The Journal of Immunology

Cutting Edge: Pivotal Function of Ubc13 in Thymocyte TCR Signaling

Masahiro Yamamoto, Shintaro Sato, Tatsuya Saitoh, Hiroaki Sakurai, Satoshi Uematsu, Taro Kawai, Ken J. Ishii, Osamu Takeuchi and Shizuo Akira

J Immunol 2006; 177:7520-7524; ;
doi: 10.4049/jimmunol.177.11.7520
http://www.jimmunol.org/content/177/11/7520

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

THE JOURNAL OF IMMUNOLOGY

CUTTING EDGE

Cutting Edge: Pivotal Function of Ubc13 in Thymocyte TCR Signaling[1]

Masahiro Yamamoto, Shintaro Sato,† Tatsuya Saitoh,* Hiroaki Sakurai,‡ Satoshi Uematsu,* Taro Kawai,† Ken J. Ishii,† Osamu Takeuchi,*† and Shizuo Akira[2]*†*

***The Ubc13 E2 ubiquitin-conjugating enzyme is essential for BCR-, TLR-, and IL-1 receptor (IL-1R)-mediated immune responses. Although Ubc13-deficient mice show defects in BCR-, TLR/IL-1R-, or CD40-mediated activation of mitogen-activated protein kinases, the function of Ubc13 in TCR-mediated signaling and responses remains uncertain. To address this, we here generated T cell-specific conditional Ubc13-deficient mice. The frequency of T lymphocytes was severely reduced in spleens from Ubc13-deficient mice. Moreover, Ubc13-deficient thymocytes displayed defective proliferation in response to anti-CD3/CD28 or PMA/ionophore stimulation. Regarding the signal transduction, although NF-κB activation was modestly affected, PMA/ionophore-induced activation of Jnk and p38 was profoundly impaired in Ubc13-deficient thymocytes. In addition, PMA/ionophore-mediated ubiquitination of NF-κB essential modulator (NEMO)/IκB kinase γ (IKKγ) and phosphorylation of TGF-β-activated kinase 1 (TAK1) were nearly abolished in Ubc13-deficient thymocytes. Thus, Ubc13 plays an important role in thymocyte TCR-mediated signaling and immune responses. The Journal of Immunology,* 2006, 177: 7520–7524.**

Activation of NF-κB and mitogen-activated protein (MAP)[3] kinases such as Jnk, p38, and Erk is a hallmark of immune receptor-mediated signaling pathways (1, 2). Recent studies demonstrate that a MAP kinase kinase kinase TGF-β-activated kinase (TAK1) is essential for NF-κB and MAP kinase activation mediated by innate immune or proinflammatory cytokine receptors such as TLRs, IL-1R, and TNFR (3, 4). On the other hand, TAK1 plays a minor role in BCR-mediated NF-κB activation in murine B cells (4). Moreover, although thymocytes from TAK1-deficient mice display severely defective NF-κB activation in response to anti-CD3/CD28 stimulation (5–7), NF-κB activation is normal in peripheral T cells (6), suggesting that TAK1-dependent and independent pathways for NF-κB activation in a cell type- or signal-specific way. Among MAP kinases, Jnk activation is most profoundly affected by TAK1 deficiency in all cell types (5–7).

Regarding the mechanism of TAK1 activation, stimulus-dependent polyubiquitination of TNFR-associated factor 6 (TRAF6) leads to phosphorylation of TAK1 that is shown to directly phosphorylate IκB kinase (IKK) complex and MAP2Ks (8, 9). The polyubiquitin chains on TRAF6 are reportedly generated by the E2 ubiquitin-conjugating enzyme Ubc13 (8, 10). Silencing of Ubc13 expression by siRNA results in defective NF-κB activation and NF-κB-dependent gene induction in HEK293 and insect cells (11, 12). However, in vivo analysis using mice lacking Ubc13 specifically in B cells, myeloid cells, and embryonic fibroblasts demonstrates almost normal NF-κB activation, despite severely reduced Jnk and p38 activation, in BCR-, IL-1R-, TLR-, or CD40-mediated signal transduction (13). Moreover, IL-1β-induced phosphorylation of TAK1 is observed, albeit with considerably delayed kinetics, in Ubc13-deficient cells (13), indicating that Ubc13 plays a minor role in NF-κB and TAK1 activation in BCR-, IL-1R-, TLR-, or CD40-mediated signaling pathways. In TCR-mediated responses, in vitro studies indicate that Ubc13 is involved in TCR-mediated NF-κB activation through NF-κB essential modulator (NEMO)/IKKγ ubiquitination (14, 15). However, whether Ubc13 plays minor or major roles in the TCR-mediated activation of NF-κB and MAP kinases in vivo remains elusive.

To assess the function of Ubc13 in T lymphocytes under physiological conditions, we generated mice carrying a modified *Ube2n* allele (the gene encoding Ubc13) specifically in T cells. As well as its effect on B cells, Ubc13 deficiency led to profound reduction in the frequency of peripheral T cells. Despite the normal cellularity in the thymus, Ubc13-deficient thymocytes showed defective proliferation in response to either

*Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, Yamada-oka, Suita, Osaka; †Exploratory Research for Advanced Technology, Japan Science and Technology Corporation, Yamada-oka, Suita, Osaka; ‡Division of Pathogenic Biochemistry, Institute of Natural Medicine, 21st Century Center of Excellence (COE) Program, Toyama Medical and Pharmaceutical University, Toyama, Japan

Received for publication July 27, 2006. Accepted for publication September 25, 2006.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by grants from Special Coordination Funds, the Ministry of Education, Culture, Sports, Science and Technology, Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists, The Uehara Memorial Foundation, The Naito Foundation, and Exploratory Research for Advanced Technology, Japan Science and Technology Agency.

[2] Address correspondence and reprint requests to Dr. Shizuo Akira, Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamada-oka, Suita Osaka 565-0851, Japan. E-mail address: sakira@biken.osaka-u.ac.jp

[3] Abbreviations used in this paper: MAP, mitogen-activated protein; TAK1, TGF-β-activated kinase 1; TRAF6, TNFR-associated factor 6; IKK, IκB kinase; NEMO, NF-κB essential modulator; SP, single positive.

0022-1767/06/$02.00

PMA/ionophore or anti-CD3/CD28. PMA/ionophore-induced activation of NF-κB as well as MAP kinases such as Jnk and p38 was affected by the Ubc13 deficiency. Furthermore, polyubiquitination of NEMO and phosphorylation of TAK1 in response to PMA/ionophore were severely impaired in Ubc13-deficient thymocytes. Our studies demonstrate that Ubc13 is essential for thymocyte TCR signaling and activation.

Materials and Methods

Reagents and mice

Agonistic anti-CD3ε and anti-CD28 were purchased from BD Pharmingen. Abs specific for phosphorylated forms of Erk (9101), Jnk (9251), p38 (9211), and IκBα (9241) were purchased from Cell Signaling Technology. Abs specific for Erk (sc-94), IκBα (sc-371), NEMO/IKKγ (sc-8330), ubiquitin (sc-8017), and actin (sc-8432) were obtained from Santa Cruz Biotechnology. Anti-Ubc13 monoclonal (37–1100) was obtained from ZYMED. Anti-phospho-TAK1 was as described previously (16). *Lck*_Cre and *Ube2n*$^{fl/fl}$ mice were as described previously (13, 17). The allele nomenclature for *Lck*_Cre and floxed Ubc13 <fl> are Tg(Lck-cre)1Jtak and Ube2n <tm1Aki>, respectively. The mice in this study were on a mixed C57BL/6 and 129P2/OlaHsd background. All animal experiments were conducted with the approval of the Animal Research Committee of the Research Institute for Microbial Diseases in Osaka University.

Western blot analysis and immunoprecipitation

After 1 h of starvation with DMEM containing 1% FBS to reduce background signals, the assay was performed as described previously (18).

In vivo ubiquitination assay

The assay was performed as described previously (13).

EMSA

Thymocytes (5×10^6) were stimulated with the indicated stimulants for the indicated periods. The assay was performed as described previously (18).

Flow cytometry

Single-cell suspensions of spleens, bone marrow, lymph nodes, and thymi were stained with FITC-, PE-, or biotin-conjugated indicated Abs. Labeled Abs were all obtained from BD Pharmingen.

Lymphocyte proliferation assay

Thymocytes (1×10^5) were cultured in 96-well plates for 72 h with the indicated concentrations of ligands. One microcurie of [^{3}H]thymidine was pulsed for the last 12 h and then ^{3}H uptake was measured in a beta scintillation counter (Packard Instrument).

Results

Generation of T cell-specific Ubc13-deficient mice

To analyze the role of Ubc13 in T lymphocytes, we inhibited Ubc13 expression specifically in the T cell lineage by crossing *Ube2n*$^{fl/fl}$ mice with transgenic mice expressing Cre under the control of proximal *lck* promoter. Southern blot analysis revealed that in thymocytes from Lck-Cre *Ube2n*$^{fl/fl}$ mice, Cre-mediated deletion resulted in a novel 1.1-kb band corresponding to the mutant *Ube2n* allele (Fig. 1, *A* and *B*). Moreover, Western blot analysis using extracts from thymocytes demonstrated that efficient loss of Ubc13 was achieved in *Lck*_Cre *Ube2n*$^{fl/fl}$ mice (Fig. 1*C*). Lck_Cre *Ube2n*$^{fl/fl}$ mice were born at the expected Mendelian ratios and presented no obvious abnormalities until at least 16 weeks of age (data not shown).

T cell development in Ubc13-deficient mice

We first examined the effect of Ubc13 deletion in thymocytes and peripheral T cells by flow cytometric analysis. The number of thymocyte and splenocytes in *Lck*_Cre *Ube2n*$^{fl/fl}$ mice was similar to that observed in control mice (control; $12.6 \times 10^7 \pm 5.51 \times 10^7$ cells vs *Lck*_Cre *Ube2n*$^{fl/fl}$; $16.3 \times 10^7 \pm 4.93 \times 10^7$ cells, per thymus; $n = 5$, and control; $8.79 \times 10^7 \pm 2.64 \times 10^7$ cells vs *Lck*_Cre *Ube2n*$^{fl/fl}$; $10.9 \times 10^7 \pm 1.92 \times 10^7$ cells, per spleen; $n = 5$). Whereas thymocyte development was comparable between control and Lck_Cre *Ube2n*$^{fl/fl}$ mice (Fig. 2*A*), CD3$^+$ T cell populations were dramatically reduced in the

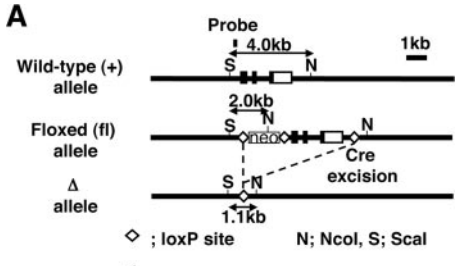

FIGURE 1. Generation of Lck_Cre *Ube2n*$^{fl/fl}$ mice. *A*, The structure of the wild-type (+), floxed (fl), or deleted (Δ) *Ube2n* gene. Black boxes denote the coding exons. Restriction enzymes: N, *Nco*I, S, *Sca*I. *B*, Southern blot analysis using genomic DNA extracted from thymocytes of indicated mice. Digested with *Nco*I and *Sca*I, electrophoresed and hybridized with the radiolabeled probe indicated in *A*. A 4.0-kb band, WT allele, 2.0 kb, floxed allele, 1.1-kb band, deleted allele (Δ). *C*, Cell lysates were subjected to immunoblot with anti-Ubc13 and anti-Erk1/2.

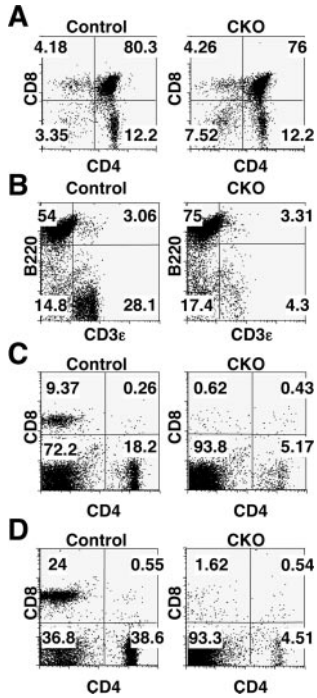

FIGURE 2. Defective peripheral T cell development in *Lck*_Cre *Ube2n*$^{fl/fl}$ mice. Flow cytometric analysis of T cell development in the thymocytes (*A*), splenocytes (*B* and *C*), and lymph node cells (*D*) from *Lck*_Cre *Ube2n*$^{fl/+}$ (control) and *Lck*_Cre *Ube2n*$^{fl/fl}$ (CKO) mice. Cells from 6- to 10-wk-old mice were stained with the indicated Abs. Percentages of positive cells within each quadrant are shown. Results are representative of three different experiments.

spleens of Lck_Cre *Ube2n*$^{fl/fl}$ mice (Fig. 2*B*). We next analyzed the frequency of CD4 SP and CD8 SP T cells in spleens and lymph nodes. In sharp contrast to control mice, the frequency of both types of cells was severely decreased in spleens of Lck_Cre *Ube2n*$^{fl/fl}$ mice (Fig. 2*C*). Moreover, we found profoundly less CD4 SP and CD8 SP T cells in lymph nodes from Lck_Cre *Ube2n*$^{fl/fl}$ mice than those in control mice (Fig. 2*D*). These results demonstrate that Ubc13 plays important roles in the maintenance or development of T cells in the periphery, but not in the thymus.

Proliferation in Ubc13-deficient thymocytes

We next analyzed whether stimulation of TCR leads to cellular activation in thymocytes. Both anti-CD3/CD28 and PMA/ionophore are known to stimulate TCR-mediated signal transduction, resulting in thymocyte proliferation (19). Control thymocytes proliferated by either anti-CD3/CD28 or PMA/ionophore stimulation in a dose-dependent fashion (Fig. 3, *A* and *B*). In contrast, Lck_Cre *Ube2n*$^{fl/fl}$ thymocytes exhibited defective growth in response to anti-CD3/CD28 or PMA/ionophore stimulation. Taken together, Ubc13 plays an important role in thymocyte proliferation in vivo.

NF-κB and MAP kinases activation in Ubc13-deficient thymocytes

Stimulation of TCR-mediated signaling pathway also activates NF-κB and MAP kinases (19). Activation of PKC-θ by PMA and ionophore led to phosphorylation of PKC-θ in both control and Lck_Cre *Ube2n*$^{fl/fl}$ thymocytes (Ref. 20 and Fig. 4*A*). We next assessed the effect of Ubc13 deficiency on NF-κB activation by EMSA. Although we found comparable NF-κB activation at a later time point (4 h) between control and Lck_Cre *Ube2n*$^{fl/fl}$ thymocytes, the early-phase NF-κB activation at 0.5, 1, and 2 h of PMA/ionophore stimulation was markedly decreased in Ubc13-deficient cells (Fig. 4*B*). Moreover, anti-CD3/CD28-induced NF-κB activation was also defective in Lck_Cre *Ube2n*$^{fl/fl}$ thymocytes at the early stage of the stimulation, albeit that at the late stage was similarly observed (Fig. 4*C*).

Next we examined MAP kinase activation by Western blot analysis. PMA/ionophore-stimulated activation of Jnk and p38 was severely and slightly impaired, respectively, in Lck_Cre *Ube2n*$^{fl/fl}$ thymocytes at the early and later time points (Fig. 4*D*), whereas the Erk activation was normal at both time points.

These results indicate that conditional ablation of Ubc13 in thymocytes as well as other cell types tested previously affects activation of Jnk and p38 (13). In addition, contrary to the previous in vivo study, Ubc13 may critically take part, at least, in the early-phase NF-κB activation in the TCR-mediated signal transduction in thymocytes.

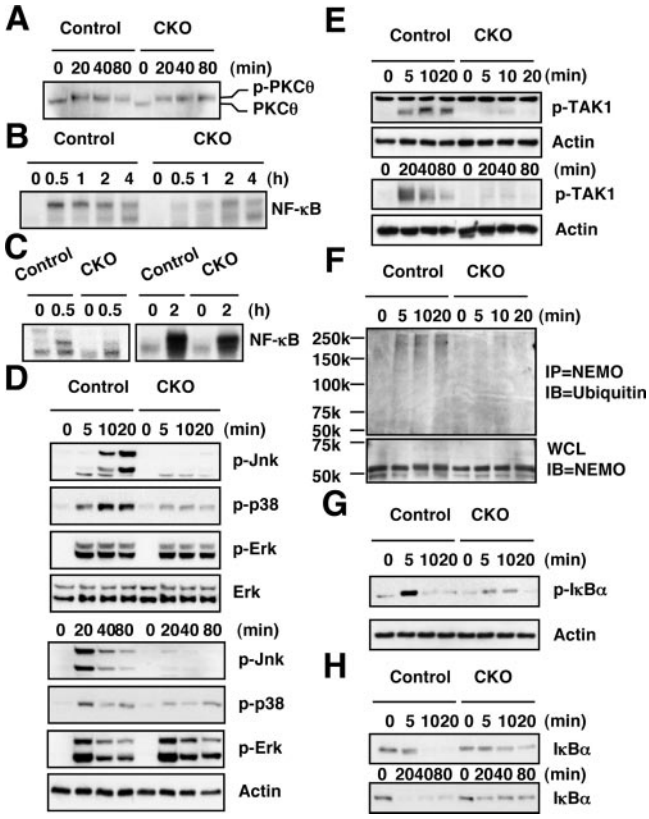

FIGURE 4. NF-κB and MAP kinases activation in *Lck*_Cre *Ube2n*$^{fl/fl}$ thymocytes. Immunoblot of phospho-PKC-θ (*A*), TAK1 (*E*), or phospho-IκBα (*F*) in thymocytes from *Lck*_Cre *Ube2n*$^{fl/+}$ (control) and *Lck*_Cre *Ube2n*$^{fl/fl}$ (CKO) mice in response to 50 ng/ml PMA/100 ng/ml ionophore for the indicated periods. Data are representative of two (*A*, *E*) or three (*F*) independent experiments. Thymocytes were stimulated with 50 ng/ml PMA/100 ng/ml ionophore (*B*) or 10 μg/ml anti-CD3/2 μg/ml anti-CD28 (*C*) for the indicated periods, and the NF-κB DNA binding activity was determined by EMSA using an NF-κB-specific probe. Data are representative of three independent experiments. Immunoblot of phospho-Jnk, p38, Erk (*C*) and IκBα (*H*) in thymocytes from *Lck*_Cre *Ube2n*$^{fl/+}$ (control) and *Lck*_Cre *Ube2n*$^{fl/fl}$ (CKO) mice in response to 50 ng/ml PMA/100 ng/ml ionophore for the indicated shorter (*upper*) and longer (*lower*) periods. Data are representative of three (*C*, *H*) independent experiments. *F*, Immunoblot of NEMO immunoprecipitates with anti-ubiquitin (*upper*) and of whole cell lysates with anti-NEMO (*lower*) from *Lck*_Cre *Ube2n*$^{fl/+}$ (control) and *Lck*_Cre *Ube2n*$^{fl/fl}$ (CKO) thymocytes stimulated with 50 ng/ml PMA/100 ng/ml ionophore for the indicated periods. Data are representative of two independent experiments.

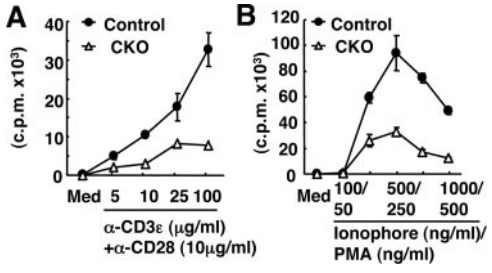

FIGURE 3. Impaired proliferation in *Lck*_Cre *Ube2n*$^{fl/fl}$ thymocytes. Thymocytes from *Lck*_Cre *Ube2n*$^{fl/+}$ (control) and *Lck*_Cre *Ube2n*$^{fl/fl}$ (CKO) mice were cultured with the indicated concentrations of anti-CD3ε/10 μg/ml anti-CD28 (*A*) or with the indicated concentrations of PMA/ionophore (*B*) for 72 h. [^{3}H]Thymidine (1 μCi) was pulsed for the last 12 h. [^{3}H]Thymidine incorporation was measured by a scintillation counter. Data are representative of three (*A*, *B*) independent experiments.

Modification of TAK1, NEMO, and IκBα in Ubc13-deficient thymocytes

Since TAK1 is required for TCR-mediated activation of NF-κB and MAP kinases in thymocytes (5–7), we tested whether Ubc13 deficiency affects PMA/ionophore-induced phosphorylation of TAK1. In sharp contrast to control cells, the phosphorylation of TAK1 in Lck_Cre *Ube2n*$^{fl/fl}$ thymocytes was severely reduced at the early and later time points (Fig. 4*E*), suggesting that Ubc13 is essential for the TCR-mediated TAK1 activation.

Table I. *Effects of Ubc13 deficiency on TCR-signaling*

Category	TAK1		MAPK		NEMO	IκBα		NF-κB
Molecules and status	p-TAK1	p-Jnk	p-p38	p-Erk	NEMO Ub	p-IκBα	IκBα Deg	NF-κB DNA binding
Compared with Control	↓ ↓ ↓	↓ ↓ ↓	↓	→	↓ ↓ ↓	↓	↓ ↓	Early ↓ ↓ Late →

→, Normal; ↓, slightly affected; ↓ ↓, modestly affected; ↓ ↓ ↓, severely affected.

In the TCR signal transduction, Ubc13 is involved in PMA/ionophore-stimulated ubiquitination of NEMO, which is required for NF-κB activation (14, 15). Therefore, we next analyzed the ubiquitination of NEMO in control and Ubc13-deficient thymocytes. In control cells, the stimulus-dependent ubiquitination of NEMO was observed in a time-dependent way (Fig. 4*F*). On the other hand, the ubiquitination in Lck_Cre *Ube2n*$^{fl/fl}$ thymocytes was almost completely disrupted, suggesting that Ubc13 plays a pivotal role in the TCR-mediated NEMO ubiquitination.

We next assessed PMA/ionophore-mediated phosphorylation and degradation of IκBα, those of which are known to be prerequisite for NF-κB activation (1). Control cells displayed IκBα phosphorylation in response to PMA/ionophore stimulation (Fig. 4*G*). Lck_Cre *Ube2n*$^{fl/fl}$ thymocytes also showed elevated levels of IκBα phosphorylation, albeit slightly decreased. Concerning IκBα degradation, PMA/ionophore stimulation led to reduction of protein levels of IκBα in Lck_Cre *Ube2n*$^{fl/fl}$ thymocytes, despite much less efficiently than control cells (Fig. 4*H*). Thus, these results demonstrate that Ubc13 is required for TAK1 phosphorylation, NEMO ubiquitination and IκBα degradation/phosphorylation in the thymocyte TCR-mediated signaling pathways.

Discussion

In the present study, we found that Ubc13 plays an important role in peripheral T cell development and TCR-mediated thymocyte proliferation. With respect to the TCR-mediated signal transduction, the Ubc13 deficiency affected activation and modification of TAK1, MAP kinases, NEMO, IκBα, and NF-κB to varying extents (Table I).

In particular, contrary to the previous finding that Ubc13 plays a minor role in the BCR-, TLR-, IL-1R-, or CD40-mediated NF-κB activation and IL-1β-mediated TAK1 phosphorylation (13), the present study demonstrates that Ubc13 is essential for the TCR-mediated NF-κB activation at the early time points and TAK1 phosphorylation. Whether the discrepancy between the previous and present study is due to different Ubc13 deletion efficiency in each conditional model or the disparity of the contribution of Ubc13 to NF-κB activation in cell type- or signal-specific ways is currently unknown. Also, we might fail to detect such defects in NF-κB activation that was observed in thymocytes, since we examined at only one point stimulation in other cell types (13).

In addition, Ubc13-deficient mice showed substantially distinct phenotype from T cell-specific TAK1-deficient mice (5–7). For instance, development of both CD4 and CD8 single positive (SP) thymocytes was severely impaired in TAK1-deficient mice, whereas that in Ubc13-deficient mice was normal. Considering that Ubc13-deficient cells still retained intact TAK1 protein, the structure, but not the kinase activity, of TAK1 might be required for regulating the thymocyte development. Whether TAK1 acts as a scaffold protein for the thymocyte development and function would be of interest in future studies.

TCR-mediated ubiquitination of NEMO was severely impaired in Lck_Cre *Ube2n*$^{fl/fl}$ thymocytes. Given that T cell-specific NEMO-deficient mice also display severe reduction of the peripheral T lymphocytes despite normal cellularity in the thymus (21), Ubc13-dependent ubiquitination of NEMO might play a role in the development or maintenance of T lymphocytes in the periphery. Moreover, activation of Jnk and p38 was also impaired in Lck_Cre *Ube2n*$^{fl/fl}$ thymocytes as observed in other conditional models. The mechanistic basis for the differential regulation of the MAP kinases and NF-κB activity by Ubc13 should be examined in the future.

In summary, we have characterized here the role of Ubc13 in T cell development and function. Moreover, our studies have demonstrated that Ubc13 is critically required for the TCR-mediated Jnk and p38 activation in thymocytes, whereas the NF-κB activation occurred in modestly Ubc13-dependent manners. Further studies using *Ube2n*$^{fl/fl}$ mice in other conditional models may reveal the nature of Ubc13 in a variety of signaling pathways.

Acknowledgments

We thank J. Takeda (Osaka University, Osaka, Japan) for providing Lck_Cre transgenic mice, K. Takeda (Kyushu University, Fukuoka, Japan) for discussions, M. Hashimoto for secretarial assistance, and N. Fukuda for technical assistance.

Disclosures

The authors have no financial conflict of interest.

References

1. Hayden, M. S., and S. Ghosh. 2004. Signaling to NF-κB. *Genes Dev.* 18: 2195–2224.
2. Chang, L., and M. Karin. Mammalian MAP kinase signaling cascades. 2001. *Nature* 410: 37–40.
3. Shim, J. H., C. Xiao., A. E. Paschal, S. T. Bailey, P. Rao, M. S. Hayden, K. Y. Lee, C. Bussey, M. Steckel, N. Tanaka, et al. 2005. TAK1, but not TAB1 or TAB2, plays an essential role in multiple signaling pathways *in vivo*. *Genes Dev.* 19: 2668–2681.
4. Sato, S., H. Sanjo, K. Takeda, J. Ninomiya-Tsuji, M. Yamamoto, T. Kawai, K. Matsumoto, O. Takeuchi, S. Akira. 2005. Essential function for the kinase TAK1 in innate and adaptive immune responses. *Nat. Immunol.* 6: 1087–1095.
5. Wan, Y. Y., H. Chi, M. Xie, M. D. Schneider, and R. A. Flavell. 2006. The kinase TAK1 integrates antigen and cytokine receptor signaling for T cell development, survival and function. *Nat. Immunol.* 7: 851–858.
6. Hong-Hsing, L., X. Min, M. D. Schneider, and Z. J. Chen. 2006. Essential role of TAK1 in thymocyte development and activation. *Proc. Natl. Acad. Sci. USA* 103: 11677–11682.
7. Sato, S., H. Sanjo, T. Tsujimura, J. Ninomiya-Tsuji, M. Yamamoto, T. Kawai, O. Takeuchi, and S. Akira. 2001. TAK1 is indispensable for development of T cells, and prevention of colitis by the generation of regulatory T cells. *Int. Immunol.* 18: 1405–1411.
8. Deng, L., C. Wang, E. Spencer, L. Yang, A. Braun, J. You, C. Slaughter, C. Pickart, and Z. J. Chen. 2000. Activation of the IκB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. *Cell* 103: 351–361.
9. Wang, C., L. Deng, M. Hong, G. R. Akkaraju, J. Inoue, and Z. J. Chen. 2001. TAK1 is a ubiquitin-dependent kinase of MKK and IKK. *Nature* 412: 346–351.

10. Hofmann, R. M., and C. M. Pickart. 1999. Noncanonical MMS2-encoded ubiquitin-conjugating enzyme functions in assembly of novel polyubiquitin chains for DNA repair. *Cell* 96: 645–653.
11. Zhou, R., N. Silverman, M. Hong, D. S. Liao, Y. Chung, Z. J. Chen, and T. Maniatis. 2005. The role of ubiquitination in Drosophila innate immunity. *J. Biol. Chem.* 280: 34048–34055.
12. Andersen, P. L., H. Zhou, L. Pastushok, T. Moraes, S. McKenna, B. Ziola, M. J. Ellison, V. M. Dixit, and W. Xiao. 2005. Distinct regulation of Ubc13 functions by the two ubiquitin-conjugating enzyme variants Mms2 and Uev1A. *J. Cell Biol.* 170: 745–755.
13. Yamamoto, M., T. Okamoto, K. Takeda, M. Sato, H. Sanjo, S. Uematsu, T. Saitoh, N. Yamamoto, H. Sakurai, K. J. Ishii, et al. 2006. Key role for the Ubc13 E2 ubiquitin-conjugating enzyme in immune receptor signaling. *Nat. Immunol.* 7: 962–970.
14. Zhou, H., I. Wertz, K. O'Rourke, M. Ultsch, S. Seshagiri, M. Eby, W. Xiao, and V. M. Dixit. 2004. Bcl10 activates the NF-κB pathway through ubiquitination of NEMO. *Nature* 427: 167–171.
15. Sun, L., L. Deng, C. K. Ea, Z. P. Xia, and Z. J. Chen. 2004. The TRAF6 ubiquitin ligase and TAK1 kinase mediate IKK activation by BCL10 and MALT1 in T lymphocytes. *Mol. Cell* 14: 289–301.
16. Singhirunnusorn, P., S. Suzuki, N. Kawasaki, I. Saiki, and H. Sakurai. 2005. Critical roles of threonine 187 phosphorylation in cellular stress-induced rapid and transient activation of transforming growth factor-beta-activated kinase 1 (TAK1) in a signaling complex containing TAK1-binding protein TAB1 and TAB2. *J. Biol. Chem.* 280: 7359–7368.
17. Takahama, Y., K. Ohishi, Y. Tokoro, T. Sugawara, Y. Yoshimura, M. Okabe, T. Kinoshita, and J. Takeda. 1998. Functional competence of T cells in the absence of glycosylphosphatidylinositol-anchored proteins caused by T cell-specific disruption of the Pig-a gene. *Eur. J. Immunol.* 28: 2159–2166.
18. Yamamoto, M., S. Yamazaki, S. Uematsu, S. Sato, H. Hemmi, K. Hoshino, T. Kaisho, H. Kuwata, O. Takeuchi, K. Takeshige, et al. 2004. Regulation of Toll/IL-1-receptor-mediated gene expression by the inducible nuclear protein IκBζ. *Nature* 430: 218–222.
19. Weil, R., and A. Israel. 2004. T-cell-receptor- and B-cell-receptor-mediated activation of NF-κB in lymphocytes. *Curr. Opin. Immunol.* 16: 374–381.
20. Sun, Z., C. W. Arendt, W. Ellmeier, E. M. Schaeffer, M. J. Sunshine, L. Gandhi, J. Annes, D. Petrzilka, A. Kupfer, P. L. Schwartzberg, and D. R. Littman. 2000. PKC-θ is required for TCR-induced NF-κB activation in mature but not immature T lymphocytes. *Nature* 404: 402–407.
21. Schmidt-Supprian, M., G. Courtois, J. Tian, A. J. Coyle, A. Israel, K. Rajewsky, and M. Pasparakis. 2003. Mature T cells depend on signaling through the IKK complex. *Immunity* 19: 377–389.