

# The TNF receptor and Ig superfamily members form an integrated signaling circuit controlling dendritic cell homeostasis

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## Abstract

Dendritic cells (DC) constitute the most potent antigen presenting cells of the immune system, playing a key role bridging innate and adaptive immune responses. Specialized DC subsets differ depending on their origin, tissue location and the influence of trophic factors, the latter remain to be fully understood. Myeloid-associated lymphotoxin- $\beta$  receptor (LT $\beta$ R) signaling is required for the local proliferation of lymphoid tissue DC. This review focuses on the LT $\beta$ R signaling cascade as a crucial positive trophic signal in the homeostasis of DC subsets. The noncanonical coreceptor pathway comprised of the immunoglobulin (Ig) superfamily member, B and T lymphocyte attenuator (BTLA) and TNFR superfamily member, herpesvirus entry mediator (HVEM) counter regulates the trophic signaling by LT $\beta$ R. Together both pathways form an integrated signaling circuit achieving homeostasis of DC subsets.

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**Keywords:** Dendritic cells; Homeostasis; TNF superfamily; Cosignaling; Lymphotoxin; Herpesvirus entry mediator

## 1. Dendritic cells

Dendritic cells (DC) originate from hematopoietic precursors and can be divided in several subsets depending on their anatomical location, surface phenotype and function. For example, two broad classes of DC are the peripheral migratory DC and the lymphoid tissue-resident DC [1]. The migratory DC, i.e. the dermal DC and Langerhans cells, can be considered as sentinels of the immune system as they form a sensing barrier in peripheral tissues at the interface with environment. The lymphoid tissue-resident DC include the splenic and thymic DC.

*Abbreviations:* *aly*, alymphoplasia; BTLA, B and T lymphocyte attenuator; HVEM, herpesvirus entry mediator; LIGHT, LT-related inducible ligand that competes for glycoprotein D binding to HVEM on T cells; LT, lymphotoxin; NIK, NF- $\kappa$ B inducing kinase; I $\kappa$ B, inhibitor of  $\kappa$ B; IKK, I $\kappa$ B kinase.

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Dendritic cells are specialized in the capture, transport, processing and presentation of antigens. In the presence of a microbial stimulus, these steps are associated with further DC differentiation (maturation) characterized by upregulation of costimulatory and major histocompatibility complex (MHC) molecules. As DC become activated they migrate to or within secondary lymphoid organs, leading ultimately to their interaction with T cells and the initiation of adaptive immune responses or tolerance depending of their state of activation [2].

In the mouse, resident DC within lymphoid organs can be further divided based on the expression of B220, CD4, CD8 $\alpha$ , CD11b, CD11c, CD24 (HSA) and CD205 (DEC-205). For example, three main subsets of CD11c<sup>hi</sup> expressing DC are present in the mouse spleen, the CD8 $\alpha$ + DC subset and the CD8 $\alpha$ - DC subset. This latter is further divided into the CD4+ and the CD8 $\alpha$ -CD4- dual negative DC subpopulations [3]. These three subsets are referred in the literature as spleen conventional DC. A fourth DC subset, the plasmacytoid DC express low levels of CD11c and the B220 markers and are also resident in the

spleen [4]. The plasmacytoid DC subset is specialized in the secretion of type I interferons in response to viral and bacterial pathogens. In draining lymph nodes, two additional DC subsets of migratory DC can be identified as CD11c<sup>lo</sup> MHCII<sup>hi</sup> and CD11c<sup>hi</sup> MHC<sup>lo</sup>. The CD11c<sup>hi</sup> CD8 $\alpha$ – DC are preferentially located in the marginal zone, and the subcapsular sinus of the lymph nodes. They secrete mostly IL-10 but only secrete low level of IL-12(p70), and are proposed to drive the development of T helper type 2 (Th2) immune responses [5]. By contrast, CD11c<sup>hi</sup> CD8 $\alpha$ + DC are located in the T cell rich areas of the spleen and lymph nodes, possess a high capacity to secrete IL-12(p70), and stimulate the development of Th1 responses [5]. They are also the most efficient DC subset to crosspresent particulate or soluble antigens as well as dying cells to CD8+ T cells *in vivo* [2].

Both spleen and lymph node lymphoid tissue-resident DC possess a half-life of about three days. Despite their rapid turnover, little is known about the factors controlling immediate precursors and homeostasis *in vivo*. In earlier studies, CD8 $\alpha$  expression was used to distinguish between “lymphoid” and “myeloid” DC. However, more recent experiments showed that different DC subsets could differentiate from both lymphoid and myeloid precursors [6,7]. CD8 $\alpha$  marker remains very useful to discriminate between subsets, but no longer defines a subset origin. Recently, the group of Shortman provided *in vivo* and *in vitro* evidence for the development of the conventional and plasmacytoid DC lineages [8,9]. The culture of mouse bone marrow cells with the ligand for the cytokine receptor Fms-like tyrosine kinase 3 (Flt3; also known as Flk2) (FLT3L) can produce DC resembling to the splenic lymphoid-tissue-resident plasmacytoid DC, and CD8 $\alpha$ + and CD8 $\alpha$ – conventional DC subsets *in vivo*. Commitment to particular DC subtypes begins mainly at the pro-DC stage. Immediate DC precursor of conventional DC can be identified in the bone marrow and lymphoid organs [10].

The cytokine FLT3L is also essential for the development and homeostasis pDC and cDC *in vivo*. Mice genetically deficient for this cytokine possess low levels of DC, similar to mice lacking STAT3 (signal transducer and activator of transcription 3), which is an important signaling molecule in the FLT3L pathway [11]. The differentiation of bone marrow cells into DC can also be achieved by granulocyte/macrophage colony-stimulating factor (GM-CSF; also known as CSF2) into cultures. However, mice deficient in GM-CSF or its receptor exhibit normal DC levels, suggesting that it does not play a critical role in the maintenance of DC subset development and homeostasis *in vivo* [12]. Together, these results revealed considerable plasticity of the mechanisms controlling DC subset development and homeostasis *in vivo*.

In this review, we will summarize the emerging molecular and cellular evidence on the crucial role of different tumor necrosis factor receptor (TNFR) superfamily members and their associated signaling pathways in

the regulation of steady state DC subtypes equilibrium *in vivo*.

## 2. Regulation of DC homeostasis by the TNFR superfamily

Lymphotoxins (LT), LT $\alpha$ , LT $\beta$  and TNF form a signaling circuit with shared receptors and ligands [13] (Fig. 1). Lymphotoxins are best known in their control of the development and the homeostasis of secondary lymphoid organs, in part by regulating chemokine expression in stromal cells. Both homotrimeric TNF and LT $\alpha$  interact with TNFR1 and TNFR2, whereas the LT $\beta$  receptor (LT $\beta$ R) binds at least two ligands, the membrane heterotrimer LT $\alpha$  $\beta$  $\beta$  and LIGHT (LT-related inducible ligand that competes for glycoprotein D binding to herpesvirus entry mediator on T cells) [14]. LIGHT also engages the herpes virus entry mediator (HVEM; TNFRS14). The LIGHT–HVEM interaction appears to induce a positive signal during T cell activation, as do its TNFR paralogs (e.g., 4-1BB, OX40, CD27) [15].

Earlier studies showed that mice deficient for *lt $\beta$ r* or its ligands *lt $\alpha$*  or *lt $\beta$*  possess reduced levels of DC in lymphoid tissues [16,17]. However, that defect was previously assumed to be a deficit in DC migration due to the disrupted architecture and the loss of chemokine expression in these mice.

Surprisingly, observations made Cyster and co-workers discovered that intrinsic LT $\beta$ R signaling in hematopoietic cells provided trophic signals necessary for the proliferation CD8 $\alpha$ – DC subsets in spleen and lymph node, independent of chemokine expression [18,19]. Moreover, the number of DC in *plt* (paucity of lymph node T cells) mice, lacking both CCL19 and CCL21 chemokines, was comparable to wild-type mice and required LT $\beta$ R signaling [20]. The lack of

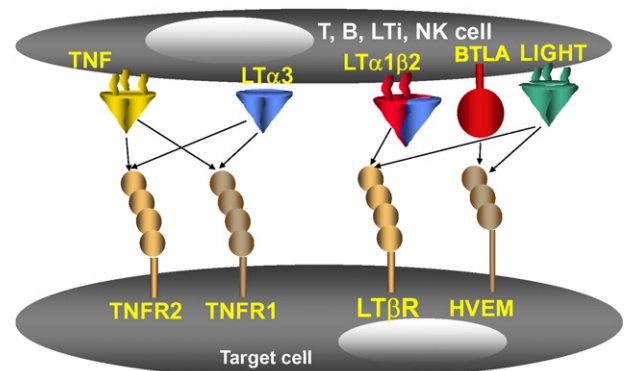


Fig. 1. Immediate TNF/LT family integrated signaling. Both homotrimeric TNF and soluble LT $\alpha$  $\beta$  interact with TNFR1 and TNFR2. LT $\beta$ R binds two ligands, the membrane LT $\alpha$  $\beta$  $\beta$  and LIGHT, yet LIGHT can also engage the herpes virus entry mediator. HVEM interacts with BTLA, a member of the Ig superfamily. The arrowed lines indicate the binding interactions among the members. Decoy receptor-3 (Dcr3) is secreted and binds LIGHT (also Fas ligand and TL1A, not shown).

TNF, which also decreases the expression of CXCL13, does not alter DC subsets *in vivo*, but participates in DC generation from bone marrow precursor cells in GM-CSF cultures suggesting the role played by TNF is redundant *in vivo* [16].

As all DC subsets express similar level of LT $\beta$ R, one wonders why the CD8 $\alpha$ <sup>+</sup> DC subset is not affected by the loss of LT $\beta$ R signaling. One explanation could come from the observation that CD8 $\alpha$ <sup>-</sup> DC subsets express higher levels of LT $\beta$ R in mice lacking the LT $\beta$ R ligand LT $\alpha_1\beta_2$ , suggesting that that LT $\beta$ R levels on CD8 $\alpha$ <sup>-</sup> DC subtypes are downregulated following engagement with the ligand [18]. Recent evidence indicates that LT $\alpha_1\beta_2$ - or LIGHT-mediated LT $\beta$ R signaling promotes bone-marrow derived DC accumulation *in vitro* and contributes also to lymphoid tissue DC proliferation *in vivo*, most likely helping to maintain their homeostasis [18,21]. Nevertheless, the remaining question is whether the LT $\beta$ R signaling promotes DC proliferation or allows cells in the CD8 $\alpha$ <sup>-</sup> DC subset to differentiate from a DC precursor within lymphoid tissues, or both.

Using a battery of mice deficient for various ligands of the TNFR superfamily, we established that LT $\alpha_1\beta_2$ -LT $\beta$ R interaction is the crucial interaction controlling the steady state cellularity of CD8 $\alpha$ <sup>-</sup> DC subtypes in the spleen. LIGHT does not play a redundant role with LT $\alpha_1\beta_2$  during steady state [19]. B and T lymphocytes are the major source of the LT $\alpha$  and LT $\beta$  in the spleens of naïve mice. B cell deficient ( $\mu$ MT) mice have been described to possess reduced level of splenic DC [22,23]. Recent observations indicated that overexpression of LT $\alpha_1\beta_2$  on B cells as well as transgenic expression of LIGHT on T cells was sufficient to promote splenic DC accumulation *in vivo* [21,23]. However, these two transgenic experimental conditions do not reflect physiological conditions, but may approximate inflammatory conditions, representing conditions where the equilibrium between the different mechanisms regulating steady state lymphoid tissue DC homeostasis is perturbed. Interestingly, a gene chip analysis on splenic DC subsets showed LT $\beta$  mRNA was the highest expressed amongst all mRNA between DC subsets [24]. This study also indicated that CD8 $\alpha$ <sup>-</sup> DC subsets that are regulated via LT $\beta$ R signaling express 5–6-fold higher LT $\beta$  mRNA levels compared to CD8 $\alpha$ <sup>+</sup> DC subset. This latter suggests that lymphoid-tissue DC could regulate their own homeostasis if they were able to express LT $\beta$  protein. However, detection of LT $\beta$  protein has been difficult. Therefore, the physiological source of LT $\alpha_1\beta_2$  playing a crucial role in steady state DC remains to be determined.

### 3. Counter regulatory mechanism for DC: the HVEM–BTLA pathway

Counter balancing pathways should exist to limit the trophic actions of LT $\beta$ R signaling in CD8 $\alpha$ <sup>-</sup> DC subsets.

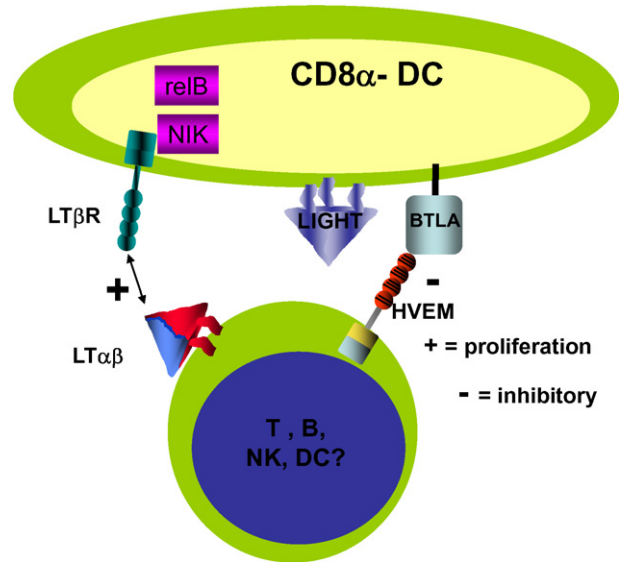


Fig. 2. Schematic model representing the regulation of DC homeostasis by a LT $\alpha\beta$  and HVEM–BTLA-integrated circuit. The specific details of the model are described in the text.

HVEM interacts with an inhibitory receptor, B and T lymphocyte attenuator (BTLA), an immunoglobulin (Ig) superfamily (Fig. 2) [25,26], known to attenuate lymphocyte activation, and thus a candidate regulator for DC. This non-canonical engagement of HVEM–BTLA induces an inhibitory signaling cascade regulating T and B cell proliferation. Following HVEM engagement, BTLA attenuates antigen-mediated signals through an ITIM/ITSM-dependent recruitment of Src homology tyrosine phosphatase-1 and -2 [27]. Biochemical analyses demonstrated that LIGHT and BTLA engage HVEM at distinct sites, suggesting that HVEM constitutes a molecular switch between positive and inhibitory signaling [28–30]. As LIGHT-deficient mice do not possess any apparent defect in DC subsets, we investigated whether the HVEM–BTLA interaction was involved in the regulation DC homeostasis. Surprisingly, BTLA-deficient mice exhibited DC profile opposite of the LT $\beta$ R deficient mice, with a specific increase in splenic CD8 $\alpha$ <sup>-</sup> DC subsets compared to control wild type mice [19]. HVEM<sup>-/-</sup> mice phenocopied the DC subset profile in BTLA<sup>-/-</sup> mice, implicating these components form a pathway regulating DC homeostasis. These results suggest that HVEM–BTLA engagement counter regulates the positive role of LT $\beta$ R signaling to maintain the steady state level of CD8 $\alpha$ <sup>-</sup> DC subsets.

The mechanism of HVEM–BTLA inhibitory signaling does not directly impact LT $\beta$ R pathway. As BTLA recruits tyrosine SHP1/2 phosphatases, it is unlikely that NIK or IKK $\alpha$ , which are serine kinases, are direct targets of BTLA activation. The proliferation activity induced through LT $\beta$ R signaling on CD8 $\alpha$ <sup>-</sup> DC subsets can be monitored by incorporation of nucleotide (bromodeoxyuridine, BrdU) in dividing DC. However, the percentage of BrdU<sup>+</sup> cells within each DC subsets in either HVEM- or BTLA-deficient mice

was similar to wild type mice [19]. This finding indicates that the inhibitory effect of HVEM–BTLA pathway does not influence the LT $\beta$ R-dependent proliferation of DC and might impact a post-mitotic phase in DC differentiation, the target of which is still unknown.

Treatment with anti-LT $\beta$ R mAb over a 14-day period not only restored DC homeostasis, but induced a DC subset profile comparable to mice lacking either HVEM or BTLA expression, characterized by an increased level of splenic CD8 $\alpha$ – DC subtypes [19]. Enforced LT $\alpha_1\beta_2$  expression in B cells also caused a specific accumulation of CD8 $\alpha$ – DC populations without affecting CD8 $\alpha$ + DC subset. Therefore, continuous pharmacological or genetically engineered triggering of LT $\beta$ R signaling, can override the physiological inhibitory action of HVEM–BTLA interaction, suggesting LT $\beta$ R signaling functions independently or is dominant to HVEM–BTLA.

The homeostasis of DC in lymphoid organs depends on the intrinsic expression of the LT $\beta$ R [18], which raised the issue of whether HVEM and BTLA expression is required in the hematopoietic or stromal compartments, or both. Mixed bone marrow chimeras revealed DC from wild type and gene deficient mice could replenish splenic DC. Both HVEM- and BTLA-deficient DC showed a substantial competitive advantage to repopulate the splenic pool of DC compared to wild type DC, clearly demonstrating the inhibitory role of the HVEM–BTLA pathway in DC proliferation [19]. However, the stromal compartment lacking HVEM or BTLA also impacted inhibitory signaling controlling DC homeostasis. Together, these results suggest that both DC–DC and DC–stromal cell interactions constitute sources of inhibitory signaling. However, the analyses of splenic DC subtypes in these mixed chimeric mice suggest an intrinsic role of HVEM in CD8 $\alpha$ + DC homeostasis, indicating a more complex picture on the role of HVEM–BTLA interaction in splenic DC subset homeostasis.

DC development and homeostasis are governed by dynamic signals. To test if the DC subset alteration in absence of LT $\beta$ R signaling was reversible, ligand (LT $\beta$ /LIGHT)-deficient mice were treated with an agonist anti-LT $\beta$ R mAb. LT-regulated DC subsets in mice lacking both LT $\beta$  and LIGHT or LT $\alpha$  were restored with the anti-LT $\beta$ R treatment, confirming intrinsic LT $\beta$ R signaling in DC is sufficient to promote proliferation and differentiation in an altered stromal microenvironment [19]. Moreover, the treatment of wild type mice with the LT $\beta$ R-Fc decoy, which neutralizes both LT $\alpha\beta$  and LIGHT, also phenocopied the DC profile in LT-deficient mice, confirming the dynamic role of LT $\beta$ R signaling in DC subset homeostasis [19]. These results demonstrate the high level of plasticity of the LT $\beta$ R-regulated mechanisms controlling DC homeostasis *in vivo*. In contrast, formation of permanent lymphoid organs, lymph nodes and Peyer's patches, requires LT $\beta$ R signaling during precise times of embryonic development. Failure of LT $\beta$ R signaling to occur on time leads to organ absence, and later LT $\beta$ R activation is insufficient to regenerate missing organs [31].

Interestingly, mice deficient in both positive and negative pathways, LT $\beta$ /LIGHT/HVEM-triple deficient mice, had decreased DC numbers, comparable to the level observed in LT $\beta$ /LIGHT-deficient mice, yet the ratio of the remaining DC subsets was reversed, comparable to wild type mice. These results emphasized that LT $\beta$ R-dependent signaling is mandatory to maintain DC homeostasis and proliferation *in vivo* [19]. BTLA-deficient mice treated with the LT $\beta$ R-Fc decoy recapitulated the phenotype observed in triple deficient mice indicating the phenotype is not an artifact, but rather without both positive and negative signaling, an LT $\beta$ R-independent, pathway maintains the balance in DC subsets, but supports fewer cells.

Recent observations from the Kaye group using a bone marrow chimeras showed that BTLA-deficient CD4+ and CD8+ T cells also possess an advantage in replenishing the spleen T cell compartment compared to wild-type T cells, indicating the existence of a common mechanism regulating both DC and T cell homeostasis [32]. Moreover, BTLA-deficient mice possess an increased CD4 to CD8 T cell ratio initiated through T cell-intrinsic signaling. Surprisingly, this observation parallels the augmented ratio of CD4 to CD8 $\alpha$  DC without intrinsic BTLA expression in DC. This finding supports the hypothesis of similar pathways coordinating T cell and DC subpopulations during non-inflammatory conditions. However, LT $\beta$ R is not expressed on T or B lymphocytes, raising the possibility that other cosignaling TNFR may be the target of counter regulation by HVEM–BTLA in T and B cells.

#### 4. Noncanonical NF- $\kappa$ B signaling cascade regulates DC homeostasis

Signaling through the TNFR has been shown to activate the RelA/p50 transcription factor through the degradation of I $\kappa$ B through the IKK $\alpha\beta\gamma$  kinase complex (Fig. 3). In contrast, LT $\beta$ R initiates not only RelA/p50 transcription factor, but also the RelB/p52 complex, but through the “noncanonical” pathway involving NF- $\kappa$ B inducing kinase (NIK) and IKK $\alpha$ , which controls p100 degradation to p52 [33]. The RelA/p50 dimer is rapidly formed by the canonical TNFR pathway controlling expression of hundreds of proinflammatory genes, as well as p100, precursor to p52. LT $\beta$ R signaling activates both RelA and RelB forms of NF- $\kappa$ B, which turns on distinct sets of genes, many involved in homeostatic processes (e.g., tissue organizing chemokines).

Allymphoplasia (aly) mice, which possess a defective NIK gene due to a naturally occurring point mutation, exhibit a DC profile similar to LT $\beta$ R-, LT $\alpha$ - and LT $\beta$ -deficient mice with a decreased splenic cDC numbers due to a specific reduction in CD8 $\alpha$ – DC subtypes [19]. This result indicates that LT $\beta$ R signaling controls DC homeostasis through the NIK-dependent activation of the “noncanonical” pathway and consistent with biochemical mechanism of LT $\beta$ R signaling. This hypothesis is strongly reinforced by

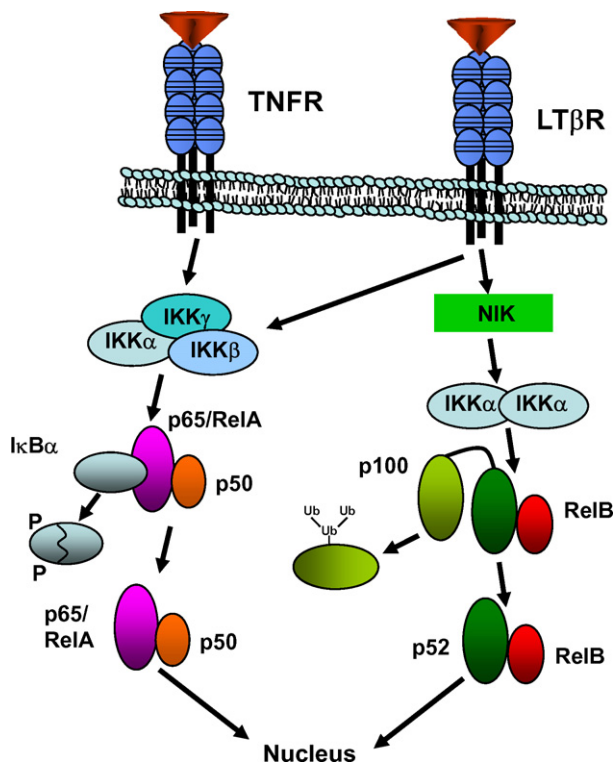


Fig. 3. TNFR and LT $\beta$ R signaling pathways for NF- $\kappa$ B activation. Signaling through TNFR activates the canonical RelA NF- $\kappa$ B pathway, which involves the  $\alpha$ ,  $\beta$  and  $\gamma$  (NEMO) subunits of the I $\kappa$ B kinase (IKK) complex, which leads to proteosomal degradation of I $\kappa$ B, releasing RelA/p50 dimer from its cytoplasmic constraint, locate to the nucleus and binding  $\kappa$ B transcriptional target sequences. LT $\beta$ R ligation activates the serine kinases NIK and IKK $\alpha$  (also a part of the canonical pathway). The phosphorylation and ubiquitin-dependent proteasomal degradation of p100 results in the processing of the precursor to the p52 subunit and accumulation of p52-RelB heterodimers to the nucleus. Signaling through the LT $\beta$ R also leads to the activation of the classical NF- $\kappa$ B pathway. TNFR signaling rapidly activates a large number of proinflammatory genes, whereas LT $\beta$ R activation of RelB takes several hours but sustained expression in the nucleus leads to different sets of activated genes involved in homeostasis, such as the chemokine, CCL21.

RelB-deficient mice that show a comparable DC subset profile to *aly* and mice deficient in components of the LT $\beta$ R pathway [34]. However, mice deficient for NF- $\kappa$ B subunits p50, p65 and c-Rel possess normal DC subsets compared to wild type mice [35]. Other genes, i.e. Krüppel family of zinc finger transcription factor Ikaros C, transcription factor PU.1, IRF-2 and IRF-4 (IFN regulatory factor-2 and -4), Notch-dependent transcription factor RBP-J, and TRAF6 (TNF receptor associated factor-6), have also been described to play a role in differentiation of CD4<sup>+</sup> DC subset [36–41]. The Ig superfamily member, CD40 and Toll-like receptors are receptors known to signal through TRAF6, yet none of those TNFR are implicated in the regulation of DC homeostasis. Another TNFR family member, TNF-related, activation-induced cytokine (TRANCE) receptor (also called RANK), which signals through TRAF6 is expressed by DC and promotes DC survival [42]. However, RANK-deficient mice do not exhibit any DC abnormalities, but are deficient in lymphoid organogenesis and osteoclast development.

The mixed chimera experiments demonstrated intrinsic expression of HVEM in DC impacted the CD8 $\alpha$ <sup>+</sup> DC subset homeostasis, however the context of this influence is not fully understood. However, CD8 $\alpha$ <sup>+</sup> DC are impacted by genetic deficiencies in IRF-8, (also called ICSBP, IFN consensus sequence binding factor), Id2 (helix-loop-helix family transcription factor inhibitor DNA binding-2), and Jak3 (Janus tyrosine kinase) [43–45]. The relationship between the function of these genes, LT $\beta$ R and HVEM–BTLA pathways and DC homeostasis remains to be explored.

## 5. DC functions in host defense regulated by TNFR superfamily

The expression of LT $\beta$ R was first thought to be restricted to stromal cells, where its function has been extensively studied. However, earlier studies already suggest the LT $\beta$ R expression by cells of the myeloid lineage [46–48]. LT $\beta$ R expression on activated pulmonary macrophages and thioglycolate-elicited peritoneal macrophages is crucial for host defense [49]. The presence of an intact LT $\beta$ R signaling in these alveolar macrophages was required to develop an efficient bactericidal effector function in response to *Mycobacterium tuberculosis*. Macrophages from *aly* mice were also shown to exhibit reduced bactericidal effector functions in response to *Listeria monocytogenes* infection [50]. Bone marrow-derived mast cells express LT $\beta$ R and triggering of LT $\beta$ R signaling using physiological or pharmacological approaches induced the production of cytokines and pro-inflammatory chemokines [51]. The group of J. Gommerman demonstrated that LT $\alpha$  $\beta$ <sub>2</sub> expression in antigen-specific T cells was required for optimal DC function [52]. Their result also indicated that DC are dysfunctional without LT $\alpha$  $\beta$ <sub>2</sub> or CD40 ligand expression on antigen-activated T cells. However, signaling through either CD40 or LT $\beta$ R restored DC function, suggesting that both pathways cooperates to optimize DC “conditioning” [52]. CD40 and LT $\beta$ R are quite similar in their mechanisms of signaling, utilizing TRAF adaptors and competent in noncanonical NF- $\kappa$ B signaling, although unlike LT $\beta$ R, CD40 activates TRAF6 pathways.

Expression of LT $\alpha$  $\beta$ <sub>2</sub> in T and B cells is crucial for the induction of adaptive immune responses against small amounts of antigen, but dispensable against some [53] but not all [54] replicating pathogens suggesting a role for LIGHT in host defense. LIGHT–LT $\beta$ R interactions may be more important in DC functions during inflammation. For example, DC derived from LIGHT-deficient mice secreted less IL-12 in response to LPS stimulation and HVEM-Fc decoy impaired IL-12 production *in vivo* leading to defective effector T cells production [55]. Several groups have also demonstrated a role for LIGHT in mediating CD8 T cell responses, particularly alloresponses [56,57].

All splenic conventional DC subsets express both HVEM and BTLA and the HVEM–BTLA interaction negatively regulates the homeostasis of CD8 $\alpha$ <sup>+</sup> DC subsets *in vivo*.

However, only a few clues are available concerning the mechanisms mediating inhibitory function in DC homeostasis. Moreover, each of these molecules exhibits a very broad pattern of cellular expression, i.e. B and T lymphocytes, NK cells, macrophages and DC, increasing the difficulty in defining a function to a specific cell lineage. So far, the functional role of BTLA has mainly been viewed from the perspective of T cells, where signaling induces a key coinhibitory signal attenuating host T cell responses. For example, HVEM–BTLA pathway regulates acceptance of partially MHC-mismatched cardiac allograft [58]. BTLA expression was also found to be highly induced on anergic CD4<sup>+</sup> T cells [59]. The treatment of mice infected with *Plasmodium berghei* ANKA with an agonist anti-BTLA mAb prevented the genesis of experimental cerebral malaria by restricting the number of T lymphocytes sequestered in the brain during infection [60]. Until recently, the hyperproliferative response of BTLA-deficient T cells to immobilized anti-CD3 was thought to reflect the loss of a negative costimulatory signals on T cells [61]. However, the observations that purified CD44<sup>lo</sup> and CD44<sup>hi</sup> CD4<sup>+</sup> and CD8<sup>+</sup> T cells are not hyperproliferative under the same conditions, suggest that the regulation of T cell activation through BTLA signaling is likely to involve binding to HVEM on a non-T cell population, presumably antigen-presenting cells. Moreover, the hyperproliferative activity of BTLA-deficient T cells is most likely caused by an increase in the memory phenotype CD8<sup>+</sup> T cells in mice lacking BTLA, rather than the loss of a coinhibitory signal [32]. Similarly, HVEM-deficient mice also exhibit increased percentages of memory-phenotype CD8<sup>+</sup> T cells, which is consistent with the idea that BTLA engagement by HVEM is responsible for the alteration in T cell subpopulation homeostasis [32]. In mixed bone marrow experiments with wild type and BTLA-deficient T cells, BTLA deficiency in T cells is responsible for the accumulation of memory-phenotype CD8<sup>+</sup> T cells [32]. This finding argues against a possible role of BTLA on antigen-presenting cells as the major cause of this phenotype. However, the increase of memory-phenotype CD8<sup>+</sup> T cells is reduced in mice lacking HVEM compared to BTLA-deficient mice. This result could be interpreted in view of the two distinct ligands for HVEM, the costimulatory LIGHT and the inhibitory BTLA, therefore influencing both positive and negative signaling in steady state T cell homeostasis. Therefore, the increase of memory-phenotype CD8<sup>+</sup> T cells in HVEM-deficient mice due to the loss of BTLA signaling is counterbalanced by the lack of LIGHT-mediated costimulation.

## 6. Concluding remarks

The homeostatic regulation of DC subsets *in vivo* is mandatory to keep the immune system informed and ready to react optimally against any invading pathogen. LT $\beta$ R-dependent signaling provides a key pathway for local

proliferation of DC that regulates the steady state numbers in lymphoid tissues. Continuous LT $\beta$ R signaling is required for maintenance of most of the DC in the spleen argues this pathway provides responsive signaling via LT $\alpha\beta$  and LIGHT expression during antigenic challenge. The presence of DC in lymphoid or nonlymphoid tissues may expand or subside in response to LT $\alpha\beta$  signaling. As LT $\alpha\beta$  is membrane-anchored, cell-to-cell contact will be required for signal transmission to DC. Expression of LT $\alpha\beta$  or LIGHT in any of several types of antigen or pathogen responding cells, including T cells, B cells, and NK cells may promote local expansion of DC to amplify adaptive immune responses. Integrated into this DC responsive pathway is a key inhibitory pathway mediated through BTLA, which counterbalances the homeostatic and effector positive role of LT $\alpha\beta$ –LT $\beta$ R interaction through its engagement with the TNFR superfamily member HVEM. This BTLA–HVEM “brake” constitutes a broad regulatory mechanism of immune responses impacting T cells, B cells, and DC. Together, these interactions form a complex integrated signaling network controlling DC subset homeostasis and may contribute to maintaining the barrier between immunity and tolerance.

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