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LIGHT (a Cellular Ligand for Herpes Virus Entry Mediator and Lymphotoxin Receptor)-Mediated Thymocyte Deletion Is Dependent on the Interaction Between TCR and MHC/Self-Peptide

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Negative selection serves as a major mechanism to maintain self-tolerance. We previously reported that LIGHT (a cellular ligand for herpes virus entry mediator and lymphotoxin receptor), a TNF family member, plays an important role in thymocyte development via promoting apoptosis of double-positive thymocytes. Here, we demonstrated that LIGHT-mediated deletion of thymocyte requires the strong interaction of TCR with MHC/self-peptide. Transgenic mice overexpressing LIGHT in thymocytes were bred with a transgenic mouse line expressing a TCR recognizing the H-Y male Ag in the context of H-2b class I MHC molecules. In male H-Y/LIGHT double-transgenic mice, more efficient negative selection of H-Y T cells occurred, and total thymocyte number was further reduced compared with H-Y/negative littermates. In contrast, the presence of LIGHT transgene had no evident impact on the thymocyte development of female H-Y/LIGHT double-transgenic mice. Taken together, LIGHT plays a role in negative selection of thymocytes via inducing the apoptosis of thymocytes bearing high affinity TCR during negative selection. *The Journal of Immunology*, 2003, 170: 3986–3993.

Cell development is tightly controlled by selective events in the thymus, namely positive and negative selection (1–6). The current model proposed that the fate of immature thymocytes is largely determined by the interaction between their TCR and MHC/peptide complexes expressed on thymic stromal cells. Thymocytes with a TCR of high avidity for MHC/peptide complexes are induced to undergo apoptosis (negative selection) to maintain central tolerance, whereas the lower avidity binding of TCR and MHC/peptide confers immature thymocytes to undergo positive selection and become single-positive (SP) \(^3\) T cells that eventually migrate into periphery and play critical roles in adaptive immunity.

Negative selection serves as a critical mechanism to maintain self-tolerance (7). The current idea of negative selection is largely based on transgenic (Tg) models of various TCR genes. Among them, H-Y Tg mouse is a well-established model that provides experimental evidence of clonal deletion and positive selection (8–10). In male H-Y Tg mice, the immature thymocytes undergo increased apoptosis due to the extremely strong interaction between MHC (H-2D\(^b\))/peptide complex and TCR, whereas in female Tg mice the interaction between MHC/peptide complex and TCR is much weaker, which leads to the positive selection of H-Y TCR\(^+\) CD8\(^+\) T cells. This model has served as a central system to elucidate the mechanisms of selection events in the development of thymocytes (11).

The existence of central tolerance implies that immature thymocytes respond differently to an encounter with Ags than do mature T cells. Optimal negative selection appears to require signals in addition to an antigenic stimulus (12–14). Our previous study has shown that LIGHT (a cellular ligand for herpes virus entry mediator (HVEM) and lymphotoxin receptor) functions as a co-stimulatory molecule for mature T cells in periphery and causes the activation and expansion of peripheral T cells (15, 16); however, the constitutive expression of LIGHT on thymocytes promotes the apoptosis of double positive (DP) thymocytes (17). In addition, blockade of endogenous LIGHT in a TCR Tg model, D7 Tg mice (18), could rescue the DP and CD8 SP thymocytes dramatically in fetal thymic organ culture (17). Therefore, these results raise the possibility that LIGHT may be involved in negative selection by regulating apoptosis process. However, it is unclear whether the LIGHT-mediated apoptosis is associated with specific interaction of TCR between its cognate MHC/peptide complex. Namely, the cell death mediated by LIGHT may be caused by a general impact on cell survival of a wide variety of immature thymocytes regardless of their TCR specificity or via clonal deletion of specific TCR\(^+\) thymocytes. Thus, we crossed LIGHT transgene into H-Y Tg system and examined thymocyte development in double-Tg mice.

Here, we show that LIGHT-mediated deletion of thymocytes is directly linked with the interaction between TCR and MHC/peptide. With the presence of H-Y Ag (H-Y/LIGHT male mice), LIGHT further promotes the apoptosis of thymocytes and the reduction of total thymocyte number. The enhanced apoptosis in H-Y/LIGHT double-Tg male mice leads to the reduction of mature CD8\(^+\) TCR\(^+\) cells in the thymus and periphery. In contrast, T cell development is largely unchanged in H-Y/LIGHT double-Tg female mice. Therefore, our study suggests that LIGHT-mediated
apoptosis of DP is associated with clonal deletion of specific TCR\(^+\) thymocytes.

**Materials and Methods**

**Mice**

LIGHT Tg mice were generated as previously described (15). The C57BL/6 (B6) Tg line was crossed to H-Y/RAG2\(^{-/-}\) mice on the B6 background (Tacoon Farms, Germantown, NY). LIGHT Tg mice (C3H) were crossed to lymphoblast \(\beta\) receptor (LT\(\beta\)R\(^+\)) and LT\(\alpha\)R\(^+\) mice for two generations. The age of the mice examined was between 5–10 wk. Animal care and use were in accordance with institutional guidelines of University of Chicago.

**Flow cytometric analysis**

Single-cell suspensions of thymocytes and splenocytes were collected and stained with PE-anti-CD8, FITC-anti-CD4 (for three-color FACS, Cy-Chrome-anti-CD4 was used), FITC-anti-V\(8.1\) (F23.1), and PE-anti-CD3 Abs (BD PharMingen, San Diego, CA) in PBS plus 0.01% NaN\(_3\) for 30 min at 4°C. FITC-labeled T3.70 Ab was obtained from eBioscience (San Diego, CA). After incubation with Abs, the cells were washed and analyzed on a FACScan (BD Biosciences, Mountain View, CA). Anti-LIGHT Ab was described previously (17). Annexin V and propidium iodide (PI) double staining for apoptosis was performed using a commercially available kit as described by the manufacturer (BD PharMingen).

**In vivo treatment protocol**

Age (5–10 wk)–and sex-matched C3H wild-type (wt; JAX) mice received three or four i.p. injections weekly of 100 \(\mu\)g of soluble murine LT\(\beta\)R-Ig or PBS. Thymi were collected 7 days after the last injection. In some cases C3H wt mice were sublethally irradiated (600 rad) and treated with fusion protein as described above. Thymocytes and splenocytes were stained with CyChrome-anti-CD4 and PE-anti-CD8 Abs (BD PharMingen) and analyzed by FACScan (BD Biosciences).

**TUNEL assay**

TUNEL staining for apoptosis was performed using a commercially available kit as described by the manufacturer (Oncoregene Research Products, San Diego, CA). The thymus samples were fixed with 10% formalin and embedded in paraﬁn. Sections were subject to TUNEL assay (Oncoregene Research Products), followed by hematoxylin counterstaining using standard methods.

**Results**

**LIGHT-mediated apoptosis is associated with TCR speciﬁcity**

To study whether TCR engagement is required for the reduction of thymocyte number in LIGHT Tg mice, we performed a further study of the role of LIGHT in thymocyte development using a conventional TCR system, H-Y Tg mice. LIGHT Tg mice were crossed with H-Y Tg mice, and the male and female progeny were analyzed for the development and maturation of thymocytes. In male H-Y/LIGHT double-Tg mice, the total number of thymocytes was signiﬁcantly reduced compared with male H-Y/NL negative littermate (H-Y/NL) Tg mice (Fig. 1A; 19.35 ± 3.98 × 10^6 in H-Y/NL mice vs 3.37 ± 1.01 × 10^6 in H-Y/LIGHT mice; \(n = 5\)). Moreover, the reduction of the DP population appeared to be more severe in H-Y/LIGHT Tg mice (24-fold reduction in DP, 8-fold in CD8 SP, 6-fold in CD4 SP). At the same time, the female H-Y/LIGHT double-Tg mice had a similar total number of thymocytes as female H-Y/NL Tg mice (Fig. 1A; 102.47 ± 8.83 × 10^6 in H-Y/NL mice vs 82.63 ± 16.1 × 10^6 in H-Y/LIGHT mice; \(n = 5\)). These data suggested that the presence of LIGHT promoted the further reduction of thymocytes in male H-Y mice, whereas it did not affect the thymocyte development largely in female H-Y mice.

Previous studies demonstrated that DP thymocytes undergo increased apoptosis in male H-Y Tg mice compared with NL. Therefore, we further tested whether the reduction of thymocytes in H-Y/LIGHT double-Tg mice was caused by enhanced apoptosis. As we expected, apoptosis was increased in male H-Y/LIGHT double-Tg mice, as shown by annexin V and PI staining (Fig. 1B).

Consistent with annexin V/PI staining, a greater number of apoptotic cells was seen in male H-Y/LIGHT Tg thymus as determined by TUNEL assay (Fig. 1C). Thus, LIGHT could further promote apoptosis in the presence of a strong interaction between MHC/peptide and TCR, while LIGHT seems to have no signiﬁcant impact on thymocyte development in the absence of such interaction, suggesting that LIGHT-mediated apoptosis is linked with TCR speciﬁcity.

**Perturbed thymocyte development in male H-Y/LIGHT double-Tg mice and unaffected thymocyte development in female H-Y/LIGHT double-Tg mice**

We examined the different populations of thymocytes by FACS. In male H-Y/LIGHT double-Tg mice, the percentages of DP and CD8 SP were further reduced in addition to the reduction of total thymocyte number (Fig. 2A). In contrast, DP and CD8 SP populations did not show any signiﬁcant change between female H-Y/NL and H-Y/LIGHT groups (Fig. 2A). We also detected the TCR expression (F23.1 Ab against H-Y \(\beta\)-chain) in thymocyte populations; the percentage of H-Y\(\beta^+\)CD8\(^+\) thymocytes was decreased in male H-Y/LIGHT double-Tg mice (Fig. 2B; 8.70% in H-Y/LIGHT mice vs 18.58% in H-Y/NL mice). In addition, the absolute number of H-Y\(\beta^+\)CD8\(^+\) thymocytes was more severely reduced (3.60 ± 0.74 × 10^6 in H-Y/NL mice vs 0.29 ± 0.09 × 10^6 in H-Y/LIGHT mice) due to the reduction of total thymocyte number in H-Y/LIGHT Tg mice. However, the percentage of the H-Y\(\beta^+\)CD8\(^-\) population appeared to be unchanged (Fig. 2B, male; 73.83% in H-Y/LIGHT mice vs 74.12% in H-Y/NL mice). A previous study demonstrated that DN thymocytes were also forced to express H-Y TCR on their surface (19). T3.70 Ab (clonotypic Ab against Tg TCR \(\alpha\)-chain) was used for triple staining of CD8/CD4/T3.70. Similar results were obtained in male Tg mice using T3.70 Ab compared with F23.1 Ab as shown in Fig. 2B. Here, we showed that the presence of LIGHT transgene caused a further reduction of H-Y\(\beta^+\)CD8\(^-\), suggesting that thymocytes with higher avidity binding were selectively deleted to a larger extent. Interestingly, the percentage of H-Y TCR\(^-\) thymocytes was enhanced in male H-Y/LIGHT double-Tg mice (Fig. 2B; CD8/F23.1, 16.38% in H-Y/LIGHT mice vs 5.42% in H-Y/NL mice), indicating that the deletion of this population (H-Y TCR\(^-\)) was not as severe as that of the H-Y TCR\(^+\) population.

Despite all the changes in male H-Y/LIGHT Tg mice, there was no obvious difference in the thymocytes of female H-Y/LIGHT Tg mice (Fig. 2B, female), which further conﬁrmed the unaffected thymocyte development in this mouse model. In female H-Y Tg mice, the percentage of Tg TCR\(\alpha^+\) thymocytes was lower than that of Tg TCR\(\beta^+\) thymocytes, suggesting rearrangements of endogenous \(\alpha\)-chains (Fig. 2B, female).

In the periphery the phenotypes were consistent with those observed in the thymus of male H-Y/LIGHT mice. The percentage of CD8\(^+\) was decreased in H-Y/LIGHT double-Tg mice compared with H-Y/NL Tg mice (Fig. 2C, male; 6.98% in H-Y/LIGHT mice vs 14.65% in H-Y/NL mice); moreover, the intensity of CD8 staining in both H-Y/LIGHT and H-Y/NL mice was reduced compared with that in wt B6 mice, as shown by the mean ﬂuorescence intensity (Fig. 2D), indicating the down-regulation of CD8 coreceptor expression in the periphery. However, the percentage of CD4\(^+\) was increased in male H-Y/LIGHT Tg mice (Fig. 2C, male); this was conﬁrmed with CD4/CD8/T3.70 staining (Fig. 2E). Consistently, all populations were unchanged in the spleen of female H-Y/LIGHT Tg mice compared with female H-Y/NL mice (Fig. 2C, female).

TCR expression of splenocytes was also assessed by FACS. The frequency of H-Y\(\beta^+\)CD8\(^+\) T cells was reduced in male H-Y/
LIGHT Tg mice (Fig. 2E, male; CD8/F23.1, 7.10% in H-Y/LIGHT mice vs 15.16% in H-Y/NL mice); in addition, the percentage of H-Y/H9252/H11001 CD8/H11002 T cells was decreased (Fig. 2E, male; CD8/F23.1, 4.95% in H-Y/LIGHT mice vs 8.87% in H-Y/NL mice). Due to the strong negative selection pressure, CD8 SP cells down-regulate their coreceptor CD8, which leads to the generation of F23.1/H11001 CD8/H11002 cells (19). To further confirm the results, we performed triple staining for CD4/CD8/T3.70. As shown in Fig. 2E, similar results were seen using T3.70 Ab compared with F23.1 Ab. In addition, the percentage of CD4+/T3.70− cells is higher in H-Y/LIGHT Tg mice than in H-Y/NL mice (Fig. 2E; CD4/T3.70, 11.35% in H-Y/LIGHT mice vs 5.31% in H-Y/NL). Again, there was no significant difference in the splenocytes of female H-Y/LIGHT Tg mice compared with H-Y/NL Tg mice (Fig. 2E, female). However, FASC staining for splenocytes from female H-Y mice showed that the percentage of Tg TCRα+/T3.70− cells is much lower than that of Tg TCRβ+(F23.1+) cells, suggesting rearrangements of endogenous α-chains in female H-Y mice (Fig. 2E, female).

FIGURE 1. LIGHT-mediated reduction of thymocyte number was directly linked with TCR specificity. A, Severe reduction of the total number of thymocytes in male H-Y/LIGHT Tg mice compared with H-Y/NL. Thymocytes were extracted, and total cell number was counted (19.35 ± 3.98 × 10^6 in H-Y/NL mice vs 3.37 ± 1.01 × 10^6 in H-Y/LIGHT mice; n = 5). A largely unaffected total number of thymocytes was seen in female H-Y/LIGHT Tg mice compared with H-Y/NL mice (102.47 ± 8.83 × 10^6 in H-Y/NL mice vs 82.63 ± 16.1 × 10^6 in H-Y/LIGHT mice; n = 5). B, Enhanced apoptosis was observed in male H-Y/LIGHT Tg mice. The number in the frame indicates the frequency of cells in the defined region. Annexin V and PI staining was performed as previously described. C, An increased number of apoptotic cells (brown staining as indicated by arrowhead) was seen in male H-Y/LIGHT Tg mice. Thymic samples were subject to TUNEL staining. The number of apoptotic cells was counted under a microscope (magnification, ×400; PHF, per high power field).

Taken together, our results suggest that the presence of the LIGHT transgene caused severe deletion of thymocytes bearing higher avidity TCR on their surface, and such deletion was directly associated with TCR specificity; furthermore, the perturbation of thymocyte development affected the distribution of peripheral T cells.

Preferential reduction of H-Y+ thymocytes in male H-Y/LIGHT double-Tg mice

A previous study (20) has shown that the H-Y αβ transgene induced strong allelic exclusion of endogenous TCR genes; however, endogenous TCR genes could still undergo rearrangement, but endogenous T cells constitute only a small fraction of thymocytes. Therefore, we examined whether the LIGHT transgene had a differential impact on H-Y transgenic T cells defined as
FIGURE 2. The distribution of different thymocyte populations was affected by the LIGHT transgene in a TCR signaling-dependent manner. A, FACS profile of thymocytes for CD4 and CD8 staining. In male H-Y/LIGHT Tg mice, the percentages of DP and CD8 SP were reduced compared with that of H-Y/NL. In contrast, the thymocyte population was largely unchanged by the LIGHT transgene in female H-Y/LIGHT Tg mice. B, FACS profile of thymocytes for CD8 and H-Y TCR staining. H-Y/H11001 CD8+ thymocytes were significantly decreased in male H-Y/LIGHT Tg mice compared with H-Y/NL determined by CD8/F23.1 and CD8/T3.70 staining. The percentage of endogenous T cells (H-Y-) was significantly increased in male H-Y/LIGHT Tg mice. No significant difference was observed between female H-Y/LIGHT Tg mice and H-Y/NL. C, FACS profile of splenocytes for CD4 and CD8 staining. CD8+ T cells were reduced significantly, while the percentage of CD4+ T cells was moderately elevated in male H-Y/LIGHT Tg mice. The total numbers of splenocytes were counted for male (62.35 ± 18.38 × 10^6 in H-Y/NL mice vs 45.93 ± 18.37 × 10^6 in H-Y/LIGHT Tg mice; n = 7) and female (77.64 ± 13.38 × 10^6 in H-Y/NL mice vs 34.0 ± 18.05 × 10^6 in H-Y/LIGHT Tg mice; n = 7) mice. D, The mean fluorescence intensity of CD8 expression on peripheral T cells was down-regulated in both H-Y/LIGHT and H-Y/NL mice compared with wt B6 mice. E, FACS profile of splenocytes for CD8 and H-Y TCR staining determined by both F23.1 and T3.70 Abs. The percentages of CD8+ H-Y+ and CD8+ H-Y- were also reduced consistently with thymocyte data. In contrast, the distribution of peripheral T cells and TCR expression were unaffected in female H-Y/LIGHT Tg mice in which the interaction between TCR and MHC/self-peptide was relatively weak. Representative data are shown, and the number in the frame indicates the frequency of cells in the defined regions.
FIGURE 3. The LIGHT transgene preferentially deleted H-Y TCR\(^+\) thymocytes. A, FACS profile of thymocytes for CD3 and H-Y TCR staining. The endogenous thymocytes defined as CD3\(^+\) H-Y\(β\) were significantly enhanced in male H-Y/LIGHT Tg mice compared with H-Y/NL mice. The total number of thymocytes was counted \((n = 5)\). B, The reduction of H-Y\(\gamma\)-expressing thymocytes was more severe than that of H-Y\(\gamma\)-expressing thymocytes. C, The percentage of H-Y\(\gamma\)-expressing T cells in male H-Y/LIGHT Tg mice was also reduced in the periphery. In contrast, the endogenous T cells constituted a much larger proportion of peripheral T cells in H-Y/LIGHT Tg mice compared with H-Y/NL mice. The total numbers of splenocytes were counted \((n = 7)\). Representative data are shown, and the number in the frame indicates the frequency of cells in the defined regions.

H-Y\(β\)-CD3\(^+\) and endogenous T cells defined as H-Y\(β\)-CD3\(^+\). Interestingly, we found that the percentage of endogenous T cells (H-Y\(β\)-CD3\(^+\)) was increased in H-Y/LIGHT Tg mice compared with H-Y/NL Tg mice and constituted a larger proportion of thymocytes (Fig. 3A). A FACS profile of wt B6 thymocytes was included as a control in which the CD3\(^+\) population constituted \(-40\%\), including CD3\(^{high}\) and CD3\(^{medium}\) populations (Fig. 3A). In light of the reduction of total thymocytes (19.35 \(±\) 3.98 \(×\) 10\(^5\) in H-Y/NL mice vs 3.37 \(±\) 1.01 \(×\) 10\(^5\) in H-Y/LIGHT mice), H-Y\(β\) transgenic thymocytes appeared to undergo more efficient deletion than endogenous thymocytes (H-Y\(\gamma\)); the deletion rate of H-Y\(β\) thymocytes was consistently much greater than that of H-Y\(\gamma\) thymocytes (Fig. 3B, 88% in H-Y\(\gamma\) mice vs 47% in H-Y\(β\) mice). These data suggested that the presence of the LIGHT transgene preferentially led to the deletion of H-Y\(β\) thymocytes. Such changes in thymocyte population occurred in the periphery; the percentage of endogenous T cells (H-Y\(β\)-CD3\(^+\)) was also higher in the splenocytes of H-Y/LIGHT mice than in those of H-Y/NL mice (Fig. 3C; 8.72% in H-Y/LIGHT mice vs 3.93% in H-Y/NL mice), probably attributable to the LIGHT-mediated deletion of H-Y\(β\)-thymocytes. Similar results were obtained using T3.70 Ab compared with F23.1 Ab. Among the F23.1\(^+\) cells, the majority of them were also T3.70\(^+\) (\(>90\%\)). It appears that the Tg TCR\(β\)-expressing cells in male H-Y mice contain very few cells with rearrangements of endogenous α-chains (Fig. 2). The pan TCR β-chain (using Ab against all Vβ-chain) and γδ TCR expression were examined for the CD3\(^+\) F23.1\(^+\) population, and the percentage of TCR\(β\)-expressing cells was essentially equal to that of CD3\(^+\) thymocytes in this population. γδ TCR constitutes an extremely minor population (\(<0.2\%\)). Taken together, our results suggested that the presence of the LIGHT transgene preferentially promoted the deletion of H-Y\(β\) thymocytes, for which TCR showed an extremely strong interaction with MHC/self-peptide, although the LIGHT transgene could also cause the deletion of endogenous T cells.

**Up-regulation of LIGHT is associated with apoptosis of H-Y\(\gamma\) thymocytes**

We tested the impact of the LIGHT transgene in negative selection, and we examined whether the expression of LIGHT will be up-regulated when thymocytes undergo enhanced apoptosis. Since male H-Y/NL thymocytes underwent strong negative selection, we stained thymocytes for the expression of LIGHT by FACS. Our data showed that H-Y/NL thymocytes indeed up-regulated LIGHT compared with wt thymocytes (Fig. 4A). The percentage of LIGHT\(\gamma\) thymocytes in male H-Y/NL mice was significantly higher than that in wt mice (Fig. 4B). The percentage of LIGHT-positive thymocytes in H-Y/NL female mice was similar to that in wt mice, since there was no enhanced negative selection in H-Y/NL female mice (data not shown). For male H-Y/NL mice, LIGHT-positive thymocytes reside in the H-Y\(β\) population, as determined by LIGHT/F23.1 or LIGHT/CD3 FACS staining, which is subject to enhanced negative selection. For wt mice, LIGHT-positive thymocytes reside in the CD3\(^{medium/high}\) population, as determined by LIGHT/CD3 FACS staining. Thus, our results suggested that the level of LIGHT expression might be associated with the extent of apoptosis in negative selection of thymocytes, at least in the Tg system.

LIGHT plays an important role in regulating the T cell development of non-Tg mice

Our results obtained from the LIGHT Tg and H-Y Tg models demonstrated the critical role of LIGHT in negative selection. To

FIGURE 4. Up-regulation of LIGHT expression was associated with enhanced negative selection. A, LIGHT expression on thymocytes was detected by anti-LIGHT Abs. Male H-Y/NL (H-Y/LIGHT\(\gamma\)) thymocytes displayed much higher LIGHT expression on their surface than wt B6 thymocytes. The total number of thymocytes was counted \((n = 5)\). B, The percentage of LIGHT\(\gamma\)-expressing thymocytes was significantly elevated in male H-Y/NL Tg mice (H-Y/LIGHT\(\gamma\); \(n = 4\)).
FIGURE 5. LIGHT is involved in the thymocyte development of non-Tg mice. A, C3H mice were sublethally irradiated and treated with LTβR-Ig three times, and thymi were collected 7 days after the last treatment. B, C3H mice were sublethally irradiated and treated with LTβR-Ig as described in Materials and Methods. Thymocyte number was significantly enhanced in the treated group compared with controls.

determine whether LIGHT plays an important role in regulating normal T cell development, the interaction between LIGHT and its receptors was disrupted by the administration of LTβR-Ig in vivo to non-Tg mice. Various strains of mice were treated once a week with LTβR-Ig for 3 wk and analyzed 1 wk later. An enlargement of thymic size and increased thymic weight were observed in the group of treated mice, but not in the untreated group. The weight of thymi in the treated group was increased 50% compared with that in the untreated one (Fig. 5A; 87.6 ± 14.9 mg in treated group vs 58.9 ± 8.4 mg in untreated group; n = 11).

Since the developing thymus may be more sensitive to fusion protein treatment than the thymus at steady state, we irradiated wt mice and treated mice with LTβR-Ig in the same way as described above. The total number of thymocytes showed a 400–500% increase in the treated group compared with the untreated group (Fig. 5B; 52.7 ± 21.4 × 10^6 in treated group vs 11.8 ± 1.3 × 10^6 in controls; p < 0.001). Thus, LIGHT is not only involved in the negative selection of Tg models, but also plays an important role in normal T cell development.

**LIGHT-mediated selection is LTβR dependent**

LIGHT binds two receptors, HVEM and LTβR, in vitro (21). To test which receptor was involved in LIGHT-mediated phenotypes in thymus, LIGHT Tg mice were crossed to the LTβR^-/- or LTα^-/- background, and the thymocyte population was examined. There was no significant difference in the total thymocyte number of wt/LTβR^+/+, wt/LTβR^-/-, and LIGHT/LTβR^-/- mice (Fig. 6A). In addition, the thymocyte FACS profile was comparable between the different groups (data not shown). Consistent with previous data, the thymocyte number in Tg/LTβR^+/+ mice was dramatically reduced (Fig. 6A). These data suggested that the presence of LTβR was required for thymic phenotypes in LIGHT Tg mice. Furthermore, the LIGHT transgene could still lead to a reduction of thymocyte number in LTα^-/- mice (LIGHT/LTα^-/-) that lack both soluble LTα3 and membrane LTα3β2 (Fig. 6B). Thus, our data indicated that the signaling via LTβR by LIGHT might have an impact on thymocyte development, which could function independently of LT.

**Discussion**

In the present study we showed that LIGHT, a member of the TNF family, plays an important role in negative selection, which requires the strong engagement of TCR and MHC/self-peptide. Negative selection is considered to be one of the major mechanisms to delete potentially autoreactive T cells. However, the mechanism of thymocyte apoptosis remains unclear. Our previous results demonstrated that overexpression of LIGHT on thymocytes promotes the apoptosis of DP thymocytes (17). Shaikh et al. (22) also showed that constitutive expression of LIGHT on thymocytes led to thymic atrophy. However, whether LIGHT-mediated apoptosis of DP thymocytes is associated with the interaction of TCR and MHC/peptide is unresolved. Here, our data suggested that up-regulation of LIGHT on thymocytes promotes the deletion of H-Y TCR^+ population preferentially; thus, LIGHT-mediated apoptosis of DP thymocytes is tightly linked with TCR specificity.

TCR signaling alone is not sufficient to induce apoptosis of thymocytes; therefore, other molecules have been suggested in the process of thymocyte deletion, including TNF/TNF receptor (TNFR) family members, costimulation molecules, coreceptors, and adhesion molecules (4). However, the role of TNF/TNFR family members remains unclear. TNFR^-/- mice displayed abnormal negative selection in certain models, but not in others (23). CD30 has been indicated to play a role in negative selection (24); however, this idea has been challenged by another study showing that CD30 is not essential for negative selection (25). Thus, it has been recently proposed that more than one member of the TNF/TNFR family is required for negative selection (26). However, this idea has been challenged by another study showing that CD30 is not essential for negative selection (25). Thus, it has been recently proposed that more than one member of the TNF/TNFR family is required for negative selection (26). Although one study (27) proposed that the dependence on CD30 for the efficient deletion of autoreactive thymocytes is determined by the concentration, affinity/avidity, and length of exposure to the deleting ligand; and clonal deletion induced by endogenous viral superantigen was significantly affected by the targeted mutation of CD28 (28). In addition, one recent study (29) showed that blockade of B7-1/B7-2 in vivo
LIGHT-mediated selection requires TCR engagement

during fetal development substantially inhibits the clonal deletion of T cells in the thymus and leads to an accumulation of T cells in the periphery, resulting in fatal multiorgan inflammation.

LIGHT belongs to the TNF family and functions as a costimulatory molecule for T cell activation and proliferation; therefore, we investigated the role of LIGHT in negative selection. Our previous data indicated that LIGHT plays a critical role in T cell development (17); however, such an impact of LIGHT could be attributed to various possibilities, such as the survival of thymocytes. In the present study we demonstrated that LIGHT-induced apoptosis preferentially affected TCR+ cells in the context of a strong interaction between TCR and MHC/peptide. In the absence of such engagement of TCR, the impact of LIGHT up-regulation was rather insignificant. Therefore, the death of DP thymocytes induced by LIGHT is closely related to TCR signaling.

The LIGHT transgene also has an impact on the development of DN thymocytes in male H-Y/LIGHT double-Tg mice. The reduction of DN thymocytes in male H-Y/LIGHT Tg mice is probably due to the following reasons. 1) In the H-Y Tg system the transgene was expressed by DN thymocytes prematurely, while in a physiological situation DN thymocytes do not express rearranged αβ TCR, which is normally expressed at the DP stage. Due to the forced expression of H-Y transgenic TCR on DN thymocytes, these cells may be sensitive to the regulation by other factors. 2) Our data suggest that the simultaneous presence of LIGHT transgene and the strong interaction between H-Y TCR and self-MHC/peptide on these DN thymocytes also enhance the deletion of this population. Thus, the phenotypes reported here are attributed to the difference in both populations, although the DP thymocyte population seems to be more significantly affected by the LIGHT transgene. The presence of the LIGHT transgene affects the development of both DP and DN populations in male H-Y/LIGHT Tg mice, since they both express H-Y TCR. However, the presence of the LIGHT transgene does not have a significant impact on DN thymocytes in female H-Y/LIGHT Tg mice; thus, LIGHT-mediated thymocyte deletion is dependent on the strong interaction between TCR and self-MHC/peptide. Our previous data from LIGHT Tg mice suggest that the presence of the LIGHT transgene does not significantly affect DN thymocytes in a non-Tg TCR system (17).

The mechanism of LIGHT-mediated apoptosis is still unclear. Our data suggest that LTβR is required for thymocyte deletion in LIGHT Tg mice. LIGHT can bind to three receptors: HVEM, LTβR, and DcR3 decoy receptor (21) (30). LTβR was mainly expressed on stromal cells; certain macrophage and dendritic cells were also positive for LTβR (31, 32). However, LTβR was not detected on T and B cells (31). The expression of LTβR has been detected in the developing human thymus, which is prominent along the trabeculae and in the medulla and corticomediullary junction (33). Therefore, LIGHT could interact with LTβR expressed on either thymic stromal cells or macrophage and dendritic cells, which play an essential role in the deletion of DP thymocytes during negative selection. The communication of signals between thymocytes and APCs may provide a novel pathway for the induction of thymocyte apoptosis. Signaling via LTβR leads to up-regulation of adhesion molecules, such as ICAM-1 and VCAM-1.

A previous study (34) reported that deletion of Ag-specific immature thymocytes by dendritic cells requires LFA-1/ICAM interactions. Thus, it is conceivable that signals can be exchanged between thymocytes and APCs initially via TCR and MHC/peptide and subsequently joined by other surface molecules and coreceptors.

We previously reported that aged LIGHT Tg mice (featured at the age of 5–7 mo) developed lymphoproliferative disease due to the expansion of T cells in the periphery, and peripheral T cells displayed hyperactivated phenotypes (15). LIGHT-induced thymocyte deletion is not attributed to peripheral activation of T cells in LIGHT Tg mice, since the mice we used for the thymocyte development study were young and showed no signs of activation in peripheral T cells (data not shown). Although LIGHT−/− mice did not show any obvious defect in the thymus (35), the process of negative selection induced by various Ags, such as viral superantigen and anti-CD3, has not been reported. The TCR Tg system may be needed to visualize Ag-specific selection events.

Previous studies suggest that up-regulation of LIGHT in peripheral T cells promotes mature T cell activation and enhances autoimmunity (15, 22). Blockade of LIGHT activity ameliorates insulin-dependent diabetes mellitus and graft-vs-host disease, indicating the essential role of LIGHT in T cell-mediated diseases (15, 16). Consistently, LIGHT−/− mice showed significant defects in the function of cytotoxicity in CD8 cells (35, 36), and targeted mutation of LIGHT prolonged the survival of cardiac transplantation (37). In the current study we showed that LIGHT caused a deletion of immature thymocytes bearing high affinity TCR. Therefore, LIGHT, as a costimulatory molecule, basically can cause the different responses for immature vs mature T cells. Up-regulation of LIGHT promotes the deletion of potentially autoreactive T cells in thymic selection, but activates mature T cells in the periphery leading to autoimmune diseases. Thus, our results indicate that immature vs mature T cells can indeed respond differently to the same stimulus, and LIGHT plays a role in the negative selection of thymocytes by inducing the apoptosis of thymocytes bearing high affinity TCR.

**References**


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