Mini Review

RANKL signaling regulates the development of the immune system and immune tolerance

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The receptor activator of the nuclear factor κB (NF-κB) ligand (RANKL), which is a member of the tumor necrosis factor (TNF) family, is crucial for the development and regulation of the immune system and also for bone formation. Previous analyses conducted on RANKL- or RANK-deficient mice have revealed that these molecules play an essential role in the development of lymph nodes but not Peyer's patches. Although the details of the mechanism by which RANKL signaling controls lymph node development have not been elucidated, it is known that this mechanism differs from that by which signals are transduced from lymphotoxin, which is another TNF family member that performs the same function. We and other researchers have recently found that RANKL signaling is crucial for the establishment of self-tolerance and the suppression of excessive immune responses. RANKL expression in keratinocytes was found to increase with ultraviolet (UV) irradiation or inflammation. Upregulated RANKL expression activates the epidermal dendritic cells (DCs) and consequently induces the proliferation of regulatory T cells, which in turn suppress autoimmune reactions. In addition, we recently found that RANKL signaling controls the development of medullary thymic epithelial cells, which play a critical role in negative selection in the thymus. The signaling pathways of the TNF receptors RANK and CD40 seem to partially overlap. These findings suggest that functional redundancies may also exist among the signaling pathways of TNFs involved in other physiological phenomena.

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Introduction

Proteins of the tumor necrosis factor (TNF) family regulate various physiological functions, including immune responses, organ development, and hematopoiesis[1]. Interactions between these proteins and their respective cognate receptors activate intracellular signaling pathways that regulate the transcriptional activation of the nuclear factor κB (NF-κB) and activator protein (AP)-1 as well as the activation of the caspase cascade[1]. Thus, proteins of the TNF family control the proliferation, survival, differentiation, and apoptosis of their target cells[1].

The receptor activator of NF-κB (RANK) and its ligand (RANKL) belong to the TNF receptor family and the TNF family respectively[2,3]. These molecules were originally identified as a cytokine and its receptor and they were considered to func-

tion in combination to enhance the survival of conventional dendritic cells (DCs)[2,3]. RANKL- and RANK-deficient mice were generated in order to determine the physiological role played by RANKL signaling[2,3]. Unexpectedly, the RANKL-deficient mice displayed no detectable abnormalities in the proportion and number of DCs in the lymphoid tissues. Instead, these mice exhibited a severe defect in bone formation, and this defect was attributed to strong inhibition of osteoclast differentiation[2-4]. Recombinant RANKL can induce the differentiation of osteoclasts from their precursor cells in the absence of osteoblasts, which are the main source of RANKL both in vivo and in *in vitro* coculture systems. Current research endeavors are rapidly uncovering the molecular mechanism underlying osteoclast differentiation, and the relevant studies have been reviewed extensively[2-4]. In brief, the binding of RANKL to RANK activates NF-κB and AP-1, and this in turn activates the expression of the nuclear factor of activated T cells (NF-ATc)1, which is a master regulator of osteoclast differentiation. This signal transduction requires TNF receptor-associated factor 6 (TRAF6). In addition to its role in osteoclast differentiation, RANKL signaling has been found to play an essential role in organ development and immune regulation. In this review, we focus on the functions of RANKL signaling in the development and regulation of the immune system, as have been uncovered by us and other researchers[5-8].

RANKL signaling regulates lymph node development

One of the most striking phenotypes observed in RANKL-deficient mice is the absence of lymph nodes[2,3]. Despite this deficit, these mice exhibit normal development of Peyer's patches. Interestingly, in contrast to mice deficient in RANKL, those deficient in lymphotoxin signaling-related molecules such as lymphotoxin α (Ltα or lymphotoxin β receptor (LtβR) lack both lymph nodes and Peyer's patches[9]. Moreover, overexpression of transgenic RANKL does not reverse the lymph node deficit in Ltα-deficient mice[10]. These reports indicate that RANKL and lymphotoxin signaling regulate the development of lymph nodes via 2 distinct mechanisms. This hypothesis was supported by the finding that a deficiency of TRAF6, a critical signal transducer for RANKL-dependent osteoclast formation, prevents the development of lymph nodes but not Peyer's patches in mice[11,12]. Furthermore, TRAF6 is not required for the transcriptional activation induced by LtβR signaling in fibroblast cell cultures[13]. These data indicate that the RANKL-RANK-TRAF6 axis controls the development of lymph nodes but not Peyer's patches. Several studies have suggested the involvement of RANKL signaling in the early stage of development wherein lymphoid tissue inducer and organizer cells interact and thus initiate lymph node development[9]. However, since these cells are very few in the lymphoid tissues of mice, the details of the molecular mechanism by which RANKL signaling regulates lymph node development in mice remain to be determined.

RANKL signaling controls the development of regulatory T cells

Foxp3-positive regulatory T (Treg) cells are crucial for maintaining self-tolerance and suppressing excessive immune responses[14]. The generation and proliferation of Treg cells require interactions between the T-cell antigen receptor and the MHC class II peptide[14]. Thus, the functional maturation or activation of MHC class II-positive antigen-presenting cells, e.g., DCs, probably plays a key role in the development and proliferation of Treg cells in the thymus and peripheral organs. However, the details of the concerned process have not been elucidated. It has been reported that RANKL signaling regulates the proliferation of Treg cells in the peripheral organs via activation of the epidermal Langerhans cells (LCs)[8]. LCs are crucial for immune responses to epidermal infections. After engulfing an antigen in the epidermis, LCs migrate to the lymph nodes to activate T cells. Loser et al. found that RANKL expression in keratinocytes is markedly upregulated after UV irradiation or inflammation due to psoriasis[8]. To assess the physiological significance of RANKL expression in the skin, mice expressing transgenic RANKL driven by the keratin 14 promoter (K14-RANKL Tg mice)[8] were also generated. As result, they found that the number of Treg cells in the skin and lymph nodes markedly increased in these mice. The expression levels of CD86 and CD205 in the DCs were also elevated. Furthermore, transfer of RANKL-stimulated DCs induced Treg cell proliferation in the peripheral organs of the mice. Interestingly, the K14-RANKL Tg mice significantly improved skin inflammation in autoimmune mice. Thus, RANKL expression in the epidermal keratinocytes might regulate the activation and maintenance of immunosuppressive LCs that control the number of Treg cells (Fig.1).

RANKL signaling regulates the microenvironment of the thymus

Self-tolerant T cells and Treg cells are produced in the thymus[6]. Several lines of evidence have indicated that the microenvironment of the thymic medulla is crucial for the establishment of central tolerance in the thymus (Fig.2)[6]. This microenvironment contains radiation-resistant stromal cells and cells of hemato-

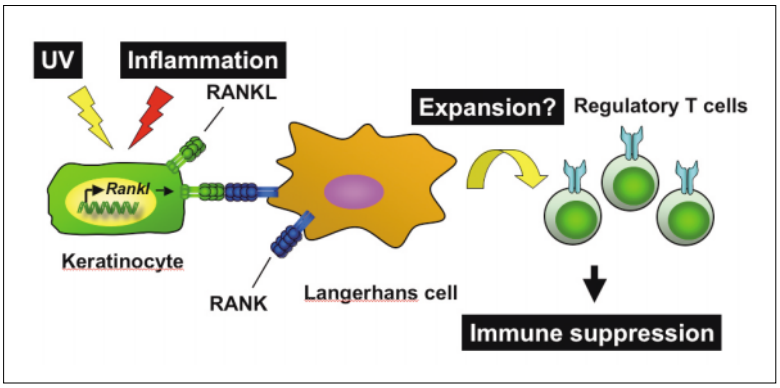

Fig.1 RANKL signaling activates epidermal DCs and thus induces the proliferation of regulatory T cells in the peripheral organs

UV irradiation or inflammation induces the expression of RANKL in keratinocytes. RANKL then activates the epidermal Langerhans cells (LC). Finally, the RANKL-stimulated LCs induce the proliferation of regulatory T cells in the peripheral organs.

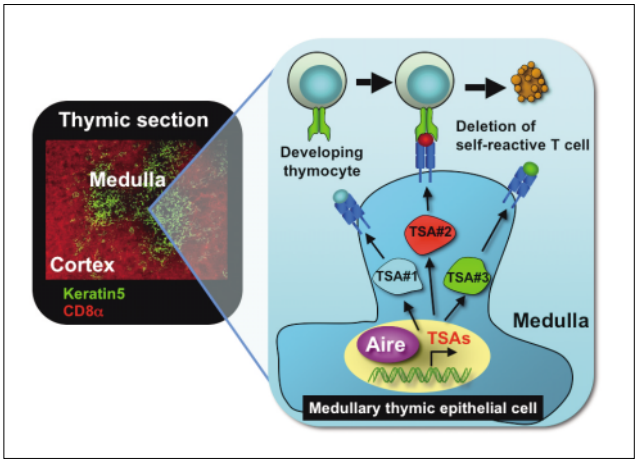

Fig.2 Negative selection is controlled by the medullary thymic epithelial cells

Left: Medullary thymic epithelial cells (mTECs) are localized in the thymic medulla. The thymic section shown was stained with anti-keratin-5 (green) to detect the medulla and with anti-CD8α (red) to detect the cortex. Right: The mTECs express peripheral tissue-specific antigens (TSA #1, #2, #3, etc.) and present them to developing thymocytes in order to eliminate self-tissue-specific T cells. The autoimmue regulator (Aire) possibly controls the expression of TSAs.

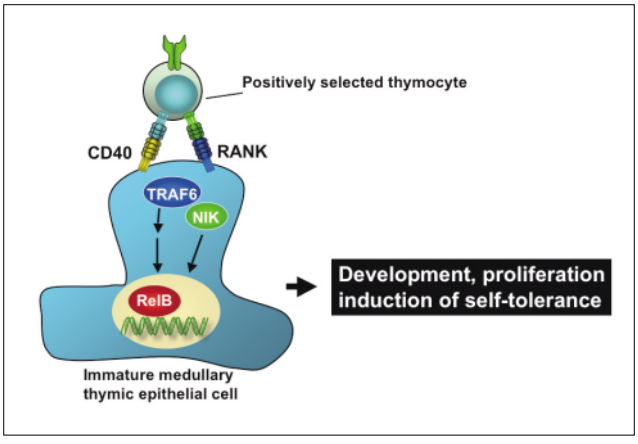

Fig.3 mTEC development is regulated by an interplay between RANK and CD40 signaling

RANK and CD40 cooperatively regulate the development of mTECs, which induce self-tolerance. The signals released by these molecules activate the signal transducers TRAF6 and NIK, and this in turn induces the translocation of RelB, a member of the NF-κB family.

poietic origin. Medullary thymic epithelial cells (mTECs), which are a subset of the radiation-resistant stromal cells localized in the thymic medulla, exhibit the unique property of "promiscuous" expression, i.e., ectopic expression of peripheral tissue-specific antigens (TSAs)[6]. It has been suggested that mTECs present TSAs and eliminate self-organ-specific T cells from the thymus, thus establishing self-tolerance (Fig.2)[6]. This hypothesis is supported by the functions of the autoimmune regulator (Aire), a molecule reported to be responsible for the development of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), which is a human autoimmune disease. Aire is strongly expressed in the nucleus of mTECs. Investigations performed on Aire-deficient mice have revealed that the expression of some TSAs is dependent on Aire. Furthermore, the autoim-

mune phenotype of Aire-deficient mice has been attributed to the lack of Aire in the thymic stroma[15]. These previous studies have delineated a potential mechanism of mTEC-dependent self-tolerance, wherein Aire regulates the promiscuous expression of TSAs.

The molecular mechanism underlying the maturation of Aire- and TSA-positive mTECs in the thymus has remained elusive. In previous studies, mice deficient in RelB or both p52 and p50 exhibited a severe defect in mTEC maturation; these findings indicate that NF-κB activation is essential for the maturation of mTECs[16,17]. The binding of TNF or Toll/interleukin (IL)-1 receptors to their respective targets activates members of the TRAF family and thus induces NF-κB activation. Among members of the TRAF family, TRAF6 is known to regulate the activation of the classical NF-κB activation pathway, in which the RelA/p50 complex is activated via IκB degradation. We previously reported that TRAF6 plays a critical role in mTEC development[18]. Another research group found that NF-κB-inducing kinase (NIK) is also essential for the signal transduction involved in mTEC development[19]. NIK is known to activate the non-classical NF-κB activation pathway, wherein the RelB/p52 complex is activated by partial degradation of p100 to p52. Therefore, the 2 NF-κB activation pathways regulated by TRAF6 and NIK are probably required for mTEC development.

The receptors that are activated upstream of TRAF6 and NIK and are involved in mTEC development have not been identified[6]. Preliminary studies have suggested that LtβR signaling is required for the expression of Aire in mTECs. However, subsequent intensive studies have revealed that LtβR signaling is minimally associated with Aire expression. The expression of transgenic CD40L is reported to cause enlargement of the thymic medulla. In addition, flow cytometric analysis has revealed that mTEC development is mildly impaired in CD40L-deficient mice. Anderson et al. recently found that RANKL signaling is involved in the expression of Aire in mTECs[20]. However, they found that mTEC development was almost completely unaffected by the absence of RANK. At least 3 TNF receptors, namely, LtβR, CD40, and RANK, appear to be involved in the development of mTECs. However, none of the mice deficient in any of these molecules were found to exhibit a severe defect in mTEC development as is observed in the case of mice lacking TRAF6 or some other molecule involved in NF-βB activation. Since the signals produced by LtβR, CD40, and RANK all induce NF-κB activation, it is possible that the roles of these receptors in mTEC development overlap to some extent.

In a recent study, we identified the some of the overlapping functions of these TNF receptors in mTEC development[5,7]. We generated RANKL/CD40 double-knockout (RANKL/CD40 DKO) mice. We found that these mice almost completely lacked mTECs expressing Aire and TSAs in the thymus[5]. Moreover, the phenotype of these mice was comparable with those of TRAF6-deficient mice, RelB-deficient mice, and *aly/aly* mice, all of which carry a functional mutation in the gene encoding NIK. Furthermore, stimulation of the fetal thymic stroma, which contains immature mTECs, with recombinant RANKL or CD40L induces mTEC maturation[5]; this induction is completely dependent on the expression of TRAF6 and functional NIK. In addition, stimulation of the fetal thymic stroma with RANKL or CD40 activates RelB, and this activation is dependent on the expression of TRAF6 and NIK[5]. Thus, it is possible that interplay between RANK and CD40 signaling induces mTEC development via TRAF6- and NIK-dependent signaling, and this in turn activates the expression of RelB and the 2 NF-κB activation pathways (Fig.3).

Perspective

RANKL signaling plays a role in establishing the microenvironment of 2 primary lymphoid organs — the bone marrow and thymus — by regulating the development of osteoclasts and mTECs. It remains to be determined whether interactions between RANKL and RANK activate the same intracellular signaling pathway in both osteoclasts and mTECs. During osteoclast differentiation, the binding of RANKL to RANK activates NFATc1; this activation is dependent on the expression of NF-κB and AP-1[4]. Although c-fos critically regulates osteoclast formation[4], it does not to seem to function similarly for mTECs. More intensive studies are necessary to clarify the differences in the pathway that induces RANKL signaling in osteoclasts and mTECs.

The molecular mechanism underlying the ectopic expression of TSAs in mTECs has not been elucidated. RANKL signaling appears to induce the expression of almost all TSAs in mTECs[6]. However, it is unlikely that RANKL directly targets the genes encoding all the TSAs. Thus, determining the molecular mechanism underlying the regulation of RANKL-induced TSA expression could be an important focus area for research on immune tolerance.

References

1) Aggarwal BB: Signalling pathways of the TNF superfamily: a double-edged sword. Nat Rev Immunol, 3: 745-756, 2003.
2) Theill LE, Boyle WJ, Penninger JM: RANK-L and RANK:

T cells, bone loss, and mammalian evolution. Annu Rev Immunol, 20: 795-823, 2002.
3) Walsh MC, Kim N, Kadono Y, Rho J, Lee SY, Lorenzo J, Choi Y: Osteoimmunology: interplay between the immune system and bone metabolism. Annu Rev Immunol, 24: 33-63, 2006.
4) Takayanagi H: Osteoimmunology: shared mechanisms and crosstalk between the immune and bone systems. Nat Rev Immunol, 7: 292-304, 2007.
5) Akiyama T, Shimo Y, Yanai H, Qin J, Ohshima D, Maruyama Y, Asaumi Y, Kitazawa J, Takayanagi H, Penninger JM, Matsumoto M, Nitta T, Takahama Y, Inoue J: The tumor necrosis factor family receptors RANK and CD40 cooperatively establish the thymic medullary microenvironment and self-tolerance. Immunity, 29: 423-437, 2008.
6) Anderson G, Lane PJ, Jenkinson EJ: Generating intrathymic microenvironments to establish T-cell tolerance. Nat Rev Immunol, 7: 954-963, 2007.
7) Hikosaka Y, Nitta T, Ohigashi I, Yano K, Ishimaru N, Hayashi Y, Matsumoto M, Matsuo K, Penninger JM, Takayanagi H, Yokota Y, Yamada H, Yoshikai Y, Inoue J, Akiyama T, Takahama Y: The cytokine RANKL produced by positively selected thymocytes fosters medullary thymic epithelial cells that express autoimmune regulator. Immunity, 29: 438-450, 2008.
8) Loser K, Mehling A, Loeser S, Apelt J, Kuhn A, Grabbe S, Schwarz T, Penninger JM, Beissert S: Epidermal RANKL controls regulatory T-cell numbers via activation of dendritic cells. Nat Med, 12: 372-1379, 2006.
9) Mebius RE: Organogenesis of lymphoid tissues. Nat Rev Immunol, 3: 292-303, 2003.
10) Kim D, Mebius RE, MacMicking JD, Jung S, Cupedo T, Castellanos Y, Rho J, Wong BR, Josien R, Kim N, Rennert PD, Choi Y: Regulation of peripheral lymph node genesis by the tumor necrosis factor family member TRANCE. J Exp Med, 192: 1467-1478, 2000.
11) Lomaga MA, Yeh WC, Sarosi I, Duncan GS, Furlonger C, Ho A, Morony S, Capparelli C, Van G, Kaufman S, van der Heiden A, Itie A, Wakeham A, Khoo W, Sasaki T, Cao Z, Penninger JM, Paige CJ, Lacey DL, Dunstan CR, Boyle WJ, Goeddel DV, Mak TW: TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. Genes Dev, 13: 1015-1024, 1999.
12) Naito A, Azuma S, Tanaka S, Miyazaki T, Takaki S, Takatsu K, Nakao K, Nakamura K, Katsuki M, Yamamoto T, Inoue J: Severe osteopetrosis, defective interleukin-1 signalling and lymph node organogenesis in TRAF6-deficient mice. Genes Cells, 4: 353-362, 1999.
13) Qin J, Konno H, Ohshima D, Yanai H, Motegi H, Shimo Y, Hirota F, Matsumoto M, Takaki S, Inoue J, Akiyama T: Developmental stage-dependent collaboration between the TNF receptor-associated factor 6 and lymphotoxin pathways for B cell follicle organization in secondary lymphoid organs. J Immunol, 179: 6799-6807, 2007.
14) Sakaguchi S, Yamaguchi T, Nomura T, Ono M: Regulatory T cells and immune tolerance. Cell, 133: 775-787, 2008.
15) Anderson MS, Venanzi ES, Klein L, Chen Z, Berzins SP, Turley SJ, von Boehmer H, Bronson R, Dierich A, Benoist C, Mathis D: Projection of an immunological self shadow within the thymus by the aire protein. Science, 298: 1395-1401, 2002.
16) Franzoso G, Carlson L, Xing L, Poljak L, Shores EW, Brown KD, Leonardi A, Tran T, Boyce BF, Siebenlist U: Requirement for NF-kappaB in osteoclast and B-cell development. Genes Dev, 11: 3482-3496, 1997.
17) Weih F, Carrasco D, Durham SK, Barton DS, Rizzo CA, Ryseck RP, Lira SA, Bravo R: Multiorgan inflammation and hematopoietic abnormalities in mice with a targeted disruption of RelB, a member of the NF-kappa B/Rel family. Cell, 80:331-340, 1995.
18. Akiyama, T., S. Maeda, S. Yamane, K. Ogino, M. Kasai, F. Kajiura, M. Matsumoto, and J. Inoue: Dependence of self-tolerance on TRAF6-directed development of thymic stroma. Science 308:248?251, 2005.
19) Kajiura F, Sun S, Nomura T, Izumi K, Ueno T, Bando Y, Kuroda N, Han H, Li Y, Matsushima A, Takahama Y, Sakaguchi S, Mitani T, Matsumoto M: NF-kappa B-inducing kinase establishes self-tolerance in a thymic stroma-dependent manner. J Immunol, 172: 2067-2075, 2004.
20) Rossi SW, Kim MY, Leibbrandt A, Parnell SM, Jenkinson WE, Glanville SH, McConnell FM, Scott HS, Penninger JM, Jenkinson EJ, Lane PJ, Anderson G: RANK signals from CD4(+)3(-) inducer cells regulate development of Aire-expressing epithelial cells in the thymic medulla. J Exp Med, 204: 1267-1272, 2007.