

# A Lymphotoxin-IFN- $\beta$ Axis Essential for Lymphocyte Survival Revealed during Cytomegalovirus Infection<sup>1</sup>

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The importance of lymphotoxin (LT)  $\beta$ R (LT $\beta$ R) as a regulator of lymphoid organogenesis is well established, but its role in host defense has yet to be fully defined. In this study, we report that mice deficient in LT $\beta$ R signaling were highly susceptible to infection with murine CMV (MCMV) and early during infection exhibited a catastrophic loss of T and B lymphocytes, although the majority of lymphocytes were themselves not directly infected. Moreover, bone marrow chimeras revealed that lymphocyte survival required LT $\alpha$  expression by hemopoietic cells, independent of developmental defects in lymphoid tissue, whereas LT $\beta$ R expression by both stromal and hemopoietic cells was needed to prevent apoptosis. The induction of IFN- $\beta$  was also severely impaired in MCMV-infected LT $\alpha^{-/-}$  mice, but immunotherapy with an agonist LT $\beta$ R Ab restored IFN- $\beta$  levels, prevented lymphocyte death, and enhanced the survival of these mice. IFN- $\alpha$  $\beta$ R<sup>-/-</sup> mice were also found to exhibit profound lymphocyte death during MCMV infection, thus providing a potential mechanistic link between type 1 IFN induction and lymphocyte survival through a LT $\alpha$  $\beta$ -dependent pathway important for MCMV host defense. *The Journal of Immunology*, 2005, 174: 7217–7225.

**H**erpesviruses and their vertebrate hosts share a long evolutionary history that is revealed by the multitude of tactics used by the virus to evade both innate and adaptive immune responses of the host. The result of this evolutionary tango is the ability of herpesviruses to establish a persistent infection without overt pathogenicity. CMV, a  $\beta$ -herpesvirus, provides an insightful model of host-virus interactions, exemplified by its extensive array of evasion strategies, from disruption of Ag-processing pathways to the modulation of cytokines, all most likely contributing to the success of CMV in establishing coexistence with its host (1). Control of murine CMV (MCMV)<sup>6</sup> requires a robust innate immune response, mediated predominately by IFNs and NK

cells, to limit viral replication in the spleen and liver (2–4). Adaptive cellular responses, characterized by CD8<sup>+</sup> and CD4<sup>+</sup> T cells, mediate viral clearance and control virion shedding from the salivary gland (5–7). Together these defenses sufficiently restrict CMV to a latent/persistent infection without palpable disease in the immunocompetent host, whereas suppression of the immune system invariably leads to viral reactivation and disseminated disease (8).

The cell death and survival activities of the TNF-related cytokines (9) may provide strong selective pressure for viruses, such as CMV, to evolve immune evasion strategies that promote host-virus coexistence (10). Not unexpectedly, several cytokines and receptors belonging to the TNF superfamily are specifically targeted by herpesviridae (11). For example, recent evidence indicates that the lymphotoxin (LT)  $\alpha$  $\beta$ -LT $\beta$ R signaling pathway may function as an antiviral effector system in the host's defense against CMV. In vitro studies have shown that LT $\beta$ R signaling induces the expression of IFN- $\beta$  in fibroblasts infected with human CMV, resulting in viral stasis in which viral replication can be curtailed without the concomitant destruction of virus-infected cells (12). Mice genetically deficient in LT $\alpha$  were found to be highly susceptible to MCMV, suggesting a potentially conserved role for this cytokine/receptor family in CMV host defense (12). However, even though the correlation between this in vitro and in vivo data is striking, the evolutionary divergence between human and mouse CMV is substantial, thus precluding any direct conclusion regarding the mechanistic role of LT in a physiologic context.

Genetic models in mice have established that the LT $\alpha$  $\beta$ -LT $\beta$ R pathway is crucial for the complex processes involved in the development (13–18) and homeostasis of lymphoid tissues (19–23) and for the differentiation of NK and NK-T cells (24–30), key effectors of innate defenses. As a result, LT-deficient mice (i.e., LT $\alpha^{-/-}$ , LT $\beta^{-/-}$ , LT $\beta$ R<sup>-/-</sup>, and the double-knockout LT $\beta$ /

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<sup>6</sup> Abbreviations used in this paper: MCMV, murine CMV; BM, bone marrow; DC, dendritic cell; EGFP, enhanced GFP; HVEM, herpesvirus entry mediator; LCMV,

lymphocytic choriomeningitis virus; LIGHT, TNF superfamily member 14; LT, lymphotoxin; p.i., postinfection.

LIGHT<sup>-/-</sup>), in which LT $\alpha\beta$  is unable to effectively activate the LT $\beta$ R, all demonstrate a complex, developmentally fixed phenotype characterized by a lack of secondary lymphoid organs, multiple defects in splenic architecture, deficiencies in the number and function of NK/NK T cells, and decreased levels of certain chemokines. However, in contrast to their innate response deficiencies, the adaptive immune system in LT-deficient mice appears largely intact, with normal T and B lymphocyte development, although impaired dendritic cell (DC) migration has been suggested (29, 31). In addition to LT $\alpha\beta$ , the LT $\beta$ R can also be activated by a second ligand LIGHT, which, in turn, is able to engage yet another receptor, the herpesvirus entry mediator (HVEM) (32). In contrast to LT-deficient mice, LIGHT-deficient mice possess a full complement of lymphoid organs and normal levels of all lymphocyte subsets (18, 33, 34), whereas constitutive transgenic expression of LIGHT has been shown to induce destructive T cell-mediated inflammatory processes and autoimmunity (35, 36). Although the role of the LIGHT-HVEM system in host defense has only recently begun to be investigated, initial reports indicate that HVEM-deficient mice demonstrate normal lymphoid tissue development and lymphocyte differentiation (61).

The shared usage of the LT $\beta$ R by at least two different cytokines, LT $\alpha\beta$  and LIGHT, as well as other potentially unknown abnormalities associated with the developmentally fixed LT-deficient phenotype, adds to the complexity of delineating the contributions of this cytokine-receptor system to host defense. In the present investigation, we identify LT as an essential effector system that contributes substantially to the induction of the IFN- $\beta$  system early during infection by MCMV. The results reveal the previously unrecognized involvement of the LT $\alpha\beta$ -LT $\beta$ R and IFN- $\beta$  pathways in promoting the survival of the adaptive immune system to this viral pathogen. Pharmacological and genetic approaches demonstrate that the LT $\beta$ R pathway is a critical effector pathway that links the pleiotropic IFN- $\alpha\beta$  response to the survival of the adaptive immune response. Thus, by counteracting the virulence of MCMV for the lymphoid compartment, this LT-IFN axis is likely to play an important role in the establishment of host-virus coexistence.

## Materials and Methods

### Mice and virus infection

Wild-type C57BL/6 (B6) mice were purchased from The Jackson Laboratory. LIGHT<sup>-/-</sup>, LT $\beta$ /LIGHT<sup>-/-</sup> (18), LT $\beta$ R<sup>-/-</sup> (17), and HVEM<sup>-/-</sup> knockout mice (61) were backcrossed 5 generations ( $n = 5$ ) to B6 mice. LT $\alpha$ <sup>-/-</sup> were backcrossed 8 generations to B6 ( $n = 8$ ) (14), LT $\beta$ <sup>-/-</sup> 10 generations ( $n = 10$ ) (16), and IFN- $\alpha\beta$ R<sup>-/-</sup> 6 generations ( $n = 6$ ) (37). All mice were used experimentally at 6–12 wk of age and were age and sex matched as closely as possible. BALB/c mice expressing a soluble murine LT $\beta$ R/human IgG1-Fc fusion protein (LT $\beta$ R-Fc) as a transgene (38) and which demonstrated serum levels  $>1.2 \mu\text{g/ml}$  as determined by ELISA were used in experiments; nontransgenic littermates served as wild-type controls. For bone marrow (BM) transfers, recipient mice (LT $\alpha$ <sup>-/-</sup>, LT $\beta$ R<sup>-/-</sup>, IFN- $\alpha\beta$ R<sup>-/-</sup>, or B6) were lethally irradiated (1000 rad) and given antibiotics in the drinking water for 3 wk postreconstitution. BM cells from donor mice were injected into the lateral tail vein or retro-orbital sinus of the irradiated recipients ( $10^7$  cells/per mouse). The chimeric mice were not used experimentally until at least 8 wk posttransfer, and all mice were bred and housed under specific pathogen-free conditions. All experiments were conducted following the guidelines of the La Jolla Institute for Allergy and Immunology's Institutional Animal Care and Use Committee.

MCMV (Smith strain) prepared in salivary glands was used to infect mice by i.p. administration, and infectious virus in organ homogenates was quantified by plaque assay on NIH 3T3 cells, as described (39). rMCMV (Smith strain) containing enhanced GFP (EGFP) under the native IE1 promoter (40) was produced and harvested, as described above.

### Cytokine assays

Cytokine gene expression was assayed by quantitative RT-PCR (Stratagene Mx 4000), as described (12). Organs from mice were solubilized in TRIzol reagent (Invitrogen Life Technologies), and total cellular RNA was isolated. The primers for detection of murine IFN $\beta$ , LT $\alpha$ , and LT $\beta$  were: IFN- $\beta$ , 5'-ctctccataactataagcag-3' and 5'-cttcagtgaggtaaggtacc-3'; LT $\alpha$ , 5'-cctcagaagcacttgacc-3' and 5'-actcttcgctcactgaggaga-3'; and LT $\beta$ , 5'-acccatagggccttgatg-3' and 5'-acgcttctcttgctcgc-3'. For determination of fold differences in mRNA levels, the cycle number, defined as relative fluorescence crossed the manually set threshold expression value, was determined using the Mx4000 analysis software. The 18S RNA was used for normalization.

### LT $\beta$ R-Fc and LT $\beta$ R Ab

Expression and purification of baculovirus-derived LT $\beta$ R-Fc have been described previously (41). Human IgG (Gammagard, clinical grade; Baxter) served as the control protein for LT $\beta$ R-Fc. All protein preparations contained  $<10$  endotoxin units/mg protein, as determined by *limulus* lysate assay. Mice were injected with purified LT $\beta$ R-Fc protein or human IgG control protein (100  $\mu\text{g}/0.5$  ml PBS, i.p.) 4 h before infection with MCMV. A rat mAb to mouse LT $\beta$ R was generated by immunizing a Sprague-Dawley rat with LT $\beta$ R-Fc protein (42). The anti-LT $\beta$ R Ab was purified by ammonium sulfate precipitation or protein G chromatography (endotoxin level  $<10$  endotoxin units/mg protein) from SCID ascites. Mice were injected with 100  $\mu\text{g}$  i.p. of anti-LT $\beta$ R Ab (3C8) or 100  $\mu\text{g}$  of isotype control Ab (BD Pharmingen) 4 h before or immediately before infection with MCMV. Statistical analysis was performed using PRISM (GraphPad).

### Microscopy

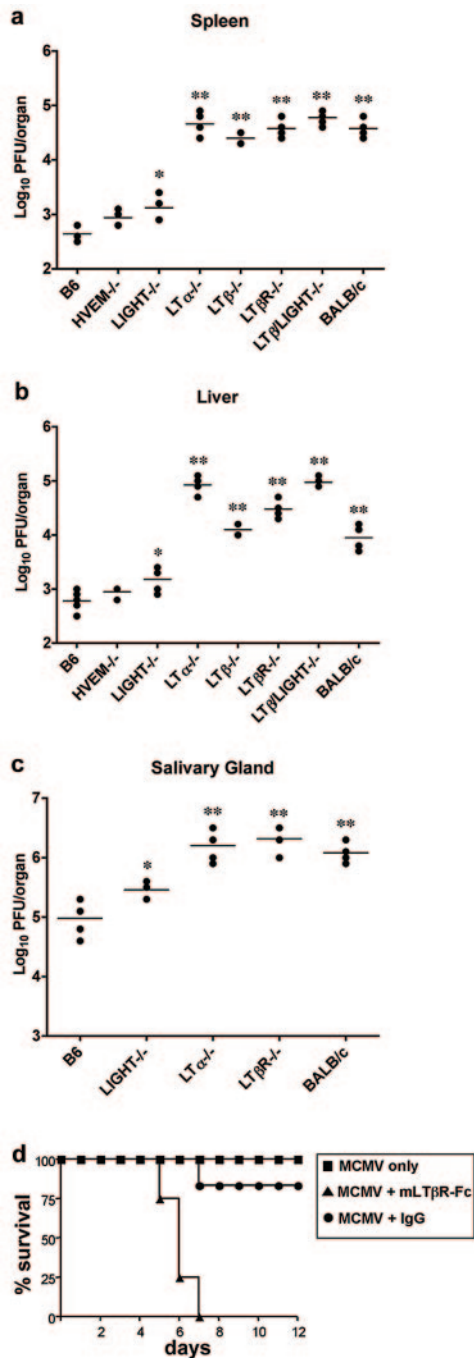
Tissues for pathologic examination were fixed in 10% buffered Formalin, embedded in paraffin, sectioned, and stained with H&E.

### Flow cytometry

Cell suspensions were prepared from spleens or livers; RBC were removed by lysis in hypotonic buffer; and mononuclear cells were isolated from perfused livers on a Percoll gradient. Cells were preincubated with anti-Fc $\gamma$ RII/III blocking reagent (2.4G2; BD Pharmingen), stained with direct fluorochrome-conjugated mAbs at 1  $\mu\text{g/ml}$  per  $5 \times 10^5$  cells, and then incubated on ice for 30 min before washing the cells three times in PBS containing 0.1% BSA and 0.01% sodium azide. The following Abs were used for lymphocyte subset detection: CyChrome RM4-4 (anti-CD4), allophycocyanin 53-6.7 (anti-CD8), CyChrome RA3-6B2 (anti-B220), PE HL3 (anti-CD11c), allophycocyanin M1/70 (anti-CD11b), PE MR5-2 (anti-V $\beta$ 8.1/8.2), and PE RR3-15 (anti-V $\beta$ 11) (BD Pharmingen). Cell death was assessed by annexin V staining (annexin V FITC; BD Pharmingen) and nuclear DNA staining by propidium iodide for subdiploid peak analysis, as described previously (43). Stained cells were analyzed on a FACSCalibur flow cytometer using CellQuest research software (BD Biosciences).

## Results

LT $\alpha$ <sup>-/-</sup> mice were shown to be highly susceptible to MCMV, requiring 100-fold less virus than wild-type B6 mice to induce a lethal infection (12). Similarly, BALB/c mice expressing LT $\beta$ R-Fc as a transgene (38) also demonstrated an increased susceptibility to MCMV infection; thus, the contribution of LT to host resistance appears to be independent of the mouse strain used. The susceptibility of LT-deficient mice to MCMV was also evident by the increased virus production at both early times (day 3) in the spleen and liver and at later times (day 12) in the salivary gland (Fig. 1). Virus titers in the spleen and liver were increased by 100-fold or more in LT $\alpha$ <sup>-/-</sup>, LT $\beta$ <sup>-/-</sup>, and LT $\beta$ R<sup>-/-</sup> mice, and in double-knockout LT $\beta$ /LIGHT<sup>-/-</sup> mice ( $p < 0.0001$ ), and by 5-fold in LIGHT<sup>-/-</sup> mice ( $p < 0.05$ ) as compared with wild-type B6 mice (Fig. 1, *a* and *b*). However, HVEM<sup>-/-</sup> mice did not show increased virus production in any of the organs examined. Similarly, virus titers in the salivary glands were also increased in all of the LT-deficient mice strains and to a lesser extent in LIGHT<sup>-/-</sup> mice (Fig. 1*c*). Together, these results indicate that while the LT $\alpha\beta$ -LT $\beta$ R pathway is clearly the dominant interaction critical for controlling replication of MCMV, LIGHT also contributes to MCMV resistance. The discordance of phenotype between LIGHT- and



**FIGURE 1.** Increased MCMV susceptibility in LT-deficient mice. *a–c*, Gene-deficient mice and wild-type mice (B6, BALB/c) were infected with  $2 \times 10^4$  PFU of MCMV. Spleens and livers were harvested at day 3 p.i. and salivary glands at day 12 p.i. Individual organs from MCMV-infected mice ( $n = 4–5$  mice/group) were assayed for infectious virus, and the mean titer per group was represented as a solid line. Data are representative of two to three similar experiments. Significance is \*,  $p < 0.05$ ; \*\*,  $p < 0.0001$ , with Student's *t* test comparing each experimental group with the B6 control mice. *d*, Wild-type B6 mice were injected with LT $\beta$ R-Fc or control human IgG and then infected with MCMV ( $2 \times 10^5$  PFU). Mice were monitored daily for morbidity and mortality.

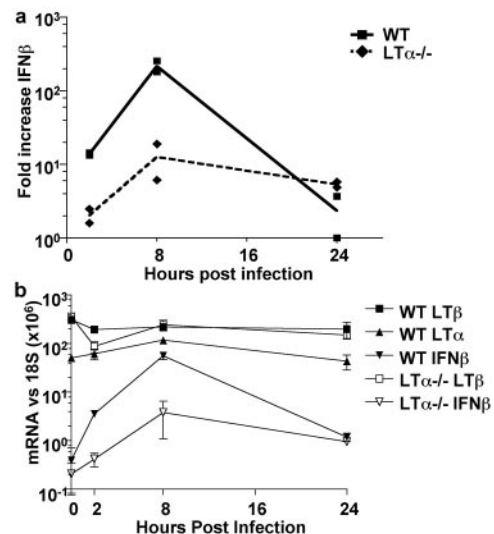
HVEM-deficient mice for virus production suggests that LIGHT is acting via the LT $\beta$ R.

LT-deficient mice lack most secondary lymphoid organs and possess a number of defects in the development and organization of the microenvironments in the spleen, as well as in the devel-

opment and maintenance of NK and NK-T cells, which could underlie their acute susceptibility to MCMV. To unambiguously distinguish between these and other potentially unrecognized developmental defects in LT-deficient mice, wild-type B6 mice were injected with LT $\beta$ R-Fc 4 h before infection with MCMV to inactivate the LT $\alpha$  $\beta$ /LIGHT ligands during infection. The LT $\beta$ R-Fc-treated B6 mice succumbed to infection with MCMV at a viral inoculum that did not cause appreciable death in control mice (Fig. 1*d*). These results indicate that developmentally fixed, LT-dependent lymphoid organ-associated defects are not solely responsible for the increased MCMV susceptibility demonstrated by LT-deficient mice. Instead, these data are consistent with the idea that LT $\beta$ R signaling can act as an effector system during MCMV infection.

The failure to control MCMV replication in LT-deficient mice may reside in the inability to activate innate defenses such as the induction of IFN- $\beta$  (12). IFN- $\beta$  induction is an essential innate defense mechanism (4) that is activated early during MCMV infection of B6 mice, with mRNA in the spleen reaching peak levels at  $\sim 8$  h postinfection (p.i.) as measured by quantitative RT-PCR (Fig. 2*a*). Strikingly, LT $\alpha$ <sup>-/-</sup> mice showed a severe defect in the induction of IFN- $\beta$  mRNA in the spleen at early times after infection, with levels of mRNA 10- to 40-fold lower than those observed in wild-type B6 mice. This result indicates that LT $\beta$ R signaling may be required to activate the expression of IFN- $\beta$  and subsequent innate defenses.

As expected, LT $\alpha$  and LT $\beta$  mRNA in the spleen of uninfected B6 mice was readily detectable, and over this time course no significant change occurred following infection with MCMV (Fig. 2*b*). Because B and T cells are the major source of LT $\alpha$  $\beta$  expression in the mouse spleen (44, 45), and DC have been proposed to be the major source of type 1 IFN in response to MCMV (46), the cross talk between lymphocytes and DC may be impaired in LT-deficient mice, prompting us to examine these cell types during MCMV infection.

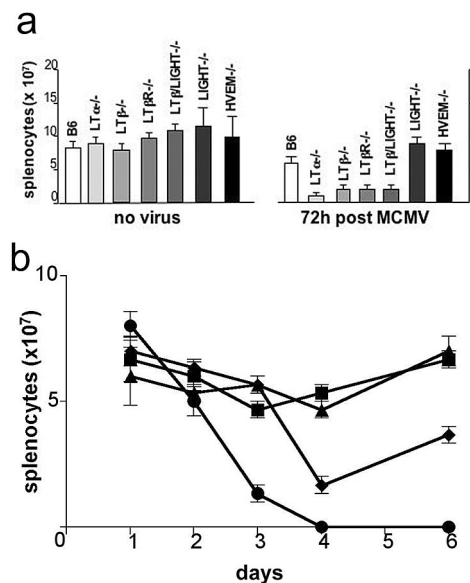


**FIGURE 2.** Expression of IFN- $\beta$  and LT $\alpha$  $\beta$  during MCMV infection. *a*, LT $\alpha$ -deficient (LT $\alpha$ <sup>-/-</sup>) or wild-type B6 (WT) mice were infected with MCMV ( $2 \times 10^5$  PFU), and spleens were harvested at indicated times for analysis of IFN- $\beta$  expression levels by real-time PCR analysis. The fold increase is relative to mock-infected mice (injected with PBS) at 8 h; basal expression levels of IFN- $\beta$  in LT $\alpha$ <sup>-/-</sup> and wild type were equivalent. *b*, LT $\alpha$ , LT $\beta$ , and IFN- $\beta$  levels in B6 and LT $\alpha$ <sup>-/-</sup> mice were measured by quantitative RT-PCR relative to 18S mRNA levels. Data are representative of two experiments ( $n = 2$  or 3 mice per group; mean  $\pm$  SEM).

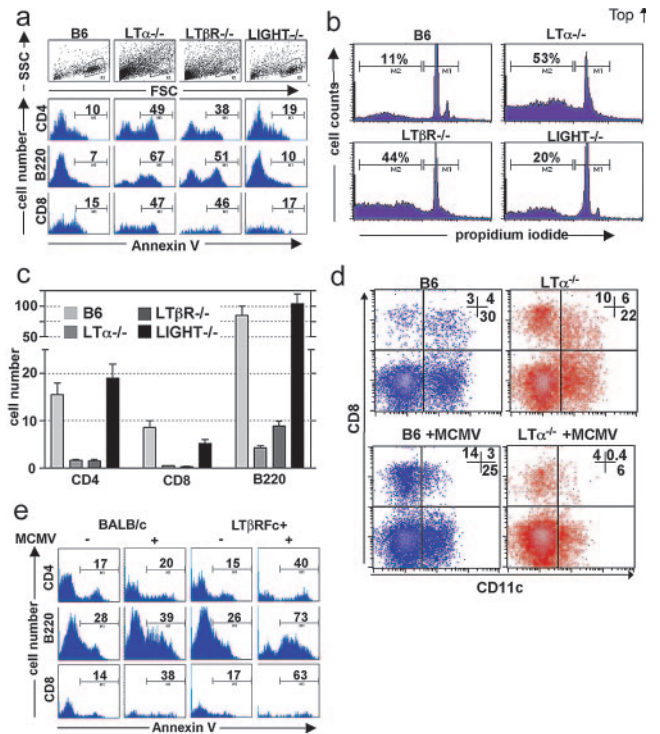
## Apoptotic collapse of the adaptive immune system

Although uninfected LT-deficient mice contain normal numbers of total splenocytes, ( $0.7\text{--}1 \times 10^8$ ), MCMV infection of LT $\alpha^{-/-}$ , LT $\beta^{-/-}$ , LT $\beta$ R $^{-/-}$ , and LT $\beta$ /LIGHT $^{-/-}$  mice induced a catastrophic loss of cells that was not seen in similarly infected wild-type B6, LIGHT $^{-/-}$ , or HVEM $^{-/-}$  mice (Fig. 3a). Moreover, the depletion of cells occurred rapidly (between days 2 and 4 p.i.) and was dependent upon the dose of virus (Fig. 3b). Flow cytometry (forward vs side scatter) revealed a large cell population characteristic of nonviable cells from MCMV-infected LT $\alpha^{-/-}$  or LT $\beta$ R $^{-/-}$  mice and, to a lesser extent, from LIGHT $^{-/-}$  mice that was not observed in control B6 mice (Fig. 4a, upper panel). Further analyses revealed that cells in the live gate from MCMV-infected LT $\alpha^{-/-}$  and LT $\beta$ R $^{-/-}$  mice showed a dramatic increase (3- to 9-fold) in staining with the apoptotic marker annexin V on CD4 $^{+}$  and CD8 $^{+}$  T cells, and on B220 $^{+}$  B cells (Fig. 4a, lower panels). Furthermore, splenocytes from LT $\alpha^{-/-}$  and LT $\beta$ R $^{-/-}$  mice displayed a significant increase in the fraction of nuclei with subgenomic levels of DNA, as detected by propidium iodide staining (Fig. 4b). Interestingly, MCMV-infected LIGHT $^{-/-}$  mice showed a significant ( $\sim 2$ -fold) increase in the percentage of annexin V-positive CD4 $^{+}$  T cells as compared with B6 mice (Fig. 4a) and a similar increase in subgenomic DNA when compared with B6 controls (Fig. 4b). Although the percentages of CD4 $^{+}$ , CD8 $^{+}$ , and B220 $^{+}$  lymphocytes were similar in all strains of mice tested before MCMV infection, a dramatic decrease in all of these lymphocyte subsets was observed in MCMV-infected LT $\alpha^{-/-}$  and LT $\beta$ R $^{-/-}$  mice, with the CD8 $^{+}$  T cell compartment demonstrating the greatest loss in cell number (Fig. 4c).

The number of hepatic DC defined by costaining with Abs to CD11c and CD8 was also decreased ( $>10$ -fold difference) in



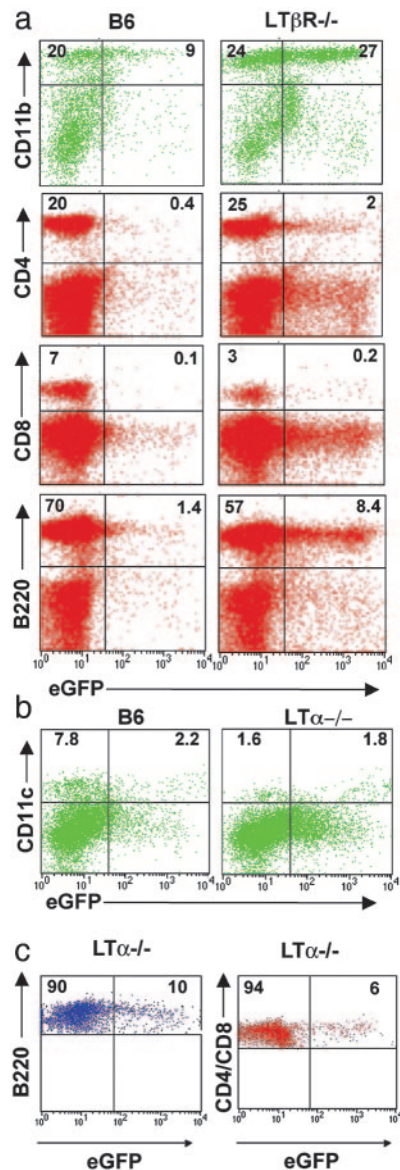
**FIGURE 3.** Splenocyte depletion following MCMV infection in LT-deficient mice. *a*, Total splenocyte counts from B6 and gene-deficient mice were determined before infection and at 72 h p.i. with  $2 \times 10^5$  PFU of MCMV. Data are the mean  $\pm$  SEM from a representative of three independent experiments ( $n = 3$  mice/group). Significance is  $p < 0.0001$  between the uninfected LT $\alpha^{-/-}$ , LT $\beta^{-/-}$ , LT $\beta$ R $^{-/-}$ , and LT $\beta$ /LIGHT $^{-/-}$  mouse groups and the MCMV-infected LT $\alpha^{-/-}$ , LT $\beta^{-/-}$ , LT $\beta$ R $^{-/-}$ , and LT $\beta$ /LIGHT $^{-/-}$  mouse groups. *b*, Spleens were harvested at 24-h intervals from B6 mice infected with MCMV at  $2 \times 10^5$  PFU ( $\blacksquare$ ) or  $2 \times 10^4$  PFU ( $\blacktriangle$ ), or LT $\alpha^{-/-}$  mice infected with  $2 \times 10^5$  PFU ( $\bullet$ ) or  $2 \times 10^4$  PFU ( $\blacklozenge$ ). The data are the mean  $\pm$  SEM from a representative of three independent experiments ( $n = 3$  mice/group).



**FIGURE 4.** MCMV induces lymphocyte apoptosis in LT-deficient mice. *a*, Light scatter profiles and histograms of splenocytes from B6 and gene-deleted mice at day 3 p.i. with  $2 \times 10^5$  PFU of MCMV. Cells were stained with anti-lymphocyte subset Abs and annexin V, and the percentages of CD4 $^{+}$ , B220 $^{+}$ , and CD8 $^{+}$  cells (gated on live cells) are indicated for each group of mice. The data are representative of four experiments ( $n = 3$  mice/group). *b*, DNA content of splenocytes from B6 and LT-deficient mice following infection with  $2 \times 10^5$  PFU of MCMV (day 3 p.i.) revealed by staining with propidium iodide. The percentage of nuclei with subdiploid DNA content is indicated. The experiment was repeated twice with similar results ( $n = 3$  mice/group). *c*, The total cell number ( $\times 10^6$ ) of lymphocyte subsets was calculated from the percentage of CD4 $^{+}$ , CD8 $^{+}$ , and B220 $^{+}$  cells determined by flow cytometry present in the spleens at day 3 p.i. with  $2 \times 10^5$  PFU of MCMV. The experiment was repeated twice with similar results ( $n = 3$  mice/group; mean  $\pm$  SEM). Significance is  $p < 0.001$  between the MCMV-infected LT $\alpha^{-/-}$  and LT $\beta$ R $^{-/-}$  groups and B6 mice for the CD4 $^{+}$ , CD8 $^{+}$ , and B220 $^{+}$  cell subsets. *d*, Flow cytometric analysis of liver mononuclear lymphoid cells isolated from uninfected and MCMV-infected B6 and LT $\alpha^{-/-}$  mice (perfused livers at day 3 p.i.). The percentages of positive cells in each quadrant are indicated; data representative of three independent experiments ( $n = 2\text{--}3$  mice/group). *e*, Annexin V staining of lymphocyte subsets from spleens of BALB/c (littermates) and LT $\beta$ R-Fc transgenic mice on a BALB/c background (uninfected (-) or infected with MCMV (+) at day 3 with  $2 \times 10^5$  PFU MCMV) is shown. The percentage of CD4 $^{+}$ , B220 $^{+}$ , and CD8 $^{+}$  cells (live cell gate) is indicated in the histograms and is representative of two independent experiments ( $n = 4$  mice/group).

MCMV-infected LT $\alpha^{-/-}$  mice as compared with wild-type B6 mice (Fig. 4d). The loss of hepatic CD11c $^{+}$ CD8 $^{+}$  DC is consistent with the ability of this DC subset in the spleen to support lytic replication of MCMV (47). Additionally, a loss of hepatic CD8 $^{+}$ CD11c $^{-}$  T cells was also seen in MCMV-infected LT $\alpha^{-/-}$  mice, as compared with similarly infected wild-type B6 mice, indicating lymphocyte death was not restricted to the spleen (also observed in the thymus; data not shown).

The cell death observed in MCMV-infected, LT-deficient mice also prompted an examination of lymphocyte viability in BALB/c mice challenged with MCMV. BALB/c mice are more susceptible to MCMV than B6 mice because they lack the *Cmv1*-encoded



**FIGURE 5.** MCMV infects macrophages and DC, but not most lymphocytes. B6 or LT-deficient mice were infected with rMCMV expressing eGFP at  $2 \times 10^5$  PFU, and 48 h later splenocytes were stained with lymphocyte subset markers: *a*, CD11b for macrophages, CD4 or CD8 for T cells, and B220 for B cells in  $LT\beta R^{-/-}$  mice; and *b*, CD11c for DC in  $LT\alpha^{-/-}$  mice. The data are representative of three experiments ( $n = 3$  mice/group). *c*, Splenocytes from MCMV-infected  $LT\alpha^{-/-}$  mice harvested at 72 h p.i. were subgated on either  $B220^+$  annexin $^+$  or  $CD4^+/CD8^+$  annexin $^+$  cells (upper left quadrants), and the percentage of eGFP $^+$  cells within these populations is shown in the upper right quadrants.

Ly-49H NK cell-activating receptor required for controlling virus replication in the spleen (48–50). MCMV infection of BALB/c mice caused a specific increase in annexin V staining of  $CD8^+$  T cells, whereas  $CD4^+$  T cells appeared unchanged and  $B220^+$  B cells showed only a fractional increase in apoptosis (Fig. 4e). In contrast, BALB/c mice expressing soluble  $LT\beta R$ -Fc as a transgene showed a striking increase in annexin V staining in all lymphocyte subsets examined (CD8, CD4, and B220) similar to LT-deficient mice, suggesting that  $LT\alpha\beta$  and LIGHT contribute to lymphocyte viability independently of *Cmv1*.

$LT\beta R$ -deficient mice infected with HSV-1 ( $\alpha$ -herpesvirus, McKrae strain) or with lymphocytic choriomeningitis virus (LCMV; arenavirus, Armstrong strain) did not show the apoptotic

**Table I.** Analysis of MCMV-induced lymphocyte death in BM-reconstituted  $LT\alpha^{-/-}$  and  $LT\beta R$ -deficient mice

Donor→Recipient <sup>b</sup>	Apoptosis (% annexin V staining) <sup>a</sup>		
	CD4	CD8	B220/CD19
<b>Ligand</b>			
B6→B6 <sup>c</sup>	22 ± 2	13 ± 3	20 ± 3
$LT\alpha^{-/-}$ → $LT\alpha^{-/-}$	84 ± 2	61 ± 5	80 ± 4
$LT\alpha^{-/-}$ →B6	84 ± 2	70 ± 3	82 ± 1
B6→ $LT\alpha^{-/-}$	23 ± 2	12 ± 1	27 ± 4
<b>Receptor</b>			
B6→B6	21 ± 4	14 ± 3	23 ± 3
$LT\beta R^{-/-}$ → $LT\beta R^{-/-}$	81 ± 3	76 ± 5	89 ± 2
$LT\beta R^{-/-}$ →B6	33 ± 5	29 ± 8	38 ± 4
B6→ $LT\beta R^{-/-}$ R $^{-/-}$	47 ± 3	40 ± 4	37 ± 3

<sup>a</sup> The percentage of apoptosis was determined by flow cytometry of T and B cell subsets costained with either anti-CD4, CD8, or B220/CD19 and annexin V. Data represent the mean ± SEM from mice in each recipient group ( $n = 3$  mice/group).

<sup>b</sup> Eight weeks following BM transfer, mice were infected with  $2 \times 10^5$  PFU of MCMV, and spleens were harvested 72 h later.

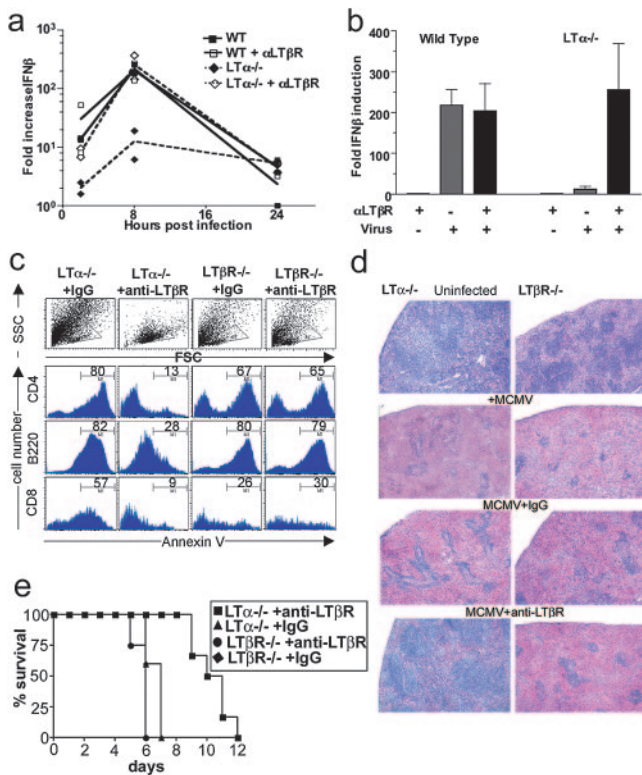
<sup>c</sup> Two mice in recipient group (mean ± range).

demise of lymphocytes observed during MCMV infection (data not shown). In addition, the proliferation and contraction of the  $V\beta 8^+$  TCR  $CD4^+$  and  $CD8^+$  subsets of T cells in response to staphylococcus enterotoxin B occurred normally in  $LT\alpha^{-/-}$  mice, as did the proliferation and viability of B cells and macrophages to LPS (data not shown). Thus, the loss of lymphocyte viability observed in LT-deficient mice during MCMV infection does not appear to be a global defect in response to all pathogens or inflammatory stimuli.

To help identify the subpopulations of cells infected with MCMV, a recombinant virus expressing EGFP under control of the IE1 promoter was used to identify infected subpopulations of cells in the spleen (40). A significant increase in EGFP $^+$  cells (27% of the cells; 3-fold) was observed in the  $CD11b^+$  cell population from  $LT\beta R^{-/-}$  mice by 2 days following infection (Fig. 5a, top panel). Moreover, EGFP fluorescence was detected in ~50% of the  $CD11c^+$  DC population in  $LT\alpha^{-/-}$  mice when compared with 20% of DC in B6 mice (Fig. 5b), consistent with previous observations that MCMV can infect macrophages and DC (40, 47). By contrast, EGFP fluorescence in either CD4 or CD8 T cell subpopulations was limited to a minor fraction of these lymphocytes from infected  $LT\beta R^{-/-}$  or B6 mice (Fig. 5a, lower panels), although a significant increase in EGFP expression occurred in 8% of the  $B220^+$  cells in  $LT\beta R^{-/-}$  mice. The increase in splenocyte infection is associated with significantly increased levels of CMV replication in the absence of LT signaling.

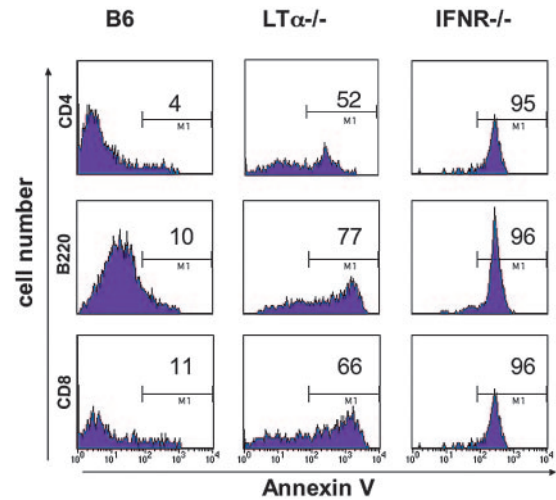
To determine whether direct viral infection contributed to lymphocyte apoptosis, gates were established to analyze the level of EGFP expression in the annexin V $^+$   $CD4/8$  and B220 subsets undergoing death. The results indicated that both annexin V $^+$  T and B cell subsets were >90% EGFP negative at day 3 following infection (Fig. 5c). This result indicates that the degree of apoptosis (>80%) is not proportional to virus-derived EGFP expression, indicating that the observed lymphocyte apoptosis does not appear to result from direct lytic infection with MCMV.

Adoptive transfers of BM from wild-type B6 (LT-sufficient) or LT-deficient donor mice into lethally irradiated LT-sufficient or LT-deficient recipients and their subsequent infection with MCMV revealed that the apoptotic phenotype was not observed when wild-type B6 BM cells were transferred into  $LT\alpha^{-/-}$  recipients. This indicates that hemopoietic cell expression of  $LT\alpha$  is required



**FIGURE 6.** LT $\beta$ R activation in vivo restores IFN- $\beta$  induction, rescues lymphocytes from cell death, and enhances survival of MCMV-infected LT $\alpha^{-/-}$  mice. LT $\alpha^{-/-}$ , LT $\beta$ R $^{-/-}$ , or B6 (WT) mice were injected i.p. with agonist anti-LT $\beta$ R or isotype control Ab (rat IgG; 100  $\mu$ g) immediately before infection with  $2 \times 10^5$  PFU of MCMV. *a*, RNA was isolated from spleens of MCMV-infected mice, and IFN- $\beta$  mRNA was determined by RT-PCR relative to the level of IFN- $\beta$  mRNA at 8 h from mock-infected mice ( $n = 2$  mice/group; data for each mouse are shown). *b*, B6 (wild-type) or LT $\alpha^{-/-}$  mice were infected with MCMV and treated with anti-LT $\beta$ R or both, and RNA was isolated from spleens harvested at 8 h p.i. The IFN- $\beta$  mRNA levels were determined by RT-PCR relative to mock-infected controls. *c*, The percentage of CD4 $^{+}$ , CD8 $^{+}$ , and B220 $^{+}$  cell subsets costained for annexin V (gated on live cells) at day 3 p.i. with MCMV for each of the treatment groups is shown. The data are representative of three independent experiments ( $n = 3$  mice/group). *d*, H&E-stained spleen sections of MCMV-infected LT $\alpha^{-/-}$  and LT $\beta$ R $^{-/-}$  mice that had been treated with the anti-LT $\beta$ R Ab or the isotype control Ab, as detailed above in *c*; 10 $\times$  magnification. *e*, LT $\alpha^{-/-}$  and LT $\beta$ R $^{-/-}$  mice were treated with the anti-LT $\beta$ R Ab or with isotype control Ab 4 h before or immediately before MCMV infection. The morbidity and mortality of the mice were observed for 12 days p.i. The data are representative of two independent experiments (4–6 mice/group). Significance is  $p < 0.01$  between the anti-LT $\beta$ R Ab-treated LT $\alpha^{-/-}$  mice and the other groups of mice.

for the protection of lymphocytes from apoptosis (Table I). Furthermore, because the LT $\alpha^{-/-}$  recipients lack secondary lymphoid organs and have defects in lymphoid tissue architecture, this result also indicates that the developmentally fixed lymphoid tissue defects do not contribute to the apoptotic phenotype. In contrast, only a partial restoration of lymphocyte viability was observed following the transfer of wild-type BM into irradiated LT $\beta$ R $^{-/-}$  recipient mice, as was the case when LT $\beta$ R $^{-/-}$  BM was transplanted into wild-type B6 recipient mice (Table I). This partial, nonreciprocal reconstitution indicates that LT $\beta$ R expression is required on both stromal and hemopoietic cells and suggests that these compartments must cooperate to fully protect lymphocytes from apoptosis during MCMV infection.



**FIGURE 7.** MCMV also induces lymphocyte apoptosis in IFN- $\alpha\beta$ R $^{-/-}$  mice. Histograms of splenocytes from B6, LT $\alpha^{-/-}$ , and IFN- $\alpha\beta$ R $^{-/-}$  mice at day 3 p.i. with  $2 \times 10^5$  PFU of MCMV. Cells were stained with anti-lymphocyte subset Abs and annexin V, and the percentages of CD4 $^{+}$ , B220 $^{+}$ , and CD8 $^{+}$  cells (gated on live cells) are indicated for each group of mice. This experiment was repeated on three separate occasions.

#### Activation of LT $\beta$ R induces IFN- $\beta$ and prevents lymphocyte death during MCMV infection

If there is a critical effector function for LT $\beta$ R signaling in host defense during MCMV infection, we reasoned that the administration of an agonist LT $\beta$ R Ab to LT $\alpha^{-/-}$  mice should restore induction of IFN- $\beta$  mRNA and protect lymphocytes from apoptosis. As predicted, the IFN- $\beta$  response in LT $\alpha^{-/-}$  mice was fully restored to wild-type levels with anti-LT $\beta$ R Ab treatment and in a similar time course (Fig. 6*a*). However, the anti-LT $\beta$ R Ab did not induce IFN- $\beta$  in the absence of virus infection, nor did it augment induction in LT-sufficient wild-type B6 mice (Fig. 6*b*). Accordingly, MCMV-infected LT $\alpha^{-/-}$  mice treated with anti-LT $\beta$ R Ab revealed a dramatic increase in total live splenic lymphoid cells as compared with mice in the other experimental groups (Fig. 6*c*, upper panel). Specifically, the percentages of apoptotic T and B cells (annexin V $^{+}$ ) decreased by 3- to 6-fold in the anti-LT $\beta$ R Ab-treated LT $\alpha^{-/-}$  mice, but not in LT $\beta$ R $^{-/-}$  mice (Fig. 6*c*, lower panels), which establishes the specificity of the anti-LT $\beta$ R Ab. In addition, H&E-stained spleen sections offered visual evidence that the anti-LT $\beta$ R Ab treatment of MCMV-infected LT $\alpha^{-/-}$  mice rescued splenic cellularity, a finding that was not observed in similarly treated MCMV-infected LT $\beta$ R $^{-/-}$  mice (Fig. 6*d*). Furthermore, virus challenge experiments demonstrated that anti-LT $\beta$ R Ab-treated LT $\alpha^{-/-}$  mice survived significantly

Table II. Analysis of MCMV-induced lymphocyte death in BM-reconstituted IFN- $\alpha\beta$ R-deficient mice

Donor $\rightarrow$ Recipient <sup>b</sup>	Apoptosis (% annexin V staining) <sup>a</sup>		
	CD4	CD8	B220/CD19
B6 $\rightarrow$ B6	14 $\pm$ 2	22 $\pm$ 2	37 $\pm$ 7
IFN- $\alpha\beta$ R $^{-/-}$ $\rightarrow$ IFN $\alpha\beta$ R $^{-/-}$	85 $\pm$ 5	94 $\pm$ 4	92 $\pm$ 3
IFN- $\alpha\beta$ R $^{-/-}$ $\rightarrow$ B6	21 $\pm$ 2	23 $\pm$ 3	36 $\pm$ 1
B6 $\rightarrow$ IFN- $\alpha\beta$ R $^{-/-}$	13 $\pm$ 2	17 $\pm$ 3	36 $\pm$ 3

<sup>a</sup> The percentage of apoptosis was determined by flow cytometry of T and B cell subsets costained with either anti-CD4, CD8, or B220/CD19 and annexin V. Data represent the mean  $\pm$  SEM from mice in each recipient group ( $n = 3$  mice/group).

<sup>b</sup> Eight weeks following BM transfer, mice were infected with  $2 \times 10^5$  PFU of MCMV, and spleens were harvested 72 h later.

longer (mean survival of 10.5 days) in response to a lethal dose of MCMV ( $2 \times 10^5$  PFU) than isotype control-treated  $LT\alpha^{-/-}$  mice (6.5 days) or anti- $LT\beta R$  Ab-treated  $LT\beta R^{-/-}$  mice (6.0 days) (Fig. 6e).

*IFN- $\alpha\beta R^{-/-}$  mice also demonstrate lymphocyte apoptosis during MCMV infection*

To determine whether type 1 IFN are involved in lymphocyte survival, mice genetically deficient for the  $\alpha$  subunit of the common type 1 receptor (IFN- $\alpha\beta R^{-/-}$ ) on a B6 background were infected with MCMV and monitored for splenocyte apoptosis and virus production. Both the  $LT\alpha^{-/-}$  mice and, in particular, the IFN- $\alpha\beta R^{-/-}$  mice exhibited a dramatic increase in the fraction of lymphocytes staining with annexin V (Fig. 7) and a corresponding decrease in total splenocyte numbers as compared with B6 mice (B6 =  $3.0 \times 10^7$  cells,  $n = 3$ ;  $LT\alpha^{-/-}$  =  $0.3 \times 10^7$  cells,  $n = 4$ ; IFN- $\alpha\beta R^{-/-}$  =  $0.7 \times 10^7$  cells,  $n = 4$ ). Virus production (day 3 p.i.) was also elevated in both  $LT\alpha^{-/-}$  ( $3 \times 10^5 \pm 0.5$  PFU/spleen;  $n = 4$ ) and the IFN- $\alpha\beta R^{-/-}$  ( $5.5 \times 10^4 \pm 0.6$  PFU/spleen;  $n = 4$ ) mice as compared with wild-type B6 mice ( $4.5 \times 10^3 \pm 1.5$  PFU/spleen;  $n = 3$ ; both  $p < 0.001$ ). Interestingly, virus production in the spleens of IFN- $\alpha\beta R^{-/-}$  mice was nearly 10-fold lower than in  $LT\alpha^{-/-}$  mice at this time point, although lymphocyte apoptosis appeared more severe in IFN- $\alpha\beta R^{-/-}$  mice, suggesting a role for type 1 IFN signaling in splenocyte survival distinct from merely restricting CMV replication and subsequent viral burden-dependent effects (Fig. 7).

BM chimeras in which wild-type BM (from B6 mice) was transferred into lethally irradiated IFN- $\alpha\beta R^{-/-}$  mice and BM from IFN- $\alpha\beta R^{-/-}$  mice was transferred into lethally irradiated wild-type B6 mice both demonstrated a significant restoration of lymphocyte viability (Table II). These results indicate that IFN- $\alpha\beta R$  expression provided by either the stromal or the hemopoietic compartments appears sufficient for preventing lymphocyte death during MCMV infection.

## Discussion

The results presented in this work establish the  $LT\alpha\beta$ - $LT\beta R$  signaling pathway as an essential effector pathway for host defense against the  $\beta$ -herpesvirus MCMV. Mice deficient in  $LT\beta R$  signaling were found to be highly susceptible to MCMV infection, as evidenced by increased mortality, enhanced virus production in target organs, impaired IFN- $\beta$  induction, and an apoptotic collapse of the adaptive immune system affecting T and B lymphocytes and DC. This striking apoptotic phenotype, in which the majority of lymphocyte death occurs without direct MCMV infection, led us to predict that the loss of a key survival factor(s) may offer a mechanistic explanation for the observed cell death. Our results, which revealed that administering an agonist  $LT\beta R$  Ab to MCMV-infected  $LT\alpha$ -deficient mice could restore induced IFN- $\beta$  to wild-type levels, prevent lymphocyte apoptosis, and extend the survival of these mice, strongly implicate type 1 IFN as key survival factors for lymphocytes during MCMV infection. That MCMV-infected IFN- $\alpha\beta R^{-/-}$  mice also exhibited the apoptotic phenotype provides further genetic evidence that lymphocyte survival requires LT-dependent activation of IFN- $\beta$  gene expression, revealing an LT-IFN axis crucial for host defense to MCMV.

In the absence of  $LT\beta R$  signaling, the host's immune system is unable to control MCMV infection, exposing the virulent potential of MCMV. Indeed, increased MCMV susceptibility appears rather selective for the  $LT\alpha\beta$ - $LT\beta R$  cytokine signaling system because genetic deficiencies in the related receptors Fas or TNFR1 do not result in increased susceptibility to MCMV (51). With regard to the role of LT in host defense against other herpesviruses,  $LT\alpha$ -

deficient mice demonstrate increased susceptibility and functionally impaired  $CD8^+$  T cell responses to HSV-1 (52), but without the significant amount of lymphocyte apoptosis observed during MCMV infection. In contrast,  $LT\alpha$ -deficient mice do not show increased susceptibility to murine  $\gamma$ -herpesvirus-68 and are effective, albeit with slightly delayed kinetics, in clearing a productive infection and controlling latency (53). These results indicate that individual pathogen-specific characteristics must figure prominently in the susceptibility profiles demonstrated by LT-deficient mice infected not only with different viruses, but with other non-viral pathogens as well. MCMV is a virus in which both innate and adaptive immune responses are needed to effectively control this infection, and the fact that both arms of the immune response appear to be compromised in LT-deficient mice most likely contributes significantly to their susceptibility to MCMV.

Increased susceptibility in LT-deficient mice to certain viral pathogens has also been attributed to developmental defects, resulting in abnormal lymphoid tissue architecture (52, 54). For example, adoptive transfer experiments demonstrated that T cell-specific responses to LCMV were impaired when wild-type splenocytes were transferred into  $LT\alpha^{-/-}$  recipients, which lack organized microenvironments, as compared with the reciprocal transfer ( $LT\alpha^{-/-}$  cells into wild-type recipients), indicating that the development of LCMV-specific  $CD8^+$  T cell responses requires intact lymphoid tissue (54). In contrast, evidence presented in this work indicates that an  $LT\beta R$  effector system, independent of lymphoid tissue architecture, can contribute significantly to MCMV host defense. For example, BM from wild-type donor mice when transferred into  $LT\alpha^{-/-}$  recipients, which possess abnormal lymphoid architecture, prevented lymphocyte apoptosis following MCMV infection, whereas the reciprocal transfer of  $LT\alpha^{-/-}$  BM into wild-type recipients did not (see Table I), indicating that the underlying developmentally dependent lymphoid tissue defects present in  $LT\alpha^{-/-}$  mice do not appear responsible for the apoptotic phenotype.

In the absence of  $LT\beta R$  signaling, MCMV infection induced the apoptotic collapse of the adaptive immune system, revealing a phenotype not previously associated with the LT signaling pathway. All of the LT-deficient mice examined exhibited significant apoptosis of T cells (both  $CD4^+$  and  $CD8^+$ ), B cells, and DC, whereas increased apoptosis in  $LIGHT^{-/-}$  mice was limited to  $CD4^+$  T cells only. Interestingly, a selective loss of  $CD8^+$  T cells was observed in BALB/c mice, which are defective in Ly-49H NK cells. These results suggest that the survival of lymphocyte subsets may depend on signaling by different subpopulations of  $LT\alpha\beta$ / $LIGHT$ -expressing cells. However, apoptosis was observed in all lymphocyte subsets when  $LT\beta R$ -Fc was genetically introduced into BALB/c mice, underscoring the potential virulence of MCMV in the absence of an LT-mediated protective mechanism for cells of the adaptive immune system. By comparison, the level of lymphocyte apoptosis in MCMV-infected normal B6 mice was usually less than  $\sim 20\%$  at the virus inoculum used, although very high doses can induce splenic necrosis in B6 mice, suggesting that deficiency in LT lowers this threshold. In LT-deficient mice, most of the apoptosis in lymphocytes appeared to occur as a bystander effect of MCMV infection because very little virus promoter-driven EGFP expression was detected in lymphocytes that had already entered the apoptotic process, as determined by annexin V staining. This conclusion is restricted by the possibility that an abortive infection without significant viral gene expression cannot be excluded. We were also unable to block lymphocyte death by treatment with Fas-Fc fusion protein in vivo (data not shown), indicating that this death receptor system does not appear to be prominently involved during MCMV infection.

The results presented in this work identify type 1 IFN as a primary survival system for T and B lymphocytes and DC in response to MCMV. Type 1 IFN are necessary for resistance to MCMV (55), and increased virus replication in the spleens of IFN- $\alpha\beta$ R<sup>-/-</sup> mice as compared with B6 controls supports this conclusion. In response to MCMV infection, IFN- $\beta$  was poorly induced in LT $\alpha$ <sup>-/-</sup> mice, and IFN- $\alpha$  mRNA accumulation was also decreased in the spleens of LT $\alpha$ <sup>-/-</sup> mice early after infection (K. Schneider and C. Benedict, unpublished observations), suggesting a generalized failure in the type 1 IFN response. However, IFN- $\beta$  mRNA was restored to wild-type levels following treatment with an agonist LT $\beta$ R Ab, indicating that the cellular elements involved in IFN- $\beta$  production are present, but lack the appropriate stimulus. Moreover, treatment with the agonist anti-LT $\beta$ R was able to restore lymphocyte viability in MCMV-infected LT $\alpha$ <sup>-/-</sup> mice consistent with the idea that LT $\beta$ R signaling, by inducing type 1 IFN during MCMV infection, is essential for lymphocyte viability. The observation that IFN- $\beta$  can enhance the survival of activated T cells in certain scenarios supports this concept (56–59). However, most compelling is the observation that IFN- $\alpha\beta$ R<sup>-/-</sup> mice also demonstrate a profound apoptotic phenotype in response to MCMV, thus providing genetic evidence that IFN- $\alpha\beta$ R signaling is critical for lymphocyte survival during MCMV infection.

These results are consistent with the idea that LT and IFN- $\alpha\beta$  function in a common pathway for lymphocyte survival, adding support to the biochemical evidence that LT $\beta$ R signaling regulates IFN- $\beta$  induction via NF- $\kappa$ B-dependent signaling (12). In the context of microbial infection, IFN- $\beta$  is a highly pleiotropic cytokine whose signaling pathways inhibit viral replication, block virus spread to neighboring cells, regulate the differentiation of NK cells, promote DC maturation, and modulate immunity (reviewed in Ref. 60). Thus, a compromised IFN- $\beta$  response in LT-deficient mice may have several distinct impacts on the overall susceptibility to MCMV. In this regard, MCMV infection of DC in LT-deficient mice may block or modulate the induction of IFN, contributing to the increased apoptosis of lymphocytes and heightened susceptibility observed in these mice. Recent studies by Biron and colleagues (46) indicate that the CD11c<sup>+</sup>CD8 $\alpha$ <sup>+</sup> DC subset is a primary target for MCMV infection in 129SvEv mice. We found that the corresponding hepatic DC subset was also specifically depleted in MCMV-infected LT $\alpha$ <sup>-/-</sup> mice, as well as an increase in the percentage of infected splenic DC, suggesting a protective role for LT in the DC compartment. The failure to activate IFN- $\beta$  expression in LT-deficient mice may also halt DC differentiation, causing a loss of the costimulatory signals required for sustained activation of T and B cells. The recruitment of DC to the spleen occurs during MCMV infection, and the plasmacytoid DC subset, a major IFN- $\alpha\beta$ -producing cell, requires IFN- $\alpha\beta$  receptor expression for this recruitment (46). Thus, a poor IFN response may affect DC recruitment, which is known to be already impaired in LT-deficient mice, due, in part, to the decreased production of CCR7-binding chemokines by stromal cells (19, 29). In addition, the expression of LT $\beta$ R on stromal cells and DC (K. Potter and C. Ware, unpublished observations) suggests that the LT $\beta$ R could provide direct antiviral signaling to these cells via IFN- $\beta$  production. Thus, the blockade or loss of IFN- $\alpha\beta$  and other lymphocyte survival factors normally induced by LT $\beta$ R signaling during the innate response to MCMV infection may significantly compromise adaptive immunity.

BM transfer experiments revealed that LT $\beta$ R expression by both radioresistant stroma and hemopoietic cells was needed to prevent lymphocyte apoptosis, demonstrating that both compartments are compromised by MCMV. These results suggest that both the stromal and hemopoietic compartments may play an im-

portant role in the production of type I IFN in response to MCMV. Interestingly, BM transfers between wild-type B6 and IFN- $\alpha\beta$ R-deficient mice revealed that expression of IFN- $\alpha\beta$ R in either compartment (stromal or hemopoietic) was sufficient to prevent lymphocyte death during MCMV infection. One interpretation of these results is that type I IFN-mediated protection acts directly on the hemopoietic compartment and indirectly on the stroma, possibly through the production of secondary survival factors downstream of IFN- $\alpha\beta$ R signaling.

The survival function of LT $\alpha\beta$  and LIGHT revealed by MCMV resembles the functional activities of their genetically linked paralogs on chromosome 19 (CD27L, 41BBL), 1 (Fas ligand, GITRL, O $\times$ 40L), and 9 (TL1A, CD30L), which provide cooperative signaling during T cell activation. It is tempting to speculate that the selective pressure responsible for the diversification of these ligands and their cognate receptors may have been a primordial herpesvirus.

Although mouse and human CMV share a common evolutionary ancestor, they have diverged with their respective host species, as evidenced by the differences in the details of their immune subversion mechanisms. The results presented in this work establish that both mouse and human CMV are controlled at least, in part, by the regulation of the type 1 IFN system through LT signaling. Thus, further delineation of the molecular mechanisms controlling this LT-IFN axis should assist in designing strategies for targeted antiviral therapies.

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## Disclosures

The authors have no financial conflict of interest.

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