Mini Review

NF-κB activation pathway in thymic epithelial cells controls establishment of self-tolerance

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Thymic epithelial cells (TEC) play pivotal roles in the establishment of self-tolerance through critical dialogue with developing thymocytes. Unique actions of NF-κB activation pathway within TECs for the establishment of self-tolerance have recently been highlighted by studies using a strain of mouse bearing a natural mutation of the NF-κB-inducing kinase (NIK) gene (*aly* mice) and gene-targeted mice of IκB kinase α (IKKα). *NIK*-mutant strain manifests autoimmunity and disorganized thymic structure with abnormal expression of Rel proteins in the stroma. The autoimmune disease seen in *NIK*-mutant mice was reproduced in athymic nude mice by grafting embryonic thymus from *NIK*-mutant mice. Similarly, an autoimmune-disease phenotype was induced in nude mice by grafting embryonic thymus from IKKα-deficient mice. The thymic microenvironment that caused autoimmunity in NIK- and IKKα-dependent manner was associated with defective processing of NF-κB2, resulting in the impaired development of thymic epithelial cells. Thus, a novel function for NIK-IKKα pathway in thymic organogenesis for the establishment of central tolerance has emerged.

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Introduction

Physical contact between thymocytes and the thymic stroma is essential for T-cell maturation and shapes the T-cell repertoire in the periphery[1]. Stromal elements that control these processes still remain elusive. Recently, much attention has been paid to the epithelial-cell component of the stroma (i.e., thymic epithelial cells; TECs), since increasing numbers of mutant and gene-targeted mice bearing structural and/or functional TEC defects have been reported, many of which are actually associated with autoimmune disease phenotypes. In this mini-review, I will focus on two transcriptional regulators within TECs, NF-κB-inducing kinase (NIK) and IκB kinase α (IKKα), which together play crucial roles in the establishment of self-tolerance by maintaining the developmental integrity of TECs, thereby preventing the development of autoimmune disease (Fig.1).

NIK and IKKα

NIK is structurally related to mitogen-activated protein kinase

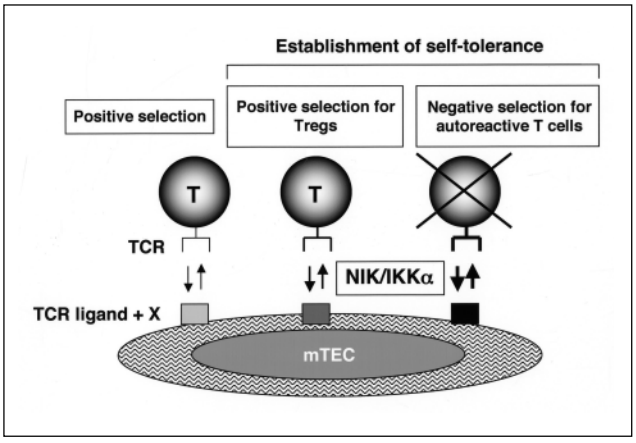

Fig.1 Transcriptional regulators, NIK and IKKα, within mTECs establish self-tolerance through critical dialogues with developing thymocytes

Two known mechanisms of self-tolerance, positive selection for Tregs and negative selection for autoreactive T cells, depending on the avidity of the interactions (depicted by the different depths of shading) between TCR and its self-ligands expressed on mTECs are shown. "X" denotes costimulatory pathways and/or undetermined molecules that might additionally contribute to this process.

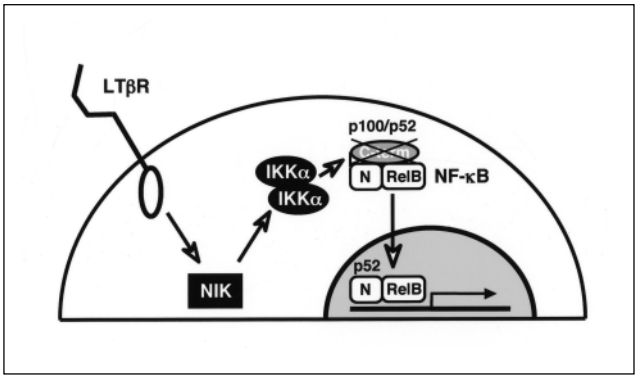

Fig.2 Alternative NF-κB activation pathway downstream of LTβR within TECs

LTβR ligation activates NIK by yet undetermined molecular mechanisms that result in the activation of IKKα homodimers. Activated IKKα catalyzes p100 of NF-κB2 through ubiquitin-dependent degradation of the C-terminus, and generates p52, a mature form of NF-κB2 consisting of the N-terminus. p52 coupled with RelB enters the nucleus to activate the target genes necessary for the differentiation of TECs. *NIK*-mutation in *aly* mice results in inability to bind to and activate IKKα.

kinase kinase (MAP3K)[2] and has been shown to phosphorylate both IKKα and IKKβ, which sequentially activate the downstream IκB proteins necessary for NF-κB activation[3]. The alymphoplasia (*aly*) strain of mouse carries a natural mutation of the *NIK* gene[4,5] in which a G855R substitution in the C-terminus of the protein results in inability to bind to IKKα [6] (Fig.2). *aly* mice have provided a unique model for the abnormal development of lymphoid organs, since they lack all lymph nodes and Peyer's patches, and development of spleen architectural features, such as germinal centers and follicular dendritic cell clusters, is disturbed[4,5,7]. This is due to defective NF-κB activation through the lymphotoxin (LT)-β receptor (LTβR)[5,6,8], which is essential for the development of secondary lymphoid organs[9]. Thymic structure is also disorganized in *aly* mice; the medulla in *aly* mice is smaller than that in control mice, and the boundary of the cortex and medulla is unclear[4,5,10,11]. Importantly, *aly* mice also serve as a model of autoimmune disease, but of unknown etiology[12]; histopathological analysis of *aly* mice has revealed chronic inflammatory changes in several organs, including the liver, pancreas, lung, salivary gland and lacrimal gland[4,11,12]. We reasoned that the autoimmune-disease phenotype seen in *aly* mice might be associated with the altered thymic microenvironment. This hypothesis was later proven to be correct, when thymic chimeras were generated (Fig.3A)[11]. *Aly* mouse thymus-grafted nude mice showed marked lymphoid cell infiltration in the liver (Fig.3D) and pancreas, accompanied by autoantibody production. Histological evaluation of the grafted thymus revealed that control thymus contained cells reactive with the lectin *Ulex europaeus* agglutinin 1 (UEA-1) (Fig.3C), whereas *aly* mouse embryonic thymus grafted into nude mice did not acquire UEA-1$^+$ cells (Fig.3E), suggesting that production of UEA-1$^+$ medullary epithelial cells requires normal NIK in the thymic stromal element. Similar results were obtained using embryonic thymus obtained from IKKα-deficient mice[13], indicating an essential function of IKKα in thymic stroma-dependent self-tolerance that cannot be compensated for by the related IKKβ subunit.

Although the exact mechanism by which NIK (and IKKα as well) regulates the thymic microenvironment required for the establishment of central tolerance is unknown, the disorganized thymic structure together with reduced *Aire* expression in mice with a mutation disrupting the *RelB* gene merits attention[14]. Because of the phenotypic similarities between *aly* mice and RelB-deficient mice[15] (multi-inflammatory lesions together with absence of UEA-1$^+$ medullary epithelial cells in the thymus), we speculate that NIK regulates the thymic microenvironment through activation of the NF-κB complex containing RelB. As

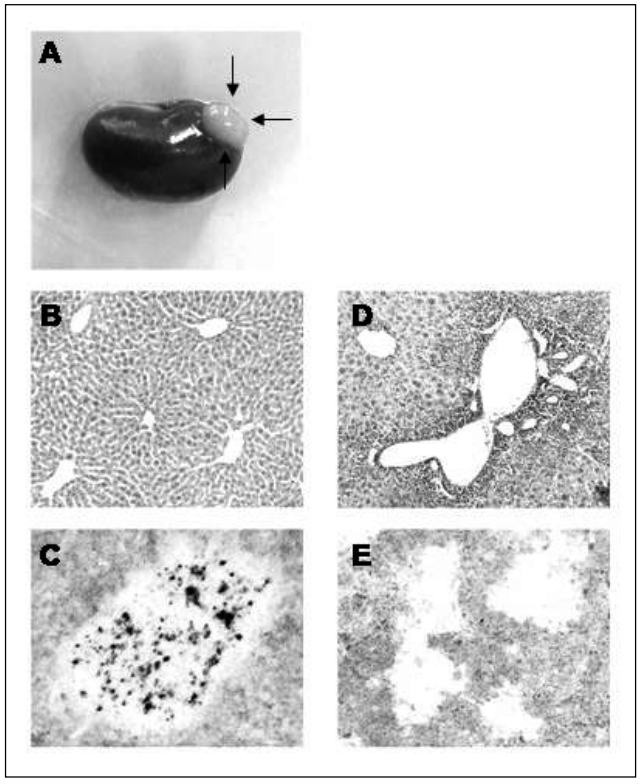

Fig.3 Thymic stromal elements in *NIK*-mutant mice are responsible for the development of autoimmunity

(A) Embryonic thymus grafted (arrows) that had developed under the renal capsule of recipient nude mice. Nude mice grafted with *NIK*-mutant mouse embryonic thymus (D), but not with control mouse embryonic thymus (B), developed an autoimmune-disease phenotype in the liver. Thymic medullas from *NIK*-mutant mice contain no UEA-1$^+$ cells (E) (stained in black in control mice; C).

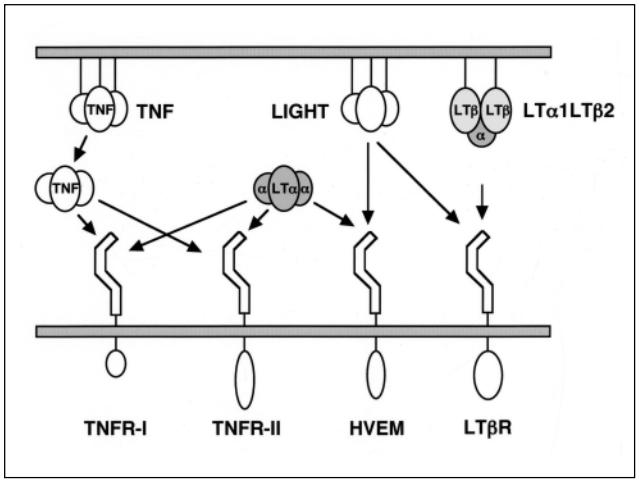

Fig.4 Ligand and receptor interactions of LT and TNF

Three distinct ligand/receptor pathways exist as follows; 1) TNF/LTα3-TNFR (type I and II), 2) LTα3/LIGHT-HVEM (*Herpes Virus Entry Mediator*) and 3) LTα1LTβ2/LIGHT-LTβR.

production of NF-κB2 (p52) is impaired in both *aly* mouse thymic stroma and IKKα-deficient thymic stroma, and NIK has been shown to be necessary for the production of natural killer T cells through the action of RelB[16,17], it is reasonable to speculate that the NIK-IKKα-related signaling pathway(s) activates the NF-κB complex in the thymic stroma consisting mainly of p52/RelB heterodimers to generate the thymic microenvironment (Fig.2). Indeed, recent two studies have demonstrated that NF-κB2 controls thymic organogenesis, thereby maintaining centrals tolerance, although there is some difference in the mechanistic explanation between the two[18,19].

LTβR expressed on TECs

Although NIK-IKKα is an essential component downstream of LTβR[5,6], and *aly* mice show structural abnormality of the thymus[4,5,10,11], the finding that LTβR-deficient mice have a disorganized thymic structure was somewhat surprising[20], because none of the preceding reports on single-gene knockout mice, either for the membrane-bound LT component (i.e., LTα and LTβ) or LIGHT (two known ligands for LTβR[21]) (Fig.4), referred to the thymic phenotypes in these mice[9,22,23]. In fact, deficiency of LTα alone was not accompanied by any obvious changes in thymic structure that would have resulted in reduced *Aire* expression at both the transcriptional[11] and protein levels (unpublished observation). LTβR-deficient mice show marked reduction of UEA-1$^+$ cells caused by both loss of the characteristic three-dimensional organization and a reduction in the absolute number of epithelial cells[20]. In contrast, LTβ-deficient mice show no significant reduction in the total mass of medullary TECs (mTECs), although changes in the UEA-1$^+$ cell distribution pattern have been pointed out. Introduction of LIGHT deficiency in LTβ-deficient mice resulted in no additional deterioration. Interestingly, LTβ/LIGHT double-deficient mice (lack of both membrane-bound LT and LIGHT) showed less severe thymic disorganization than LTβR-deficient mice, suggesting that LTβR might have additional ligand(s) other than membrane-bound LT and LIGHT[20,24]. Alternatively, in the light of the fact that LTβ-deficient mice show less profound phenotypes of lymph-node genesis (i.e., presence of mesenteric lymph nodes) compared with LTα-deficient mice[25,26], it is possible that LTα/LIGHT double-

Table 1 Autoimmune pathogenesis in mice deficient for NIK and IKKα in TEC

	NIK-mutant mice	IKKα-deficient mice
Thymic structure assessed by immunohistochemistry	Defective development of mTECs	Defective development of mTECs in grafted thymus
Thymic self-antigen gene expression detected by RT-PCR	Dramatically reduced in total thymus, but not in individual TECs	Not assessed
Production and function of Tregs	Reduced, but functionally competent	Not assessed
NF-κB2 processing in TECs	Defective	Defective
Possible mechanisms for the breakdown of central tolerance	Defective NF-κB activation required for the development of mTECs	Defective NF-κB activation required for the development of mTECs

deficient mice might show quite equivalent thymic phenotypes to those of LTβR-deficient mice.

LTβR-deficient mice show some signs of autoimmunity; their serum contains autoantibodies against several organs (i.e., stomach, pancreas and salivary gland)[20]. A role of LTβR in mTEC development seems to be a likely explanation for the autoimmune phenotypes of LTβR-deficient mice, similar to seen in both *aly* mice and IKKα-deficient mice (see below for further discussion).

LTβR signaling

NIK-IKKα constitutes an essential component downstream of LTβR for secondary lymphoid organogenesis[6]. It is therefore reasonable to speculate that NIK-IKKα also plays important roles in thymic organogenesis through the action of LTβR signaling. However, given that *aly* mice show more profound reduction and disorganization of mTECs than LTβR-deficient mice[20], it is possible that in this process NIK-IKKα is additionally acting downstream of other receptor(s) beyond LTβR. One hint in the search for such receptor(s) involved in NIK-IKKα-dependent thymic organogenesis is impaired processing of NF-κB2 in thymic stroma from *aly* mice[11] and IKKα-deficient mice[13]. This alternative NF-κB activation pathway[27,28] was originally demonstrated in hemopoietic cells from *aly* mice[7], and subsequently characterized for LTβR[29] (Fig.2). Another signal that involves the generation of p52 from a precursor p100 might represent an additional NIK-IKKα-dependent pathway that could fill the gaps of thymic phenotypes between *aly* mice and LTβR-deficient mice.

NF-κB activation within TECs and autoimmunity

It is now clear that LTβR/NIK-IKKα is not the only NF-κB-activating axis that regulates thymic organogenesis. TRAF6 (tumor necrosis factor receptor (TNFR)-associated factor 6) has also been demonstrated to be essential for organization of the thymic microenvironment[30]. TRAF6, an adaptor molecule that transduces signals from members of the TNF superfamily and Toll/IL-1 receptor family, activates NF-κB and activating protein 1 (AP1)[31]. Similarly to *aly* mice and IKKα-deficient mice, TRAF6-deficient thymus-grafted nude mice show marked lymphoid cell infiltration in multiple organs. In contrast to *aly* mice and IKKα-deficient mice, however, NF-κB2 processing in TECs from TRAF6-deficient mice is not impaired. Instead, RelB expression in TECs is severely reduced. TRAF6-dependent RelB expression has been confirmed by the recovery of RelB expression following the introduction of TRAF6 into TRAF6-deficient TECs. Thus, deficiency of NIK/IKKα and TRAF6 merges at the point where p52/RelB complex formation is disturbed, although this does not mean that NIK/IKKα and TRAF6 cooperate together within TECs in order for this heterodimeric complex to be formed. Rather, NIK/IKKα and TRAF6 probably regulate NF-κB activation independently in this process, because TRAF6 deficiency does not affect NF-κB activation downstream of LTβR[30]. The upstream receptor(s) responsible for TRAF6-dependent thymic organogenesis is currently unknown.

In addition to negative selection, self-tolerance is maintained by another mechanism involving immunoregulatory T cells (Treg)[32], and *Foxp3*, a transcription factor that is genetically defective in an autoimmune disease known as IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome), is a key regulator for the development of Tregs[33-35]. *aly* mice[11] and TRAF6-deficient mice[30], both of which show abnormal development of TECs, have reduced numbers of Tregs. Requirement of NIK/TRAF6 for the production of Tregs might provide a clue as to how the production of Tregs is controlled

through the interactions with TECs, as illustrated in Fig.1.

Concluding remarks

Table 1 shows a general phenotypic comparison between *NIK*-mutant *aly* mice and IKKα-deficient mice. Autoimmune disease is a pathological condition in which the immune system turns on itself and causes serious damage to host tissues through as yet unknown mechanisms[36]. Breakdown of self-tolerance is considered to be the key event in initiating the disease process, and an understanding the pathogenesis involved is crucial for developing a suitable therapeutic approach. For this reason, it is essential to know how self-tolerance is established within the organized thymic microenvironment. With the advent of thymic organogenesis using thymic precursor cells[37,38], it may be feasible to manipulate the thymic microenvironment through the modulation of NF-κB activation pathways, thereby controlling the processes for the establishment of self-tolerance.

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