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CD8 T Cell Responses to Lymphocytic Choriomeningitis Virus in Early Growth Response Gene 1-Deficient Mice

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Previous in vitro work has implicated a role for transcriptional factor early growth response gene 1 (EGR1) in regulating immune responses. However, the in vivo role of EGR1 in orchestrating T cell responses has not been studied. To investigate the importance of EGR1 in T cell immunity, we compared Ag-specific CD8 T cell responses between wild type (+/+ ) and EGR1-deficient (EGR1−/−) mice following an acute infection with lymphocytic choriomeningitis virus (LCMV). These studies revealed that the expansion of LCMV-specific CD8 T cells was substantially reduced in EGR1−/− mice, as compared with +/+ mice. The reduced numbers of LCMV-specific CD8 T cells in EGR1−/− mice were not due to an intrinsic T cell defect per se because purified EGR1-deficient T cells exhibited normal proliferative response to anti-CD3 stimulation in vitro, and underwent normal activation and expansion in response to LCMV upon adoptive transfer into T cell-deficient mice. Furthermore, adoptive transfer of CD8 T cells bearing a transgenic TCR into EGR1−/− mice showed that EGR1 deficiency in non-CD8 T cells impaired CD8 T cell expansion in vivo following an LCMV infection. Further investigations on accessory cells showed that bone marrow-derived dendritic cells from EGR1−/− mice did not exhibit detectable impairment to prime Ag-specific CD8 T cell responses in vivo. However, in LCMV-infected mice, EGR1 deficiency selectively impaired the maturation of CD8α−/− plasmacytoid dendritic cells. Taken together, our findings suggest that EGR1 might promote expansion of CD8 T cells during an acute viral infection by modulating the cues in the lymphoid microenvironment. The Journal of Immunology, 2004, 173: 3855–3862.

During T cell activation, TCR signaling initiates a genetic program that is associated with altered expression of thousands of genes, which culminates in the clonal expansion and differentiation of naive T cells into effector and memory cells (1, 2). Of the several transcripts induced in the immediate early phase of activation (without de novo protein synthesis), many are transcription factors that in turn regulate the second wave of gene expression in the ensuing early phase. The early growth response genes (ERGs),3 namely EGR1, EGR2, EGR3, and EGR4, comprise a family of immediate early genes that encode DNA-binding proteins with highly homologous zinc finger domains (3–6). The expression of ERGs is induced in diverse cell types in response to mitogenic, differentiation, and apoptotic stimuli (7). Despite the ubiquitous nature of the expression of EGR proteins, EGR-regulated genes exhibit cell type-specific expression (7). There is compelling evidence that EGR1, the prototypic member of the EGR family, might play an important role in the normal development and functioning of the immune system (8). In the adult mouse, EGR1 is expressed at high levels in the thymus, and several studies have ascribed an important role for EGR1 in regulating the maturation of T cells in the thymus (9–13). EGR1 expression has been shown to promote the differentiation of macrophages, and EGR1 is readily induced in macrophages upon exposure to LPS (14–17). In both T and B lymphocytes, EGR1 is rapidly induced in response to signaling via the Ag receptors (1, 18–20). Interestingly, CD28 signaling alone up-regulated the expression of EGR1 in naive T cells (1). Additionally, EGR1 has been implicated in the expression of IL-2, IL-2R, TNF, ICAM-1, and CD44 (21–26). Taken together, these findings provide a strong precedent that EGR1 might be important in regulating T cell responses. However, the importance of EGR1 in T cell activation in vivo is not known. In this study, we have sought to decipher the role of EGR1 in the development of Ag-specific CD8 T cell responses following an acute infection of mice with lymphocytic choriomeningitis virus (LCMV). Our studies show that EGR1 activity promotes clonal expansion of virus-specific CD8 T cells in vivo. Further studies demonstrated the importance of EGR1 expression in the maturation of CD8α−/− plasmacytoid dendritic cells and supporting a lymphoid microenvironment that maximizes the expansion of CD8 T cells during an acute LCMV infection.

Materials and Methods

Mice

The EGR1-deficient mice (EGR1−/−) on the C57BL/6 background were kindly provided by J. Milbrandt (Washington University, St. Louis, MO) (27). Heterozygous EGR1+/− mice were bred under specific pathogen-free conditions at University of Wisconsin. Homozygous null EGR1−/− and littermate wild-type (+/+ ) mice were used in our experiments at 6–8 wk of age. The use of P14 TCR transgenic mice on the C57BL/6/Thy-1.1 background has been described previously (28). TCR-deficient mice (T cell −/−) on the C57BL/6 background (29) were purchased from The Jackson Laboratory (Bar Harbor, ME). All animals were used in accordance with the strict guidelines of the institutional animal care committee.

Virus

Mice were infected with 2 × 107 PFU of the Armstrong strain of LCMV by i.p. injection (30). Infectious LCMV in the tissues was quantitated by a plaque assay using Vero cells (30).
were stained with MHC I (H-2Db) tetramers and quantitated 8 days later. Splenocytes with LCMV, and virus-specific CD8 T cells were infected directly ex vivo using LCMV-infected (LCMV-infected targets) and uninfected (control targets) MC57G cells as target cells in a standard $^{51}$Cr release assay. The data are the mean of three mice/group ± SD.

Cytotoxicity assay

The MHC class I-restricted LCMV-specific cytotoxic activity in the spleen was assessed directly ex vivo using MC57G cells as target cells (30).

Detection of LCMV-specific CD8 T cells using MHC I tetramers

The preparation and use of MHC class I tetramers (D^b) specific to the LCMV epitopes nuclear protein (NP) 396–404, gp33–41, and gp276–286 have been described previously (31). Briefly, single cell suspensions of splenocytes were stained with allophycocyanin-labeled MHC tetramers, PE-labeled anti-CD8, and FITC-labeled anti-CD44 Abs. In some experiments, anti-Thy-1.1 and anti-Thy-1.2 Abs were used in conjunction with anti-CD8 Abs and MHC I tetramers. Following staining, cells were acquired using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA), and data were analyzed using CellQuest software (BD Biosciences). All Abs were purchased from BD Pharmingen (La Jolla, CA).

Intracellular cytokine staining

Splenocytes were stimulated for 5 h with various LCMV CTL epitope peptides in vitro, and the number of epitope-specific cytokine-producing CD8 T cells was quantitated by flow cytometry, as described elsewhere (31).

T cell proliferation assays

T cells were purified from the spleens of naive +/+ and EGR1–/– mice using T cell enrichment columns (R&D Systems, Minneapolis, MN). As assessed by flow cytometry, following enrichment, T cells were >90% pure. A total of $10^7$ T cells was cultured in vitro in 96-well plates with or without plastic-immobilized anti-CD3 Abs (2C-11), as described before (32, 33). Cultures were pulsed with $[^{3}H]$thymidine (0–24 and 24–48 h); cells were harvested onto Unifilter plates (PerkinElmer Life Sciences, Boston, MA) using Filtermate cell harvester (Packard Bioscience, Meriden, CT); and radioactivity was measured using TopCount Microplate Scintillation and Luminescence Counter (Packard Bioscience). To assess IL-2R expression on T cells, 48 h after stimulation with anti-CD3, cells were stained with anti-CD4, anti-CD8, and anti-CD25 Abs (BD Pharmingen). Stained cells were analyzed for IL-2R expression using flow cytometry, as described above. IL-2 levels in the culture supernatants were measured using the Quantikine Immunoassay kit (R&D Systems).

Adaptive transfer of T cells from naive +/+ and EGR1–/– mice into T cell-deficient mice

T cells were purified from spleens of uninfected +/+ and EGR1–/– mice, as described above; T cell recovery was 7–8 × 10^6 T cells/spleen. Following purification, 7–8 × 10^6 +/+ or EGR1–/– T cells derived from individual spleens were adoptively transferred into TCR-deficient mice by i.v. injection. Three hours after cell transfer, mice were infected with LCMV-Arm, and virus-specific CD8 T cell responses were quantitated 8 days later.

Adaptive transfer of P14 transgenic CD8 T cells into +/+ and EGR1–/– mice

T cells were purified from spleens of uninfected +/+ and EGR1–/– mice, as described above; T cell recovery was 7–8 × 10^6 T cells/spleen. Following purification, 7–8 × 10^6 +/+ or EGR1–/– T cells derived from individual spleens were adoptively transferred into TCR-deficient mice by tail vein injection. Four hours after cell transfer, these mice were infected with LCMV-Arm, and virus-specific CD8 T cell responses were quantitated 8 days after infection (28).

Preparation and use of bone marrow-derived dendritic cells

Dendritic cells were generated in vitro from bone marrow cells of +/+ and EGR1–/– mice, as previously described (34). Briefly, erythrocyte-depleted bone marrow cells were cultured in the presence of GM-CSF (20 ng/ml;
Sigma-Aldrich, St. Louis, MO) for 6–8 days. To induce maturation, cells were treated with LPS (500 ng/ml) 2 days before harvest. Following harvest, CD11c−/− mature dendritic cells were purified using anti-CD11c-coated magnetic beads (Miltenyi Biotec, Auburn, CA); dendritic cells so obtained were >99% pure and expressed uniformly high levels of MHC II, CD80, and CD86. Purified mature dendritic cells were pulsed with LCMV-CTL epitope peptide gp33–41, and ~8 × 10^5 cells were adoptively transferred into C57BL/6 mice. Priming of gp33–41-specific CD8 T cells was assessed 7 days after dendritic cell transfer.

**Flow cytometry**

Dendritic cells were purified from the spleens, as described previously (35, 36). Briefly, single cell suspensions of splenocytes were prepared by digestion with collagenase D (Roche Applied Sciences). Subsequently, dendritic cells were purified from the splenocytes using magnetic beads, as described above, and stained with allophtocyanin-labeled anti-CD11c, PerCP-labeled anti-B220, FITC-labeled anti-CD8, and PE-labeled anti-CD80 (B7.1) or anti-I-A^b or anti-H-2D^d. To analyze for CD86 expression, splenocytes were stained with allophtocyanin-labeled anti-CD11c, PerCP-labeled anti-B220, PE-labeled anti-CD8, and FITC-labeled anti-CD86 (B7.2) Abs (BD Pharmingen). Following staining, cells were acquired on a FACSCalibur flow cytometer (BD Biosciences), and data were analyzed, as described above.

**Quantitation of NO production in vitro**

To measure NO production by macrophages, 1 × 10^6 splenocytes were stimulated with LPS (Sigma-Aldrich) in 96-well plates, and nitrate concentration in the supernatant was measured 24 h later using Griess reagent system (Promega, Madison, WI).

**Statistical analysis**

Data were analyzed using the Systat statistical analysis software (version 10.2; Chicago, IL). Groups were compared by the Student’s t test, and significance was defined at p ≤ 0.05.

**Results and Discussion**

**EGR1 promotes expansion of CD8 T cells during an acute LCMV infection**

Infection of immunocompetent mice with LCMV elicits a potent CTL response that is associated with a massive expansion of virus-specific CD8 T cells (31); the peak of the anti-LCMV CD8 T cell response is attained on day 8 postinfection (PI). To examine the role of EGR1 in regulating the activation and expansion of CD8 T cells, we compared Ag-specific CD8 T cell responses between +/+ and EGR1−/− mice following an acute LCMV infection. As shown in Fig. 1, on day 8 PI, the splenocytes from +/+ mice exhibited strong MHC I-restricted cytotoxic activity directly ex vivo. The CD8 T cell-dependent cytotoxic activity in the spleens of EGR1−/− mice was lower than in +/+ mice. To more precisely quantify CD8 T cell activation and expansion in vivo, we used MHC class I tetramers to enumerate CD8 T cells specific to the D^b-restricted LCMV epitopes NP396–404, gp33–41, and gp276–286. As illustrated in Fig. 2A, CD8 T cells specific to all of the three epitopes were readily detected in the spleens of both +/+ and EGR1−/− mice. However, the frequencies and total number of LCMV-specific CD8 T cells in the spleens of EGR1−/− mice were significantly lower than in +/+ mice (Fig. 2). It is worth mentioning that the total numbers of CD8 and CD4 T cells in the spleens of uninfected +/+ and EGR1−/− mice were similar (data not shown). Previous work has shown that EGR1 might regulate CD44 expression in lymphocytes (25). However, LCMV-specific CD8 T cells in EGR1−/− mice expressed high levels of CD44, which were comparable to activated CD8 T cells in +/+ mice.

We also quantitated LCMV-specific CD8 T cells in the spleens by intracellular staining for IFN-γ. LCMV-specific CD8 T cells from both +/+ and EGR1−/− mice produced high levels of IFN-γ upon stimulation ex vivo; flow cytometric assessment of staining intensities revealed that the levels of IFN-γ produced by LCMV-specific CD8 T cells from +/+ and EGR1−/− mice were comparable (data not shown). Previous work has shown that EGR1 might regulate CD44 expression in lymphocytes (25). However, LCMV-specific CD8 T cells in EGR1−/− mice expressed high levels of CD44, which were comparable to activated CD8 T cells in +/+ mice.

**Results and Discussion**

**FIGURE 3.** LCMV-specific cytokine-producing CD8 T cells in EGR1-deficient mice. Eight days following infection with LCMV, splenocytes were stimulated ex vivo with the indicated LCMV CTL epitope peptides, and the number of IFN-γ-producing CD8 T cells was determined by intracellular cytokine staining. The data are the mean of four mice/group ± SD and representative of three independent experiments.

**FIGURE 4.** Effect of EGR1 deficiency on the expansion of CD8 T cells in the nonlymphoid organs. On the eighth day after infection with LCMV, mononuclear cells were isolated from the liver, and CD8 T cells specific to the indicated epitopes were enumerated by staining with MHC I tetramers and anti-CD8 Abs. The data are the mean of three mice/group ± SD.
Abs.
A
SD.
B
CD8 T cells was examined by flow cytometry. The histograms in B are gated on CD8 T cells; the dotted and solid lines represent staining for IL-2R on unstimulated and anti-CD3-stimulated CD8 T cells, respectively. C, Seventy-two hours after stimulation with anti-CD3, IL-2 levels in the culture supernatants were quantitated by an ELISA, and the data are the mean of three mice/group ± SD.

In addition to lymphoid organs, LCMV-specific effector CD8 T cells can be detected in significant numbers in nonlymphoid organs such as liver and lung (39). Therefore, it was important to investigate the role of EGR1 in regulating the number of effector CD8 T cells in the nonlymphoid organs following an acute LCMV infection. Data in Fig. 4 show that similar to spleen, the number of LCMV-specific CD8 T cells in the livers of EGR1−/− mice was lower (but not statistically significant) than in +/+ mice. Taken together, these data suggested that EGR1 activity is required for optimal expansion of CD8 T cells in the lymphoid organs during an acute LCMV infection. Despite lower expansion of CD8 T cells, EGR1−/− mice resolved LCMV infection by day 8 PI (data not shown).

Effect of EGR1 deficiency on responses of T cells in vitro and in vivo
It is well documented that TCR signaling induces the expression and/or activity of EGR1 in T cells (18, 20). Furthermore, EGR1 has been shown to be important in the induction of IL-2 and IL-2R expression in T cells in vitro (21–24). It has been shown previously that splenocytes from EGR1−/− mice exhibit normal proliferative responses to stimulation with Con A and anti-CD3 Abs (27). In this study, we sought to determine whether reduced expansion of LCMV-specific CD8 T cells in EGR1−/− mice was due to an intrinsic T cell defect that is independent of APCs. To this end, we first compared the proliferation of purified T cells from uninfected +/+ and EGR1−/− mice in response to stimulation with anti-CD3 in vitro. As shown in Fig. 5A, the proliferative responses of T cells from EGR1−/− and +/+ mice to anti-CD3 stimulation were comparable. Additionally, the level of IL-2R expression on activated CD8 T cells from EGR1−/− mice was similar to +/+ T cells (Fig. 5B). Upon anti-CD3 stimulation, T cells from EGR1−/− mice secreted higher levels of IL-2, as compared with +/+ T cells (Fig. 5C). Taken together, these data demonstrated that EGR1 deficiency did not significantly affect anti-CD3-induced proliferation of T cells at least in vitro.

It could be argued that in vitro stimulation of T cells with anti-CD3 (Fig. 5) may be too strong and hence not physiological. To address this concern, we devised an adoptive transfer approach to compare the responses of +/+ and EGR1−/− T cells in vivo. Purified T cells obtained from the spleens of uninfected +/+ or EGR1−/− mice were transferred into T cell−/− mice and subsequently infected with LCMV. Eight days after infection, LCMV-specific CD8 T cell responses were quantitated by intracellular cytokine staining. Data in Fig. 6 show that adoptively transferred donor T cells from both +/+ and EGR1−/− mice underwent activation and clonal expansion in response to LCMV infection in the recipient T cell−/− mice. In LCMV-infected T cell−/− mice, the magnitude of expansion of EGR1−/− T cells was comparable to those of +/+ T cells. Taken together, data presented in Figs. 5 and 6 show that T cells from EGR1−/− mice might not have an intrinsic defect to undergo activation and expansion in vitro and in vivo.

Expansion of adoptively transferred transgenic CD8 T cells is impaired in EGR1-deficient mice
The lower expansion of virus-specific CD8 T cells in LCMV-infected EGR1−/− mice (Fig. 2) could be due to a defect in CD8 T cells and/or non-CD8 T cells. However, as shown in Figs. 5 and 6, T cells from EGR1−/− mice exhibited normal activation and expansion in vitro and in vivo. To further examine the role of EGR1 in non-CD8 T cells in regulating the expansion of CD8 T cells
during an acute LCMV infection, we performed an adoptive transfer experiment using P14 CD8 T cells that express a transgenic TCR specific to the D\(^\alpha\)-restricted LCMV epitope gp33–41. The goal of this experiment was to compare the expansion of EGR1-expressing Ag-specific CD8 T cells in an EGR1-deficient vs EGR1-sufficient environment during an acute LCMV infection. To this end, we transfected equal number of purified naive Thy-1.1/P14 CD8 T cells into +/- or EGR1+/− recipient mice, which were subsequently infected with LCMV. It is worth noting that the donor P14 CD8 T cells express the congenic Thy-1.1 marker and the T cells in the recipient mice express the Thy-1.2 marker. As shown in Fig. 7, upon LCMV infection, the adoptively transferred P14 CD8 T cells showed remarkable expansion and constituted ~40% of the splenocytes in +/- recipient mice on day 8 PI. In contrast, the expansion of P14 CD8 T cells was significantly reduced in EGR1+/− recipient mice (Fig. 7). These data suggested that EGR1 expression in non-CD8 T cells promotes the expansion of virus-specific CD8 T cells during an acute LCMV infection.

**Effect of EGR1 deficiency on dendritic cells and macrophages**

The primary CD8 T cell response to LCMV is largely independent of CD4 T cells and B cells (40, 41), but lower expansion of LCMV-specific CD8 T cells in EGR1+/- mice might be due to lack of EGR1-dependent effects in professional APCs, the dendritic cells and macrophages. Therefore, it was important to focus our efforts on characterizing the role of EGR1 in regulating dendritic cells and macrophages. To this end, we generated mature bone marrow-derived dendritic cells (CD11c+ve/MHC IIhigh) from +/- and EGR1+/− mice, and compared their ability to induce CD8 T cell responses to the immunodominant epitope gp33–41. Seven days after adoptive transfer of +/- and EGR1+/− Ag-pulsed dendritic cells into naive C57BL/6 mice, the priming of Ag-specific CD8 T cells was evaluated by intracellular cytokine staining. As shown in Fig. 8, the induction of CD8 T cell responses by dendritic cells from EGR1+/− mice and +/- mice was comparable. These data showed that EGR1 deficiency had no detectable effect on the ability of in vitro generated dendritic cells to stimulate CD8 T cell responses in vivo. However, it is worth emphasizing about the distinct possibility that the in vitro culture conditions and supplements that were used during the derivation of dendritic cells might have overcome the requirement for EGR1 in dendritic cell maturation. Therefore, it was important to examine the effect of EGR1 deficiency on LCMV-induced dendritic cell maturation in vivo; dendritic cell maturation induced by LCMV infection is associated with an up-regulation in the expression of costimulatory molecules (42). Distinct sets of CD11c+ve dendritic cells have been described in the lymphoid system of mice based on morphology, lineage, cytokine production, and cell surface phenotype (43, 44). Although the expression of B220 has been used to classify murine dendritic cells into plasmacytoid (B220+ve) and nonplasmacytoid (B220−ve) subtypes, CD8α expression seems to distinguish between lymphoid (CD8α+ve) and myeloid (CD8α−ve) dendritic cells. Plasmacytoid and CD8α−ve dendritic cells have been shown to play an important role in early response to viral infections (45, 46). We investigated the effect of EGR1 deficiency on LCMV-induced maturation of subsets of CD11c+ve dendritic cells (distinguished based on B220 and CD8α expression) in the spleen on days 2 and 3 PI. Maturation of dendritic cell subsets was assessed by quantitating the expression levels of costimulatory (CD40, CD80, and CD86) and MHC (MHC I and MHC II) molecules by flow cytometry. As shown in Fig. 9, the expression levels of costimulatory and MHC molecules on CD11c+veCD8α+veB220+ve and CD11c+veCD8α+veB220−ve dendritic cell subsets were largely similar between naive +/- and EGR1−/− mice. As compared with preinfection levels, 2 days after LCMV infection, the expression levels of CD40, CD80, and MHC II (I-A\(^b\)) in particular were strongly up-regulated on both subsets of dendritic cells in +/- mice. Strikingly, in the EGR1−/− mice, the induction of costimulatory and MHC molecules was substantially attenuated preferentially in the CD11c+veCD8α+veB220+ve subset, but not in the CD11c+veCD8α+veB220−ve subset. Akin to day 2 PI, on the third day after infection, EGR1 deficiency blunted the up-regulation of costimulatory and MHC molecules selectively in the CD11c+veCD8α+veB220+ve subset of dendritic cells (data not shown). On both days 2 and 3 PI, less remarkable differences were noted when CD8−ve dendritic cells were compared to +/- and EGR1+/− mice (data not shown). Taken together, these data suggested that EGR1 might play a role in regulating the LCMV-induced in vivo maturation of the CD8−ve plasmacytoid dendritic cells. Surface receptors, including TLR, cytokine receptors, TNF receptors, and Fc receptors, induce dendritic cell maturation (47). Although induction of EGR1 by TLR4 signaling has been reported (48), it is not known whether TLR4-induced dendritic cell maturation is EGR1 dependent. The relevance of suboptimal maturation of CD8−ve plasmacytoid dendritic cells to the size of the CD8 T cell response to LCMV in EGR1−/− mice is debatable and...
warrants further investigation. Nonetheless, at the least, this finding is indicative of the importance of EGR1 in orchestrating the cellular and molecular events in the lymphoid microenvironment that might regulate the antiviral CD8 T cell response.

We also compared LCMV-induced activation of macrophages in the spleen of +/- and EGR1–/– mice. On day 3 PI, macrophages (CD8α–/–CD11c–/–CD11b+/+) from LCMV-infected EGR1–/– mice expressed slightly lower levels of CD80 and CD86, as compared with +/- mice (data not shown). Previous work has shown that splenic macrophages in LCMV-infected mice are primed to produce NO in vitro (49). Next, we quantitated LPS-induced NO production by macrophages from the spleens of LCMV-infected +/- and EGR1–/– mice on day 8 PI. Data in Fig. 10 show that macrophages from both +/- and EGR1–/– mice produced similar levels of NO in response to LPS stimulation, which indicated that EGR1 deficiency did not significantly affect NO production by activated macrophages.

![Figure 8](http://www.jimmunol.org/) Priming of CD8 T cells by bone marrow-derived dendritic cells from EGR1-deficient mice. Mature dendritic cells were generated from the bone marrow cells of +/- and EGR1–/– mice, as described in Materials and Methods. Purified dendritic cells derived from +/- and EGR1–/– mice were pulsed with the LCMV epitope peptide gp33–41 and adoptively transferred into C57BL/6 mice. Seven days after dendritic cell transfer, priming of gp33–41-specific CD8 T cells was assessed by intracellular cytokine staining. Dot plots in A are gated on total splenocytes that were stimulated with the gp33–41 peptide, and the numbers are the percentages of IFN-γ-producing CD8 T cells among splenocytes; IFN-γ-producing CD8 T cells were not detected in unstimulated cultures. The graph (B) shows the total number of IFN-γ-producing gp33–41-specific CD8 T cells in the spleen. Data are the mean of four to five mice/group ± SD and representative of two independent experiments.

![Figure 9](http://www.jimmunol.org/) Effect of EGR1 deficiency on the LCMV-induced expression of costimulatory and MHC molecules on dendritic cells. Two days after LCMV infection, CD11c–/– dendritic cells were purified from the spleens of +/- and EGR1–/– mice. Purified dendritic cells were stained with anti-CD11c, anti-CD8α, anti-B220, and anti-CD80, or anti-CD86, anti-CD40, anti-MHC II (I-Ab), or anti-MHC I (H-2D) Abs, and analyzed by four-color flow cytometry. The histograms are gated on the indicated subsets of dendritic cells (CD11c–/–CD8α–/–B220–/– or CD11c–/–CD8α–/–B220–/–) and show the expression levels of CD80, CD86, CD40, MHC I, and MHC II. The numbers are the mean fluorescent intensities of staining for the indicated molecules in LCMV-infected mice. The numbers in parentheses are the mean fluorescent intensities of staining in naive uninfected mice.
FIGURE 10. Effect of EGR1 deficiency on NO production by macrophages in LCMV-infected mice. On day 8 after LCMV infection, splenocytes from +/+ and EGR1−/− mice were stimulated with the indicated concentrations of LPS for 24 h, and nitrite levels in the culture supernatants were determined using the Griess reagent.

In summary, we have provided evidence genetic for the first time that transcriptional regulator EGR1 plays an important role in promoting activation and expansion of Ag-specific CD8 T cells during an acute LCMV infection. We also show that reduced expansion of LCMV-specific CD8 T cells in EGR1−/− mice is not due to an intrinsic T cell defect, but a sequel to a lack of EGR1-dependent cues in the lymphoid microenvironment.

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