Mini Review

Regulation of host immune responses by nuclear IκB proteins

Masahiro Yamamoto, and Kiyoshi Takeda*

Department of Microbiology and Immunology, Graduate School of Medicine, Osaka University, Osaka, Japan

Nuclear factor-κB (NF-κB) plays an essential role in optimal activation of host immune systems, which is conserved from insects to mammals. Various microbial components and host-derived inflammatory cytokines activate NF-κB, leading to activation of the host immune system. Since excessive activation of NF-κB is harmful to the host, its activity is finely regulated at multiple steps in immune signaling pathways. One mechanism to prevent NF-κB activation is conducted by cytoplasmic IκB family proteins. Cytoplasmic IκBs have been shown to interact with NF-κB subunits in the cytoplasm of unstimulated cells. On stimulation, IκBs are rapidly degraded in a ubiquitin-proteasome dependent manner, allowing liberated NF-κB to translocate into the nucleus and activate the transcription of genes encoding various immune mediators. After the translocation of NF-κB from the cytoplasm to the nucleus, nuclear proteins structurally similar to cytoplasmic IκBs participate in the regulation of NF-κB activity as co-activators or -inhibitors through association with NF-κB subunits. For that reason, the regulatory IκB-like nuclear molecules are known as 'nuclear IκB proteins'. In this review, we will discuss the physiological function of the nuclear IκB proteins, IκBζ, IκBNS, and Bcl-3 in the context of innate and adaptive immune responses.

Rec.1/8/2008, Acc.8/12/2008, pp516-521

*Correspondence should be addressed to:
Kiyoshi Takeda, Department of Microbiology and Immunology, Graduate School of Medicine, Osaka University, 2-2, Yamada-oka, Suita, Osaka, 565-0871, Japan. Phone: +81-6-6879-3982, Fax: +81-6-6879-3989, e-mail: ktakeda@ongene.med.osaka-u.ac.jp

Key words TLR, IκB, inflammation, two-step gene induction, MyD88

Introduction

Activation of the host immune system requires a set of transcription factors including NF-κB, AP-1, C/EBP, and interferon regulatory factors, that positively regulate the expression of genes involved in various immune responses such as proinflammatory cytokine production, proliferation, and up-regulation of surface molecules in a variety of immune cells[1-6]. Among the transcriptional activators, NF-κB family proteins are well known for their role in the regulation of innate and adaptive immune responses. In unstimulated cells, NF-κB is sequestered in the cytoplasm by associating with inhibitory proteins called IκB. Activation and regulation of NF-κB is tightly controlled by the IκB proteins that mask the nuclear localization signal of NF-κB, preventing its translocation into the nucleus in the unstimulated state. On stimula-

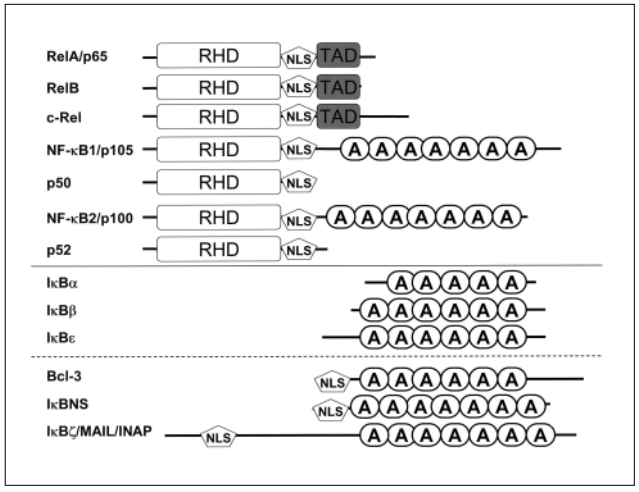

Fig.1 NF-κB and IκB family proteins

RelA/p65, RelB, and c-Rel possess trans-activation domain in the C-terminal portions. Distinct from cytoplasmic IκB proteins, Bcl-3, IκBNS, and IκBζ contain nuclear localization signal. RHD: Rel homology domain. NLS: Nuclear localization signal. TAD: Trans-activation domain. A: Ankyrin motif.

tion by various host immune mediators such as proinflammatory cytokines including tumor necrosis factor (TNF) or interleukin (IL)-1 superfamily proteins, or a number of microbial components such as Toll-like receptor (TLR) ligands, signaling cascades downstream of the receptors are activated, leading to phosphorylation of IκB proteins by IκB kinase complex including α, β, and γ subunit of IκB kinases, which are shown to be required for NF-κB activation through phosphorylation of cytoplasmic IκBs[3], and subsequent degradation of the phosphorylated IκB proteins. This allows NF-κB to translocate into the nucleus for the transcription of target genes. As for the structural character, IκB family proteins harbor ankyrin repeats that are shown to be required for interaction with NF-κB subunits[3] (Fig.1).

IκB-like proteins possessing ankyrin repeats are found not only in the cytoplasm but also in the nucleus, where they are called 'nuclear IκB proteins'. To date, three nuclear IκB proteins IκBζ, IκBNS, and Bcl-3 have been cloned and characterized by *in vitro* biochemical and *in vivo* genetic analysis in terms of the host immune response (Fig.1). Compared with cytoplasmic IκB proteins, nuclear IκB proteins containing nuclear localization signals in the N-terminal portions were found to be bound with p50 or p52 subunit of NF-κB to modulate gene expression in the nucleus. In this review, we summarize recent findings on nuclear IκB proteins with particular emphasis on the immunological aspects[7-9].

IκBζ

IκBζ (also known as INAP or MAIL) was originally identified as a gene that is specifically induced by LPS (a TLR4 ligand) or IL-1 stimulation[10-12]. Unlike other IκB family members, IκBζ is preferentially induced in response to TLR ligands, but not TNF, which activates NF-κB and MAP kinases as in TLR-mediated pathways. *In vitro* studies showed that overexpression of IκBζ inhibits NF-κB activation. On the other hand, ectopic expression of IκBζ promoted LPS-induced IL-6 production in a cell line. IκBζ-deficient mice were generated to examine its physiological role in TLR-mediated immune responses[13]. IκBζ-deficient mice showed defective IL-6 production in response to all TLR ligands and IL-1, but not to TNF. Regarding the IL-6 promoter, the NF-κB site is responsible for the positive effect of IκBζ, which specifically interacts with the p50 subunit of NF-κB. In addition, microarray analysis comparing gene expression profiles of LPS-stimulated wild-type and IκBζ-deficient macrophages demonstrated that a subset of LPS-inducible genes, such as IL-12 p40, GM-CSF and G-CSF, were severely affected by IκBζ deficiency. The LPS-induced transcription of IκBζ occurs earlier than the transcription of these genes. In addition, LPS-induced expression of these genes requires de novo protein synthesis, indicating that these genes are secondary response genes. Furthermore, LPS-induced expression of genes that are induced early, like IκBζ, was not impaired in IκBζ-deficient cells. Thus, some TLR-mediated responses are regulated by a two-step mechanism: firstly, primary response genes are induced early after TLR stimulation; then, the primary response gene product IκBζ mediates induction of secondary response genes (Fig.2).

Also, IκBζ-deficient mice exhibited pathological changes in the conjunctiva characterized by heavy lymphocyte infiltration into the submucosa and loss of goblet cells in the conjunctival epithelium[13,14]. The inflammatory symptoms found in IκBζ-deficient mice are reminiscent of Stevens-Johnson syndrome and/or cicatricial ocular pemphigoid, in which patients experience loss of goblet cells and infiltration of lypmphocytes into the conjunctival tissues over the cornea[14,15]. In addition, IκBζ/MAIL-deficient mice showed atopic dermatitis-like skin lesions with higher concentrations of serum IgE, suggesting a role for IκBζ in the negative regulation of the *in vivo* immune response[16]. Indeed, TNF-α production in LPS-induced septic shock was augmented and prolonged in IκBζ-deficient mice, indicating that IκBζ may function as a negative regulator of TNF-α production in cell types other than macrophages. The apparent contradiction observed in *in vitro* and *in vivo* analysis using IκBζ-deficient mice might be possibly due to the difference of the cell-

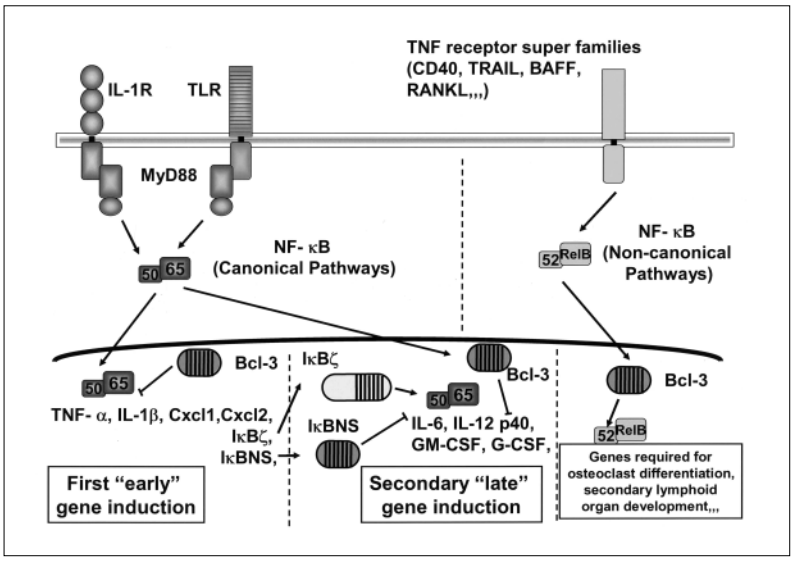

Fig.2 Role of nuclear IκB proteins in signal transduction in response to inflammatory stimuli

IκBζ and IκBNS act positively and negatively, respectively, in TLR/IL-1R-mediated MyD88-dependent secondary "late" immune responses. Bcl-3 suppresses p50-dependent gene expression and promotes p52-dependent responses.

type specific regulation of NF-κB gene expression by IκBζ, since overexpression of IκBζ inhibits or activates NF-κB-dependent gene expression in 293T cells or 3T3 fibroblast cells, respectively[10,11]. Further detailed analysis may reveal the precise role of IκBζ as a positive or negative regulator of NF-κB-dependent gene expression.

In terms of the mechanisms how IκBζ potentiates expression of a subset of LPS-inducible genes, IκBζ-deficient macrophages showed defective induction of trimethylation of histone H3 on the promoter of IκBζ-regulated genes, suggesting the role of IκBζ in the nucleosome remodeling induced by LPS[17].

IκBNS

IκBNS was originally cloned as a gene rapidly induced by TCR stimulation in thymocytes[18]. IκBNS also contains ankyrin repeats as well as IκBζ or Bcl-3 and is localized in the nucleus. Overexpression of IκBNS by a retrovirus in thymocytes promoted cell death, suggesting the involvement of IκBNS in thymocyte development *in vivo*. Moreover, ectopic expression of IκBNS abrogated PMA/ionophore-induced NF-κB DNA binding activity and NF-κB-dependent luciferase reporter activity, suggesting a role for IκBNS as a negative regulator of NF-κB.

Independently, gene expression analysis of colonic lamina propria (CLP) macrophages, which constitutively produce IL-10 and are therefore considered to have an important role in the maintenance of colonic mucosal immunological homeostasis, revealed the specific expression of IκBNS in CLP macrophages, but not in peritoneal macrophages, suggesting a potential role for IκBNS in intestinal mucosal immunity[19]. In addition, LPS stimulation up-regulated the mRNA expression of IκBNS. Overexpression of IκBNS in a macrophage cell line inhibited LPS-induced IL-6 production at the transcriptional level through association with the p50 subunit of NF-κB. Moreover, upon LPS stimulation, IκBNS was recruited to the κB site of IL-6, but not the TNF-α, promoter[19].

Further studies were conducted by generation of IκBNS-deficient mice[20,21]. Not only IL-6, but also other secondary response genes whose induction has been shown to be severely impaired in IκBζ-deficient cells, were conversely up-regulated in IκBNS-deficient cells in response to LPS[20]. Moreover, LPS-induced NF-κB DNA binding activity and the recruitment of NF-κB to the promoter of secondary response genes were also prolonged in IκBNS-deficient cells. Given that IκBNS proteins like IκBζ are shown to be recruited to the promoter of the secondary late-phase gene in response to LPS and both binds the p50 subunit of NF-κB[10,13,17,18], IκBζ and IκBNS might have opposite functions especially in the regulation of LPS-mediated secondary late-phase gene induction (Fig.2). However, the molecular mechanisms of

the opposite function in detailed are currently unknown.

In addition, IκBNS-deficient mice were highly susceptible to LPS-induced septic shock and to dextran sodium sulfate-induced colitis, demonstrating the role of IκBNS in limiting TLR-mediated immune responses and the maintenance of intestinal homeostasis *in vivo*[20]. On the other hand, thymocytes from IκBNS-deficient mice produced less IL-2 and exhibited impaired proliferation after TCR stimulation[21]. In addition, *in vitro* studies demonstrated that the positive function of IκBNS was mediated through the κB site on the IL-2 promoter. This suggests that, whether IκBNS and IκBζ act positively or negatively for NF-κB-dependent gene expression might depend on the cell type expressing these genes.

Bcl-3

Bcl-3 was the first identified molecule among the nuclear IκB proteins and was originally cloned as a proto-oncogene for chronic lymphatic leukemia[22,23]. Subsequent *in vitro* studies revealed that Bcl-3 was bound with p50 or p52 and its overexpression resulted in p50 or p52-mediated gene expression or gene suppression in various conditions[24-27], suggesting that Bcl-3 acts as a activator or an inhibitor of NF-κB-dependent gene expression through association with the p50 or p52 subunit of NF-κB. Bcl-3-deficient mice were generated and analyzed extensively in terms of adaptive immune responses[28,29]. Bcl-3-deficient mice showed impaired formation of germinal centers and disrupted splenic structures along with reduced numbers of splenic follicular B cells and marginal zone macrophages. The phenotypes found in Bcl-3 is similar to those found in NFκB2-deficeint mice in terms of the impaired development of lymphoid organ tissues and Bcl-3 are shown to be bound with p52 subunit of NF-κB, suggesting that Bcl-3 may function as an activator of p52/NFκB2 *in vivo* (Fig.2). Parasite-induced Th1 immune responses were retarded in Bcl-3-deficient mice *in vivo*, however, Bcl-3-deficient naïve T cells were able to differentiate normally into Th1 cells in *ex vivo* culture condition, indicating that Bcl-3-dependent non-T cell factor(s) may regulate parasite-induced Th1 differentiation[29]. Moreover, Bcl-3-deficient T cells are defective in Th2 differentiation and the production of Th2 cytokines such as IL-4, IL-5 and IL-13, due to a T-cell-intrinsic decrease in GATA-3 transcription factor expression, which is consistent with the finding that Bcl-3 can transactivate the gata-3 promoter, indicating a distinct role for Bcl-3 in the regulation of Th1 and Th2 differentiation[31].

Bcl-3 was also identified by microarray-based gene expression analysis as a gene induced by stimulation of an anti-inflammatory cytokine IL-10[32], treatment of which has been shown to diminish NF-κB-DNA binding activity. Ectopic expression of Bcl-3 also impaired NF-κB-DNA binding activity and resulted in reduced production of TNF-α, but not IL-6, in response to LPS in macrophages where LPS stimulation leads to the selective recruitment of Bcl-3 to the promoter of TNF-α, but not IL-6, by currently unknown mechanisms[32]. Moreover, Bcl-3-deficient macrophages were defective in IL-10-mediated suppression of LPS-induced TNF-α production, demonstrating the physiological function of Bcl-3 in innate immune cell populations[32]. In addition, Bcl-3-deficient mice and cells were found to be hypersensitive to TLR activation and unable to control responses to lipopolysaccharides, suggesting that Bcl-3 plays a role in negative regulation of TLR-mediated immune responses. Bcl-3 promoted the ubiquitination of p50 to facilitate the degradation, resulting in limitation of the strength of TLR responses[33].

Conclusions and Perspectives

Given that the three nuclear IκB proteins possess ankyrin repeats to interact with NF-κB subunits, structurally they should be classified as 'inhibitors' of NF-κB. However, distinct from the cytoplasmic IκB family proteins that simply inhibit the translocation of NF-κB subunits to the nucleus and prevent NF-κB-dependent gene expression, the nuclear IκB proteins IκBζ, IκBNS, and Bcl-3 act not only as suppressors but also activators of NF-κB-dependent gene expression, In vitro studies demonstrate that these nuclear IκB proteins interact with the p50 or p52 subunits of NF-κB. Considering that only p50/p52 doubly-deficient mice, but not singly-deficient mice, exhibit severely defective immune disorders such as osteopetrosis, some immunological phenotypes may be compensated for in mice lacking only one nuclear IκB protein (Fig.2)[34]. Further studies using mice devoid of two or all three nuclear IκB proteins may be useful to clarify and discover new and detailed physiological aspects of the nuclear IκB proteins in the future.

In conclusion, nuclear IκBs IκBz, IκBNS, and Bcl-3 play various roles in inflammatory responses through p50 or p52 subunit of NF-κB. The more detailed and precise molecular mechanisms that clarify the role of nuclear IκB proteins should be examined by *in vitro* and *vivo* studies in the future.

Acknowledgements

We thank members of Takeda's lab for helpful discussion, and M. Yasuda for secretarial assistance. This work was supported by Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labor and Welfare, as well as the Tokyo Biochemical Research Foundation, the Cell Science Research Foundation, the Yakult

Bio-Science Foundation, the Osaka Foundation for Promotion of Clinical Immunology, the Sumitomo Foundation, the Sankyo Foundation of Life Science, Mochida Memorial Foundation for Medical and Pharmaceutical Research, and Takeda Science Foundation.

References

1) Yamamoto M, Takeda K, Akira S: TIR domain-containing adaptors define the specificity of TLR signaling. Mol Immunol, 40: 861-868, 2004.
2) Akira S, Uematsu S, Takeuchi O: Pathogen recognition and innate immunity. Cell, 124: 783-801, 2006.
3) Ghosh S, Karin M: Missing pieces in the NF-κB puzzle. Cell, 109(Suppl), S81-S96, 2002.
4) Shaulian E, Karin M: AP-1 as a regulator of cell life and death.Nat Cell Biol, 4: E131-E136, 2002.
5) Poli V: The role of C/EBP isoforms in the control of inflammatory and native immunity functions. J Biol Chem, 273: 29279-29282, 1998.
6) Honda K, Taniguchi T: IRFs: master regulators of signalling by Toll-like receptors and cytosolic pattern-recognition receptors. Nat Rev Immunol,6: 644-658, 2006.
7) Ghosh S, May MJ, Kopp EB: NF-B and Rel proteins: evolutionarily conserved mediators of immune responses. Annu Rev Immunol, 16: 225-260, 1998.
8) Wessells J, Baer M, Young HA, Claudio E, Brown K, Siebenlist U, Johnson PE: Bcl-3 and NF-B p50 attenuate lipopolysaccharide-induced inflammatory responses in macrophages. J Biol Chem, 279: 49995-50003, 2004.
9) Akira S, Takeda K: Toll-like receptor signalling. Nat Rev Immunol, 4: 499-511, 2004.
10) Yamazaki S, Muta T, Takeshige K: A novel IκB protein, IκB-ζ, induced by proinflammatory stimuli, negatively regulates nuclear factor-κB in the nuclei. J Biol Chem, 276: 27657-27662, 2001.
11) Kitamura H, Kanehira K, Okita K, et al: MAIL, a novel nuclear IκB protein that potentiates LPS-induced IL-6 production. FEBS Lett, 485: 53-56, 2000.
12) Haruta H, Kato A, Todokoro K: Isolation of a novel interleukin-1-inducible nuclear protein bearing ankyrin-repeat motifs. J Biol Chem, 276: 12485-12488, 2001.
13) Yamamoto M, Yamazaki S, Uematsu S, et al: Regulation of Toll/IL-1-receptor-mediated gene expression by the inducible nuclear protein IκBζ. Nature, 430: 218-222, 2004.
14) Ueta M, Hamuro J, Yamamoto M, Kaseda K, Akira S, Kinoshita S: Spontaneous ocular surface inflammation and goblet cell disappearance in IκBζ gene-disrupted mice. Invest Ophthalmol Vis Sci, 46: 579-588, 2005.
15) Kawasaki S, Nishida K, Sotozono C, Quantock AJ, Kinoshita S: Conjunctival inflammation in the chronic phase of Stevens-Johnson syndrome. Br J Ophthalmol, 84: 1191-1193, 2000.
16) Shiina T, Konno A, Oonuma T, Kitamura H, Imaoka K, Takeda N, Todokoro K, Morimatsu M: Targeted disruption of MAIL, a nuclear IκB protein, leads to severe atopic dermatitis-like disease. J Biol Chem, 279: 55493-55498, 2004.
17) Kayama T, Ramirez-Carrozzi VR, Yamamoto M, Mizutani T, Kuwata H, Iba H, Matsumoto M, Honda K, Smale ST, Takeda K: Class-specific regulation of pro-inflammatory genes by MyD88 pathways and IκBζ. J Biol Chem, 283: 12468-12477, 2008.
18) Fiorini E, Schmitz I, Marissen WE, Osborn SL, Touma M, Sasada T, Reche PA, Tibaldi EV, Hussey RE, Kruisbeek AM, Reinherz EL, Clayton LK: Peptide-induced negative selection of thymocytes activates transcription of an NF-kappa B inhibitor. Mol Cell, 9: 637-648, 2002.
19) Hirotani T, Lee PY, Kuwata H, et al: The nuclear Iκ B protein IκBNS selectively inhibits lipopolysaccharide-induced IL-6 production in macrophages of the colonic lamina propria. J Immunol, 174: 3650-3657, 2005.
20) Kuwata H, Matsumoto M, Atarashi K, et al: IκBNS inhibits induction of a subset of Toll-like receptor-dependent genes and limits inflammation. Immunity, 24: 41-51, 2006.
21) Touma M, Antonini V, Kumar M, Osborn SL, Bobenchik AM, Keskin DB, Connolly JE, Grusby MJ, Reinherz EL, Clayton LK: Functional role for IκBNS in T cell cytokine regulation as revealed by targeted gene disruption. J Immunol, 179: 1681-1692, 2007.
22) Ohno H, Takimoto G, McKeithan TW: The candidate proto-oncogene bcl-3 is related to genes implicated in cell lineage determination and cell cycle control. Cell, 60: 991-997, 1990.
23) Bours V, Villalobos J, Burd PR, Kelly K, Siebenlist U: Cloning of a mitogen-inducible gene encoding a kappa B DNA-binding protein with homology to the rel oncogene and to cell-cycle motifs. Nature, 348: 76-80, 1990.
24) Bours V, Franzoso G, Azarenko V, Park S, Kanno T, Brown K, Siebenlist U: The oncoprotein Bcl-3 directly transactivates through kappa B motifs via association with DNA-binding p50B homodimers. Cell, 72: 729-739, 1993.
25) Nolan GP, Fujita T, Bhatia K, Huppi C, Liou HC, Scott ML, Baltimore D: The bcl-3 proto-oncogene encodes a nuclear I kappa B-like molecule that preferentially interacts with NF-kappa B p50 and p52 in a phosphorylation-depen-

dent manner. Mol Cell Biol, 13: 3557-3566, 1993.
26) Wulczyn FG, Naumann M, Scheidereit C: Candidate proto-oncogene bcl-3 encodes a subunit-specific inhibitor of transcription factor NF-kappa B. Nature, 358: 597-599, 1992.
27) Franzoso G, Bours V, Park S, Tomita-Yamaguchi M, Kelly K, Siebenlist U: The candidate oncoprotein Bcl-3 is an antagonist of p50/NF-kappa B-mediated inhibition. Nature, 359: 339-342, 1992.
28) Lenardo M, Siebenlist U: Bcl-3-mediated nuclear regulation of the NF-kappa B trans-activating factor. Immunol Today, 15: 145-147, 1994.
29) Franzoso G, Carlson L, Scharton-Kersten T, Shores EW, Epstein S, Grinberg A, Tran T, Shacter E, Leonardi A, Anver M, Love P, Sher A, Siebenlist U. Critical roles for the Bcl-3 oncoprotein in T cell-mediated immunity, splenic microarchitecture, and germinal center reactions. Immunity, 6: 479-490, 1997.
30) Schwarz EM, Krimpenfort P, Berns A, Verma IM: Immunological defects in mice with a targeted disruption in Bcl-3. Genes Dev, 11: 187-197, 1997.
31) Corn RA, Hunter C, Liou HC, Siebenlist U, Boothby MR. Opposing roles for RelB and Bcl-3 in regulation of T-box expressed in T cells, GATA-3, and Th effector differentiation. J Immunol, 175: 2102-2110, 2005.
32) Kuwata H, Watanabe Y, Miyoshi H, Yamamoto M, Kaisho T, Takeda K, Akira S. IL-10-inducible Bcl-3 negatively regulates LPS-induced TNF-alpha production in macrophages. Blood, 102: 4123-4129, 2003.
33) Carmody RJ, Ruan Q, Palmer S, Hilliard B, Chen YH: Negative regulation of toll-like receptor signaling by NF-κB p50 ubiquitination blockade. Science, 317: 675-678, 2007.
34) Franzoso G, Carlson L, Xing L, Poljak L, Shores EW, Brown KD, Leonardi A, Tran T, Boyce BF, Siebenlist U: Requirement for NF-κB in osteoclast and B-cell development. Genes Dev, 11: 3482-3496, 1997.