CD26 in lymphocyte development/differentiation and OVA-induced airway inflammation

S. Yan[1], D. Marguet[2], W. Reutter[1] and H. Fan[1*]

[1]Institut für Molecularbiologie und Biochemie, Charite´ Universitätsmedizin Berlin
[2]Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Marseille, France
**: Correspondence to H. Fan, Institut für Molecularbiologie und Biochemie, CBF, Charite´ Universitätsmedizin Berlin, Arnimallee 22, 14195-Berlin-Dahlem, Germany, *e-mail: hua.fan@charite.de*

Summary

CD26 gene knockout mice with C57BL/6 background were used to study the role of CD26 deficiency in the immune system. $CD26^{-/-}$ mice display an apparently normal phenotype. However, in their spleen lymphocyte population the percentage of $CD4^+$ T cells is lower, and that of NK cells is higher, than that in $CD26^{+/+}$ mice. In peripheral blood, $CD26^{-/-}$ mice present a conspicuously decreased proportion of $CD4^+$NKT lymphocytes. After stimulation with Pokeweed mitogen *in vivo*, a reduced IL-4, IL-2 and delayed IFN-γ production in sera of $CD26^{-/-}$ mice were demonstrated, which further resulted in decrease sera immunoglobulin IgG (both subclasses IgG1 and IgG2a) concentration in $CD26^{-/-}$ mice. The reduced IgG concentration in $CD26^{-/-}$ mice was also presented upon antigen (ovalbumin) immunization. OVA-challenge resulted in enhanced tissue response in the lung of $CD26^{-/-}$ mice.

Introduction

CD26 (EC. 3.4.14.5), a widely distributed multifunctional type II plasma membrane glycoprotein, is involved in different biological processes. As dipeptidyl peptidase IV, this enzyme associated with the processing of variety of physiological active peptides including some chemikines, cytokines, neuropeptides and hormones (1). On human T cells, CD26

exhibits a costimulatory function and plays a crucial role in immune regulation via its ability to bind adenosine deaminase and mediate signaling by interaction with CD45 (2). Its role in diseases, such as type II diabetes, HIV infection, malignant transformation and transplantation rejection, has been indicated (3). However, the definite functions of CD26 *in vivo* have not yet been elucidated. To address this question, the lymphocyte sub-populations and immune response in $CD26^{-/-}$ mouse were investigated.

Materials and Methods

Homozygous $CD26^{-/-}$ mice on C57BL/6 background and wild type mice were used in this work. After immunization of the animals *in vivo* with pokeweed mitogen (PWM, 40 mg/mouse, i.p.) or ovalbumin (OVA, 20 mg/mouse, i.p.), the immune responses were investigated. Subpopulations of spleen lymphocytes (MSLs) and peripheral blood lymphocytes (PBLs) were analyzed by FACScan cytometry. Cytokine production and Immunoglobulin concentrations were determined by ELISA kit (R&D System, Minneapolis, USA).

Results:

1. *The percentage of $CD4^+$ cells is significantly lower, while that of NK cells is higher in spleen lymphocytes of $CD26^{-/-}$ mice.* $CD26^{-/-}$ mice display an apparently normal phenotype. However, the percentage of $CD4^+$ cells in MSLs of $CD26^{-/-}$ mice is 23% lower than that in MSLs of $CD26^{+/+}$ mice (17.89 ± 2.75% vs. 23.06 ± 2.33%, P<0.001). In contrast, the proportion of spleen $NK1.1^+CD3^-$ (NK) cells was elevated by 67% in $CD26^{-/-}$ mice (4.93 ± 1.47% vs. 2.95 ± 0.78%; P<0.001). This suggests an involvement of CD26 expression in development, maturation and migration of $CD4^+$ and NK cells (4).
2. *The percentage of $CD4^+$ NKT cells is significantly lower in pe-*

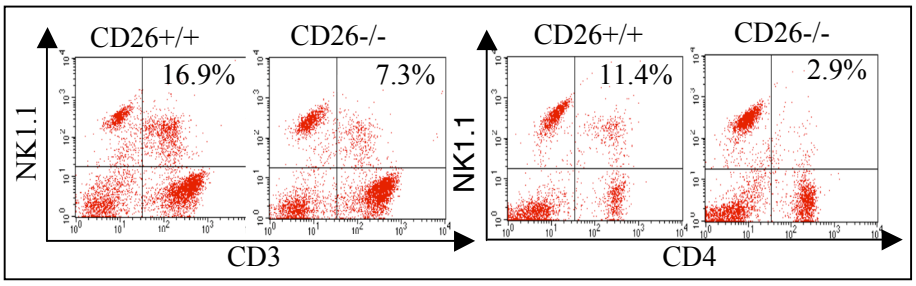

Fig. 1. Flow cytometry assay of NKT and $CD4^+$NKT cells in MPBLs populations. Data were shown as one representative of seven experiments.

ripheral blood lymphocyte population of CD26$^{-/-}$ mice. In peripheral blood lymphocytes (PBLs), no difference was found in the percentages of CD3$^+$ (also two main subsets CD4$^+$ and CD8$^+$ lymphocytes), CD19$^+$, or NK cells between CD26$^{-/-}$ and CD26$^{+/+}$ mice. However, the percentage of NKT cells in CD26$^{-/-}$ mice was decreased to 40% of that in CD26$^{+/+}$ mice (6.5 ± 1.5% vs. 16.5 ± 3.0 %, P<0.001) (Fig. 1). Further analysis of NKT subsets showed that the decreased NKT lymphocytes were NK1.1$^+$CD4$^+$ (CD4$^+$ NKT) cells, which represented only 22% of that in CD26$^{+/+}$ mice (2.7 ± 0.4% vs. 12.1 ± 1.9%; P<0.001) (Fig. 1). CD4$^+$ NKT cells are thymus- and CD1d-dependent (5). The significant decrease of CD4$^+$ NKT cells in CD26$^{-/-}$ MPBLs indicates an involvement of CD26 in the development of NKT in thymus (6).

3. Reduced IL-4, IL-2, and delayed IFN-γ production in sera of CD26$^{-/-}$ mice immunized by PWM. The influence of CD26 deficiency on the immune response was examined first by cytonkine secreting in mouse sera after PWM stimulation *in vivo*. Th1 cytokines IL-2 and IFN-g, Th2 cytokines IL-4 and IL-5 were measured. Injection of PWM induced a rapid elevation of these cytokines in sera. IL-2, IL-4, IL-5 and IFN-g concentrations peaked at 2 h, 4 h, 4 h and 12 h after immunization, respectively. All these cytokines dropped down to basic level after 36 h upon immunization. As shown in Fig 2A, in sera of CD26$^{-/-}$ mice the maximal value of IL-4 (at 4 h) was 38% (182 vs. 471 pg/ml, P<0.01), and that of IL-2 (at 2 h) about 50% (395 vs. 794 pg/ml, P<0.05) of that in CD26$^{+/+}$ mice. The maximum of IFN-g production was not affected, however, a delayed IFN-g increase was observed in CD26$^{-/-}$ mice. At 2 h after immunization, CD26$^{-/-}$ mice secreted only half the amount of IFN-g as compared with CD26$^{+/+}$ (210 vs. 409 pg/ml, P<0.05). No significant difference was observed in IL-5 production. NKT cells are one of the earlier players involved in cytokine production and immune

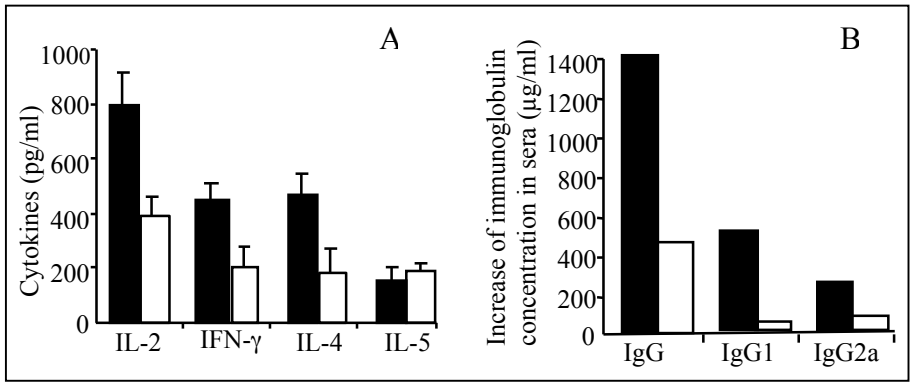

Fig. 2. Cytokine and immunoglobulin concentrations in mice sera after stimulation by PWM. The values represented the mean of six mice at each time point.

response initiation. After stimulation *in vivo*, NKT cells produce large amount of both IL-4 and IFN-γ. Thus, the decreased NKT cells in peripheral blood of knockout mice may produce much less IFN-γ and IL-4 upon stimulation (6).

4. CD26$^{-/-}$ mice produced markedly less IgG after immunization by PWM or Ovalbumin. The reduced IL-4 and IL-2 production combined delayed IFN-g secretion by CD26$^{-/-}$ MSLs prompted us to examine whether differentiation and immunoglobulin production of B lymphocyte were dependent on the expression of CD26. Mice were immunized with PWM, and the concentrations of different immunoglobulin classes in sera were measured by ELISA at different times after immunization. No significant difference was found on IgM and IgE concentration. However, CD26$^{-/-}$ mice showed significantly lower IgG concentrations (Fig. 2B). On day 6, the increase of serum IgG concentrations in CD26$^{+/+}$ mice was 1400 μg/ml, while in CD26$^{-/-}$ mice it was only 450 μg/ml. Also the concentrations of both immunoglobulin isotypes IgG1 and IgG2a were significantly lower in CD26$^{-/-}$ mice (Fig. 2B). Until day 6 after immunization, IgG1 production in CD26$^{-/-}$ mice increased barely, and IgG2a production elevated slightly (65 μg/ml), in comparison with CD26$^{+/+}$ mice which showed marked increases in IgG1 and IgG2a production (491 μg/ml, and 236 μg/ml, respectively) (6).

In agreement with the result from PWM stimulation, upon ovalbumin immunization, the markedly reduced is IgG (total IgG and subclasses IgG1 and IgG2a). Although OVA can induce high circulating IgE, but no difference was observed. Aerosol challenge with OVA resulted in markedly enhanced tissue response in the lung of CD26$^{-/-}$ mice.

Therefore, the reduced concentrations of these cytokines in MPBLs of CD26$^{-/-}$ mice correspond to the disturbed humoral response of these animals. The impaired class switching to IgG1 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.

Conclusion

CD26 contributes to the regulation of development, maturation and migration of CD4^{+}T, NK and NKT cells, and of cytokine secretion, T-cell dependent antibody production and immunoglobulin isotype switching of B-cells.

References

(1) Franco R et al., 1998, *Immunol. Rev.* 161, 27-42.
(2) Morimoto C. and Schlossman SF
(3) Meester et al., *Immunol. Tody* 1999, 20, 367-75.
(4) Fan H et al., 2003, *Adv Exp Med Biol.*;524, 165-74.

(5) Brutkiewicz RR, Sriram V. *Crit Rev Oncol Hematol*.2002, 41, 287-98.
(6) Yan S et al., 2003 *Eur. J. Immunol*. 33, 1519-27.

This work is supported by Deutsche Forschungsgemeinschaft (SFB366) and by the Graduiertenkolleg 276/2