7 Summary

1. We presented the purification of rat CD26/DPPIV and elucidation of its three-dimensional structure by cryo-TEM. Homogenous dimeric rat CD26 with enzyme activity was purified by two-step chromatography, mAb-conjugated affinity chromatography and SE-FPLC. Its structure was determined by cryo-TEM and single particle analysis at a resolution of ~14 Å. The reconstruction confirms that the protein exists as a dimer, as predicted earlier. Since there are structural analogies to the serine peptidase prolyl oligopeptidase (POP), docking calculations of the two structures were performed. Although the docking showed a similar spatial organization (catalytic domain, β-propeller, distal opening, central cavity) the detailed comparison revealed clear discrepancies. The most marked difference is a second (lateral) opening in CD26/DPPIV, which would enable direct access to the catalytic site. We therefore assume that substrate selectivity and binding rate are most probably driven by different mechanisms in CD26/DPPIV and POP.

2. In order to investigate the role of CD26 in the immune system, CD26 gene knockout mice with C57BL/6 background were used to study the immune response after stimulation with PWM and with ovalbumin, respectively. $CD26^{-/-}$ mice display an apparently normal phenotype. But in their spleen lymphocyte population, the percentage of $CD4^+$ T cells is lower and that of NK cells is higher than in $CD26^{+/+}$ mice. In their peripheral blood, $CD26^{-/-}$ mice presented a conspicuously decreased proportion of $CD4^+$ NKT lymphocytes. *In vitro,* compared with $CD26^{+/+}$ mice, the PWM-stimulated IL-4 production in $CD26^{-/-}$ mice was decreased by 60 – 80% in the supernatants of spleen lymphocytes, whereas the production of IL-10 and IFN-γ was increased. No significant differences were found in the production of IL-2, IL-5, IL-6 and IL-13 between deficient and wild type mice. After immunization of mice with PWM *in vivo,* serum concentrations of total IgG, IgG1, IgG2a and IgE were markedly lower in $CD26^{-/-}$ mice than those in $CD26^{+/+}$ mice, while no difference was found in IgM production. Further analysis of cytokine concentrations *in vivo* revealed that a reduced IL-4, IL-2 and delayed IFN-γ production in sera of $CD26^{-/-}$ mice contributed to the decrease of PWM-induced immunoglobulin production. After

ovalbumin immunization *in vivo*, both IgM and IgG (total IgG and subclasses IgG1 and IgG2a) production were lower in $CD26^{-/-}$ mouse. The difference of IgG production between two strains was obvious. But IgE production was not affected. Aerosol challenge with OVA resulted in a much more severe eosinophilia and inflammation in the lung of $CD26^{-/-}$ mice, which was caused by markedly increased local Th2 cytokines IL-4, IL-5 and IL-13. These results indicate that CD26 contributes to the regulation of development, maturation and migration of $CD4^{+}$T, NK and NKT cells, of cytokine secretion, of T-cell dependent antibody production and immunoglobulin isotype switching of B cells. Its expression in the lung is crucial for regulation of allergen-mediated eosinophilia and local inflammation.

3. For better understanding of the underlying molecular mechanisms of CD26 function, the inducible expression system of CD26/GFP fusion protein was successfully established in Jurkat cells. CD26/GFP fusion protein was first constructed and expressed in CHO cells. This fusion protein possessed both fluorescent properties of GFP and enzyme activity of CD26. CD26/GFP DNA was further cloned into expression vector of Tet-on system. Recombinent plasmid pTRE2/CD26/GFP was transfected into Jurkat cells. In this system, the expression of CD26/GFP fusion protein was well regulated by doxycycline. In the absent of doxycline, no expression was observed at all; in contrast, the addition of doxycline induced a high expression of the fusion protein.

8 Outlook

1. Structure analysis of CD26 with a non-hydrolyzing peptide (e.g. resistant analogues of GLP-1 or GIP) to elucidate the mechanism underlying its specific enzyme activity.

2. Interaction of CD26/DPPIV with CD45, ADA and HIV-tat, and gp120 by cryo-TEM or crystallization.

3. Systematical investigation of the influence of CD26/DPPIV on NKT cell development.

4. Further clarification of CD26/DPPIV functions in pathogenesis of asthma by application of exogenous CD26 or inhibitor of its enzyme activity.

9 Abbreviations

ADA	Adenosine deaminase
Alum	Alu-Gel-S suspension
BAL(F)	Bronchoalveolar lavage (fluid)
BCA	Bicinchoninic acid
BSPL	Brain-specific dipeptidyl peptidase-like protein
CD	Cluster of differentiation
ConA	Concanavalin A
DIGs	The detergent-insoluble glycolipid-enriched domains, lipid rafts
DPPIV	Dipeptidyl peptidase IV
ECM	Extracellular matrix
ELISA	Enzyme-linked immunosorbent assay
FACS	Fluorescence-activated cell sorting
FAP-α	Fibroblast activation protein alpha
FCS	Fetal calf serum
FITC	Fluorescein isothiocyanate
GFP	Green fluorescent protein
GIP	Glucose-dependent insulinotropic polypeptide
GLP	Glucagon-like peptide
IFN	Interferon
IL	Interleukin
IP-10	Inflammatory protein-10
kDa	Kilodalton
LPS	Lipopolysaccharide from Escherichia coli 0111:B4
MACS	Magnetic cell sorting
MAPK	Mitogen-activated protein kinase
MCP	Monocyte chemoattractant protein
MDC	Monocyte derived chemokine

Mr.	Relative molecular weight
MBPL	Mouse peripheral blood lymphocytes
MSL	Mouse spleen lymphocytes
NAALADase	N-acetylated α-linked acidic dipeptidase
NK	Natural killer cells
NKT	$NK1.1^{+}CD3^{+}$ cells
OVA	Ovalbumin
PAGE	Polyacrylamide gel electrophoresis
PHA	Phytohemagglutinin
POP	Prolyl oligopeptidase
PWM	Pokeweed mitogen
QPP	Quiescent peptidyl peptidase
RANTES	Regulated on activation normal T cell expressed and secreted
R-PE	R-phycoerythrin
RT-PCR	Reverse transcriptase polymerase chain reaction
SDF-1	Stromal derived factor-1
SDS	Sodium dodecylsulfate
SE-FPLC	Size-exclusion fast protein liquid chromatography
TEM	Transmission electron microscope

10 Curriculum Vitae

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Education and Qualifications

1978-1983	Primary School Botou, Hebei Province
1983-1989	No. 1 Middle School Botou, Hebei Province
09.1989-07.1993	Bachelor student in department of Biology, Hebei University
11.1991	Awarded the scholarship for excellent students
07.1993	Graduated as 'Bachelor of Science' from Hebei University
09.1996-06.1999	Master student in the department of Microbiology in Nanjing Agricultural University
06.1998-02.1999	Carrying out part of research work for Master dissertation in Shanghai Institute of Materia Medica, Academia Sinica
12.1998	Conferred 'Professor Fan Qingsheng Microbiology Scholarship'
06.1999	Awarded 'Prize for Excellent Research Paper — Structure of antibiotic in the mycelia of *Streptomyces hygroscopicus* NND-52' in 6th meeting of Microbiological Society of Jiangsu Province, China
06.1999	Graduated as 'Master of Science' from Nanjing Agricultural University
11. 1999-Present	Ph.D. student in Institute für Molecularbiologie and Biochemie, Freie Universität Berlin
09.2002	FEBS International Summer School on Immunology, Ionian Village, Western Peloponese, Greece
07.2003	EMBO Conference / FEBS Advanced Course on protein phosphorylation and protein phosphatases, Barcelona, Spain Supported by FEBS young travel fund
12.2003	Awarded ‘Prize for Excellent Research Paper — Disturbed distribution of lymphocyte population and severe OVA-induced airway inflammation in CD26$^{-/-}$ mice‘ in Charté-Campus Benjamin Franklin

Work experience

07.1993-09.1996, working as a lecturer in department of Biochemistry, Medicine High School Handan, China.

11 Publications

Original papers:

1. Yan, S., Marguet, D., Dobers, J., Reutter, W. and Fan, H. 2003. Deficiency of CD26 results in a change of cytokine and immunoglobulin secretion after stimulation by PWM. *Eur. J. Immunol.*, 33 (6): 1519-1527.
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