible due to unspecific inhibition of DPPIV-like molecules, such as QPP, DPP8, and attractin. This unspecific inhibition was also demonstrated in a series of papers (Duke-Cohan et al., 1995; Jacotot et al., 1996; von Bonin et al., 1998; Chiravuri et al., 1999). Therefore, our results suggest that the enzymatic activity may be partially involved in immunoregulation, whereas additional effects unrelated to the target protease activity should be carefully considered in case of application of inhibitors.

In conclusion, our results, based on CD26 gene knock out mice, indicate that CD26 is involved in development, maturation, and migration of $CD4^{+}$T, NK and NKT cells, in cytokine secretion, and in T-cell dependent antibody production and isotype switching of B cells. Its expression in the lung is crucial for regulation of eosinophilia and local inflammation.