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TNF Receptor 1 Mediates Dendritic Cell Maturation and CD8 T Cell Response through Two Distinct Mechanisms

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TNF-α and its two receptors (TNFR1 and 2) are known to stimulate dendritic cell (DC) maturation and T cell response. However, the specific receptor and mechanisms involved in vivo are still controversial. In this study, we show that in response to an attenuated mouse hepatitis virus infection, DCs fail to mobilize and up-regulate CD40, CD80, CD86, and MHC class I in TNFR1–/– mice as compared with the wild-type and TNFR2–/– mice. Correspondingly, virus-specific CD8 T cell response was dramatically diminished in TNFR1–/– mice. Adaptive transfer of TNFR1-expressing DCs into TNFR1–/– mice rescues CD8 T cell response. Interestingly, adoptive transfer of TNFR1-expressing naive T cells also restores DC mobilization and maturation and endogenous CD8 T cell response. These results show that TNFR1, not TNFR2, mediates TNF-α-driven DC maturation, as follows: a direct effect through TNFR1 expressed on immature DCs and an indirect effect through TNFR1 T cell response to mouse hepatitis virus in vivo. They also suggest two mechanisms by which TNFR1 mediates TNF-α-stimulated TNF-α-driven DC maturation. These results show that TNFR1, not TNFR2, mediates TNF-α-stimulated TNF-α-driven DC maturation and CD8 T cell response to mouse hepatitis virus in vivo. They also suggest two mechanisms by which TNFR1 mediates TNF-α-stimulated TNF-α-driven DC maturation.

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Initiation of a T cell response requires T cell–dendritic cell (DC) interactions through two sets of receptors, as follows: TCR recognition of Ag in the form of peptides presented by the MHC molecules and binding of the costimulatory molecule CD28 on naive T cells to its ligands CD80 (B7.1) and CD86 (B7.2) expressed on mature DCs (1–3). DCs are sentinels and normally reside in the tissues in an immature state without expressing CD80 and CD86 (4). Following microbial infection, immature DCs take up and process Ags to present them as peptides with MHC (4, 5). Immature DCs are also activated to express the costimulatory molecules through pathogen recognition receptors, such as TLR (6), which recognize evolutionarily conserved microbial components or pathogen-associated molecular patterns (7). By the time DCs migrate from the site of infection to the draining lymph nodes, they have matured, present antigenic peptides, and express costimulatory molecules (8, 9). In the draining lymph node, the mature DCs activate cognate naive T cells by providing two stimulating signals (9).

In addition to pathogen-associated molecular patterns, cytokines can also stimulate DC maturation and expression of CD80 and CD86 (10). TNF-α and its family member lymphotoxin (LT) α are potent cytokines secreted by activated macrophages and T cells (5, 11, 12) and exert pleiotropic effects on inflammation and immunity through two receptors, TNFR1 (p55) and TNFR2 (p75), which are ubiquitously expressed by most nucleated cells (13, 14). Treatment of DCs with TNF-α, but not LTα, in vitro leads to upregulation of surface expression of MHC class I, class II, CD80, and CD86, and an enhanced T cell stimulatory activity (15). Transgenic expression of TNF-α in DCs results in elevated levels of CD40, CD80, and ICAM-1 expression and induction of a stronger MLR (16). In TNF-α-deficient (TNF-α–/–) mice, DC maturation is impaired and T cell response is diminished following infection with a replication-deficient recombinant adenovirus (rAd) (17). Adoptive transfer of Ag-primed wild-type DCs into TNF-α–/– mice rescues T cell response. Although in LTα–/– mice the number of DCs in the spleen was reduced, the observed deficiency is likely due to a lack of the membrane-bound LTα/β, but not soluble LTα, which signals through LTβR (18–21). Furthermore, the defective DC maturation and CD8 T cell responses in TNF-α–/– mice suggest that LTα does not replace TNF-α function in these processes. Together, these results suggest a critical role of TNF-α, but not LTα, in DC maturation in vivo.

The role of TNF-α/TNFFR in DC maturation and T cell response, however, is more complex. In contrast to the diminished T cell response to rAd in TNF-α–/– mice (17), following Mycobacterium infection, Ag-specific CD8 T cells, as identified by peptide–MHC tetramer staining, are significantly elevated at 14 and 27 dpi postinfection (dpi) (22). Similarly, following acute or chronic infections with lymphocytic choriomeningitis virus (LCMV), although the magnitude of virus-specific CD8 T cell response is similar between wild-type and TNFR1–/– or TNFR2–/– mice 8 dpi, the response is much greater in TNFR1–/– and TNFR2–/– double-knockout mice (23, 24). Seven days postinfluenza virus...
infection, virus-specific CD8 T cell response is enhanced in TNFR2−/− mice (25). These apparently contradictory results of CD8 T cell responses to different infections in TNF-α−/−, TNFR1−/−, TNFR2−/−, and TNFR1−/− and TNFR2−/− double-knockout mice raise at least two questions, as follows: What is the likely cause contributing to the different effects of TNF-α and TNFR on CD8 T cell responses to different infections in knockout mice? Through which receptor does TNF-α mediate DC maturation in vivo? Consistent with a much less important role of LTα in DC maturation, LTα−/− mice make delayed, but effective CD8 T cell response to influenza virus infection (26), and the defective CD8 T cell responses to LCMV most likely result from abnormal lymphoid architecture (27).

Further complicating the delineation of the role of TNF-α/TNFR in DC maturation is the observation that T cells are required for efficient DC maturation, including CD80 and CD86 expression. In RAG1−/− mice, which are deficient in T cells, the number of DCs is significantly reduced, and the residual DCs are defective in expressing CD80 and CD86 and activating naive T cells (28). Both the deficiency in DC number and function are restored by adoptive transfer of naive T cells into the RAG1−/− mice (29). Recently, B7-H1 (PD-L1) expressed by naive T cells is shown to condition in DC maturation, LTα−/− knockout mice raise at least two questions, as follows: What is the direct effect through TNFR1 expressed on immature DCs and TNFR deficiencies on CD8 T cell responses to different infections.

**Materials and Methods**

**Viruses, mice, and infection**

Wild-type MHV-A59, referred to as A59/WT, was engineered to express enhanced GFP (eGFP), ISQAVHAAHAEINEAGR (OVA253-262), and SIYRYGYGL (SIY), referred to as RA59/GOS. The recombinant RA59/GOS was constructed by replacing open reading frame (ORF) 4 in A59/WT with a sequence coding for eGFP–OVA–SIY fusion protein through targeted RNA recombination (31, 32). Briefly, a 960-bp DNA fragment containing OVA–SIY sequences was cloned in frame into the 3′ end of eGFP. The DNA fragment encoding eGFP–OVA–SIY fusion protein was then cut out and cloned into pmHS4 plasmid (32, 33) via SalI and NotI sites, referred to as pMH54-eGFP–OVA–SIY. In vitro transcription using pMH54-eGFP–OVA–SIY plasmid as a template led to production of corresponding RNA that was flanked by A59/WT sequences. To construct pMH54–eGFP–OVA–SIY, WT with a sequence coding for eGFP–OVA–SIY fusion protein through and in vitro transcribed RNA led to generation of recombinant MHV, in which ORF4 of A59/WT was replaced by eGFP–OVA–SIY. The correct recombinant RA59/GOS was identified by eGFP expression and sequencing, and plaque was purified twice on 17Cl-1 cells. Both A59/WT and RA59/GOS viruses were amplified and titrated by plaque assay in 17Ct-1 cells (34). MHV-A59, IFV, pMH54 plasmid, 17Cl-1, and AK-D cell lines were all provided by R. Baric (University of North Carolina, Chapel Hill, NC).

Further complicating the delineation of the role of TNF-α/TNFR in DC maturation and CD8 T cell response is enhanced in the absence of TNFR1, but not TNFR2. The impaired CD8 T cell response is associated with an impaired DC maturation and mobilization and can be corrected by adoptive transfer of wild-type DCs into TNFR1−/− mice. Furthermore, DC mobilization and maturation and CD8 T cell response in TNFR1−/− mice are also restored by adoptive transfer of TNFR1-expressing naive T cells. These findings suggest that TNFR1 mediates DC maturation through two mechanisms, as follows: a direct effect through TNFR1 expressed on immature DCs and an indirect effect through TNFR1 expressed on naive T cells. Our results also suggest a possible explanation for the divergent effects of TNF-α and TNFR deficiencies on CD8 T cell responses to different infections.
selection using anti-CD4– or anti-CD8–conjugated magnetic beads. Purified cells were counted and analyzed for CD3 by flow cytometry. Purified T cells (∼95% CD3+) were injected i.v. into Thy1.2+ TNFR1−/− mice (5–8 × 10⁶ per recipient in 200 μl PBS).

Statistical analyses

Statistical significance between groups was assessed by Student t test using Prism 5 software (GraphPad Software). A p value <0.05 was considered significant.

Results

Construction and characterization of recombinant MHV

Although a CD8 epitope, RCQIFANI (37), has been identified in wild-type MHV-A59 (A59/WT), its binding to MHC class I Kb is weak. Kb tetramer loaded with RCQIFANI failed to stain CD8 T cells in the spleen of MHV-infected C57BL/6 (B6) mice (data not shown). To facilitate studying T cell responses to A59/WT, we constructed a recombinant MHV-A59 expressing eGFP fused to a CD4 epitope, ISQA VHAAHAEINEAGR (OVA323–339), and a CD8 epitope, SIYRYYGL (SIY). The recombinant MHV, referred to as RA59/GOS, was constructed by targeted RNA recombination replacing a nonessential gene, ORF4, with sequence encoding eGFP–OVA–SIY fusion protein (Fig. 1A, see Materials and Methods). Recombinant virus was selected via eGFP-positive cells (Fig. 1B) and further plaque purified.

The recombinant RA59/GOS virus was further characterized for its replication in vitro and infection in mice. Murine fibroblasts 17Cl-1 were inoculated with 5 × 10⁵ PFU of either A59/WT or RA59/GOS (multiplicity of infection [MOI] = 1), and virus titer in the culture supernatants was measured every 4 h for the next 24 h. The titer of A59/WT increased steadily, reaching a peak level of 10⁹ PFU/ml at ~20 h postinfection (Fig. 1C). In contrast, the titer of RA59/GOS only reached 10⁶ PFU/ml 24 h postinfection, indicating a reduction of replication capacity by ~100-fold. Furthermore, B6 mice were inoculated i.p. with A59/WT or RA59/ GOS, and virus titers were measured in the liver 1, 3, and 5 dpi. Following inoculation with 5 × 10⁵ PFU A59/WT, virus was readily detected in the liver at all three time points, peaking at 3 dpi (Fig. 1D). In contrast, RA59/GOS virus was detected in the liver of inoculated mice only at 1 dpi and only when 1 × 10⁶ PFU was used for the inoculation. No virus was detected at 5 or 5 dpi or when lower doses of virus were inoculated. Correlating with virus replication, elevated levels of serum alanine aminotransferase (ALT) were detected in B6 mice infected with A59/WT virus at 1, 3, and 5 dpi (Fig. 1E), whereas no elevated ALT level was detected in RA59/GOS-inoculated mice at any day or virus doses. Taken together, these results suggest that RA59/GOS is an attenuated virus with dramatically reduced capacity to replicate in cultured cells and mice.

RA59/GOS induces CD8 T cell responses in a dose-dependent manner

To study CD8 T cell response to MHV infection, we inoculated B6 mice with 10², 10⁴, or 10⁶ PFU RA59/GOS. At 7 dpi, SIY-specific

FIGURE 1. Construction and characterization of recombinant MHV-A59 expressing an eGFP–OVA–SIY fusion protein. A, Schematic diagram of recombinant MHV. Targeted RNA recombination was used to replace ORF4 of MHV-A59 with a sequence encoding eGFP–OVA–SIY fusion protein. The eGFP–OVA–SIY fusion gene was first cloned into pMH54 plasmid via SalI and NotI sites, and then transcribed into RNA for recombination with fMHV in AK-D cells. See Materials and Methods for details. B, eGFP fluorescence of RA59/GOS-infected cells. The 17Cl-1 cells were seeded on cover glass in a six-well plate, followed by RA59/GOS infection at MOI of 1. Eight hours postinfection, cells were fixed and visualized by fluorescence microscopy (original magnification ×50). Bright field and fluorescent images of the same area are shown. C, Comparison of replication of wild-type and recombinant MHV-A59 in cultured cells. The 17Cl-1 cells were infected in triplicates with A59/WT or RA59/GOS at MOI of 1. Culture supernatants were harvested every 4 h and assayed for virus titer by plaque assay. The mean virus titer ± SD of triplicate samples is shown. Representative results from one of three experiments are shown. D and E, Comparison of virus titers and ALT levels in B6 mice infected with A59/WT or RA59/GOS. B6 mice were inoculated i.p. with A59/WT (5 × 10⁵ PFU/mouse) or the indicated doses of RA59/GOS. PBS-injected mice were used as control. At 1, 3, and 5 dpi, sera and livers were harvested for assaying ALT levels and virus titers, respectively. Virus titers (D) and ALT levels (E) are shown as mean ± SD of five to six mice per group. Representative results from one of three experiments are shown. ND, not detectable.
CD8 T cells were identified in the spleen and liver by staining with anti-CD8 Ab and a H-2Kb-Ig fusion protein, the Kb component of which was loaded with SIY peptide (SIY-Kb), followed by flow cytometry. When mice were infected with A59/WT virus, a background level (0.1–0.2%) of SIY-positive CD8 T cells was detected in the spleen and liver (Fig. 2A). Similar background level of SIY-positive CD8 T cells was detected in the spleen and liver of RA59/GOS-infected mice when H-2Kb-Ig fusion protein used for staining was not loaded with SIY peptide (Fig. 2A, far right panel). In contrast, the frequency of SIY-positive CD8 T cells was increased at least 10-fold over the background level in the spleen and liver of RA59/GOS-infected mice that were stained with both anti-CD8 and SIY-Kb. Notably, the frequency of SIY-Kb–positive CD8 T cells increased with the increasing inoculation virus doses. Similarly, the total numbers of SIY-positive CD8 T cells in the spleen and liver increased with the increasing inoculation virus doses (Fig. 2B). In addition, a significant fraction of SIY-Kb–positive CD8 T cells from both the spleen and liver was induced to express IFN-γ or TNF-α by SIY peptide in vitro (Fig. 2C). These results suggest that attenuated RA59/GOS virus can cause infection in mice, but probably does not replicate significantly, resulting in functional CD8 T cell responses proportional to the doses of inoculating virus.

**Defective CD8 T cell response to RA59/GOS in TNFR1-deficient mice**

To investigate the effect of TNFR in CD8 T cell response to MHV, we compared SIY-specific CD8 T cell responses to RA59/GOS among B6 mice, B6 mice deficient in TNFR1 (TNFR1−/−), and B6 mice deficient in TNFR2 (TNFR2−/−). At 7 dpi, similar frequency and number of SIY-specific CD8 T cells were detected in the spleen and liver of B6 and TNFR2−/− mice (Fig. 3A, 3B). However, only background level of SIY-specific CD8 T cells was detected in the spleen and liver of TNFR1−/− mice.

To exclude the possibility that CD8 T cell response in TNFR1−/− mice was delayed, the frequency and total number of SIY-specific CD8 T cells were measured in the spleen and liver 11 dpi. Again, whereas similarly robust SIY-specific CD8 T cell responses were detected in the spleen and liver of B6 and TNFR2−/− mice, no significant SIY-specific CD8 T cell response was detected in TNFR1−/− mice (Fig. 3C, 3D). These results show that TNFR1, but not TNFR2, is required for efficient CD8 T cell response to MHV infection.

**Impaired DC mobilization and maturation in TNFR1-deficient mice following RA59/GOS infection**

Because of the critical role of DCs in mediating CD8 T cell responses, we determined DC numbers and maturation status in TNFR1−/− mice following RA59/GOS infection. At 3 dpi, cells from spleen and liver of B6 and TNFR1−/− mice were enumerated and analyzed for CD11c+ plus CD40, CD80, CD86, or MHC class I. Compared with CD11c+ cells from spleen or liver of B6 mice, a significantly lower percentage of CD11c+ cells from spleen and liver of TNFR1−/− mice expressed CD40, CD80, CD86, or class I (Fig. 4A, 4B; p < 0.05). More dramatically, whereas the number of CD11c+ DCs was similar in the spleen or liver between PBS-injected B6 and TNFR1−/− mice (Fig. 4C), the number of CD11c+ DCs was increased ~2-fold in both spleen and liver of B6 mice following RA59/GOS infection. However, the number of CD11c+ DCs was not increased at all in the spleen and liver of TNFR1−/− mice following RA59/GOS infection. Thus, in the absence of TNFR1, DC mobilization and maturation are impaired following RA59/GOS infection.

Next, we determined whether SIY-specific CD8 T cell response to RA59/GOS infection can be rescued by transfer of TNFR1-expressing DCs into TNFR1−/− mice. GM-CSF–induced bone marrow DCs from B6 mice were adoptively transferred into TNFR1−/− mice, followed by infection with RA59/GOS and analysis at 7 dpi. Without DC transfer, virtually no SIY-specific CD8 T cells were detected in the spleen or liver of TNFR1−/− mice. With DC transfer, SIY-specific CD8 T cells were detected in the spleen and liver of TNFR1−/− mice (Fig. 5). Statistically, there was no difference in both the frequency and the number of SIY-specific CD8 T cells in the spleen or liver between B6 mice and TNFR1−/− mice that were transferred with DCs. These results...

**FIGURE 2.** RA59/GOS induces CD8 T cell responses in a dose-dependent manner. Groups of B6 mice were infected i.p. with different doses of RA59/GOS virus or with 5 × 10^6 PFU of A59/WT virus. At 7 dpi, cells from spleen and liver were enumerated and analyzed for CD3, CD8, SIY-Kb, and 7AAD (A, B). Alternatively, cells were stimulated with SIY peptide or PMA plus ionomycin (PMA + I) or without any stimulation (Control) for 5 h and stained for CD8, SIY-Kb, and intracellular IFN-γ or TNF-α (C). Kb indicates the same stains except that H-2Kb-Ig fusion protein was not loaded with SIY peptide. A, Representative SIY-Kb (or Kb) versus CD8 staining profiles of CD3+CD8+ live cells (7AAD−) from spleen and liver are shown. The number indicates percentage of SIY-Kb−positive cells among CD8+ cells. B, Comparison of mean ± SD of SIY-Kb+ CD8+ cells in the spleen (left panel) and liver (right panel) of four mice per group. Data from one of two similar experiments are shown. *p < 0.05. C, Intracellular staining of IFN-γ and TNF-α gating on SIY-Kb+ and CD8+ T cells in the spleen and liver. Representative data from two independent experiments are shown.
show that the impaired DC maturation and mobilization is a major factor contributing to the defective CD8 T cell response to RA59/GOS infection in TNFR1−/− mice.

Transfer of TNFR1-expressing T cells also rescues endogenous CD8 T cell response to RA59/GOS in TNFR1-deficient mice

We also tested whether TNFR1-expressing T cells can rescue CD8 T cell response to RA59/GOS infection in TNFR1−/− mice. Total T cells were purified from lymph nodes of Thy1.1+ B6 mice. Purified Thy1.1+ T cells (>95% CD3+) were transferred into TNFR1−/− mice on Thy1.2+ background (5 × 10^6 cells per recipient), followed by RA59/GOS infection and analysis for Thy1.1, CD8, and SIY-Kb at 7 dpi. In both spleen and liver, <0.5% of live cells were transferred Thy1.1+ T cells (Fig. 6A). As expected, without T cell transfer, the frequency and number of SIY-specific endogenous (Thy1.1+) CD8 T cells were minimal in the spleen or liver of TNFR1−/− mice. With T cell transfer, significant levels of SIY-specific Thy1.1+ CD8 T cells were detected in the spleen and liver of TNFR1−/− mice (Fig. 6B, 6C). Statistically, there was no difference in both the frequency and the number of SIY-specific Thy1.1+ CD8 T cells in the spleen or liver between B6 mice and TNFR1−/− mice transferred with T cells. Furthermore, when purified CD4 and CD8 T cells were transferred separately into TNFR1−/− mice, endogenous CD8 T cell responses to MHV were also elevated to similar levels as in B6 mice (Fig. 6D). These results show that transfer of TNFR1-expressing T cells into TNFR1−/− mice also restores the endogenous CD8 T cell response to RA59/GOS infection.

FIGURE 3. Defective CD8 T cell response to RA59/GOS in TNFR1-deficient mice. A and B, B6, TNFR1−/− (R1), and TNFR2−/− (R2) mice were inoculated i.p. with 1 × 10^6 PFU RA59/GOS or the same volume of PBS. At 7 dpi, cells from spleen and liver were enumerated and analyzed for CD3, CD8, SIY-Kb, and 7AAD, as in Fig. 2. A, Representative SIY-Kb versus CD8 staining profiles of CD3+CD8+ live cells from spleen and liver. B, Comparison of mean ± SD of SIY-Kb+ CD8+ cells in the spleen and liver of four mice per group. Combined data from two experiments are shown. C and D, B6, TNFR1−/−, and TNFR2−/− mice were infected and analyzed, as in A and B, except analysis was done 11 dpi. Data shown are from three to four mice per group.

FIGURE 4. DC maturation and mobilization are impaired in TNFR1-deficient mice, and the impaired DC response is restored by adoptive transfer of TNFR1-positive T cells. B6 mice, TNFR1−/− mice, and TNFR1−/− mice that were transferred with purified CD3+ T cells (TNFR1−/− + T or R1 + T) 1 d earlier were inoculated with 1 × 10^6 PFU RA59/GOS virus or the same volume of PBS. Three days later, cells from spleen and liver were enumerated and analyzed for CD11c plus CD40, CD80, CD86, or MHC class I. A, Comparison of CD40, CD80, CD86, and MHC I expression by CD11c+ cells from B6 mice (histograms with solid lines), TNFR1−/− mice (shaded histograms), and TNFR1−/− mice injected with T cells (histograms with dotted lines). B, Comparison of mean fluorescence intensity of CD40, CD80, CD86, and MHC I by CD11c+ cells from the spleen (upper panel) or liver (lower panel) from B6 mice, TNFR1−/− mice, and TNFR1−/− mice injected with T cells (histograms with dotted lines). C, Comparison of the total numbers of CD11c+ cells in the spleen (upper panel) and liver (lower panel) of B6 mice, TNFR1−/− mice (R1), and TNFR1−/− mice injected with T cells (R1+T). *p < 0.05, **p < 0.01.
T cells.

later, mice were infected with 10^6 PFU RA59/GOS virus, and the frequency and the number of SIY-specific CD8 T cells recipient). One day later, mice were infected with 10^6 PFU RA59/GOS virus, and the frequency and the number of SIY-specific CD8 T cells were analyzed in the spleen and liver at 7 dpi, as in Fig. 2. GOS virus, and the frequency and the number of SIY-specific CD8 T cells in TNFR1-deficient mice by adoptive transfer of TNFR1-expressing T cells. Total, CD4+, and CD8+ T cells were transferred with purified CD8 T cells. Data shown are from one of two independent experiments.

**TNFR1-expressing T cells restore DC mobilization and maturation in TNFR1-deficient mice**

We further determined whether DC mobilization and maturation in TNFR1−/− mice were restored by adoptive transfer of TNFR1-expressing T cells. Purified Thyl.1+ T cells were transferred into TNFR1−/− mice (5 × 10^6 cells per recipient), followed by RA59/GOS infection and analysis for CD11c, CD40, CD80, CD86, and MHC I at 3 dpi. As shown in Fig. 4A and 4B, DCs from T cell–transferred TNFR1−/− mice upregulated CD40, CD80, CD86, and MHC I to the similar levels as those in B6 mice. In addition, the numbers of DCs in the spleen and liver were restored to the similar levels as those in B6 mice (Fig. 4C). These results show that transferred T cells rescue the virus-specific CD8 T cell response in TNFR1−/− mice through DC activation and mobilization.

**Discussion**

Studies have shown a critical role of TNF-α in DC maturation both in vitro and in vivo (12, 15–17). Although LTα also binds to TNFR1 and TNFRII, its effect on DC accumulation and/or homeostasis in the spleen is primarily through signaling via LTβR by membrane LTα/β, but not soluble LTα (18–21). Consistently, LTα−/− mice produce delayed, but effective CD8 T cell responses to influenza virus infection (26), and the defective CD8 T cell responses to LCMV most likely result from abnormal lymphoid architecture (27). Furthermore, the defective DC maturation and CD8 T cell responses in TNF-α−/− mice suggest that LTα does not replace TNF-α function in these processes. These findings suggest that signaling through TNF by soluble LTα is unlikely for DC maturation or CD8 T cell responses. Nevertheless, because LTα−/− mice lack both soluble LTα and membrane LTα/β and because there is no reagent for selectively blocking soluble LTα, current studies have not conclusively ruled out an essential involvement of soluble LTα in DC maturation and CD8 T cell responses.

Results presented in this work reveal two distinct mechanisms by which TNFR stimulates DC maturation and initiates T cell response following MHV infection. First, our findings suggest that TNF-α...
can stimulate DC maturation in vivo directly through TNFR1 expressed on immature DCs. Although maturation of DCs is impaired in TNF-α−/− mice following infection with a replication-defective rAd (17), which receptor mediates TNF-α’s effect on DC maturation in vivo was not identified. Our observation that DC maturation and CD8 T cell response are impaired in TNFR1−/−, but not TNFR2−/−, mice suggests that it is TNFR1 that mediates DC maturation in vivo. Our findings are consistent with and further extend the studies showing that TNF-α stimulates DC maturation in vitro via TNFR1 (38). Furthermore, because of complex cell–cell interactions in vivo, previous studies did not address whether TNF-α can directly stimulate DC maturation through TNFRs expressed on immature DCs. For example, T cell response to rAd in TNF-α−/− mice is restored by adoptive transfer of Ag-primed mature wild-type DCs. Because TNF-α is secreted primarily by activated macrophages and T cells (5, 11, 12), the transferred DCs are unlikely to secrete TNF-α to activate maturation of endogenous DCs to restore T cell response. It is more likely that the transferred DCs bypass endogenous DCs and directly activate T cell response. Like TNF-α−/− mice, TNFR1−/− mice have normal numbers of DCs in the spleen and liver and normal T cell and macrophage development. Unlike TNF-α−/− mice, TNF-α is still present in TNFR1−/− mice. Because only the transferred DCs can respond to TNF-α in TNFR1−/− mice, restoration of DC8 T cell response in TNFR1−/− mice by adoptive transfer of bone marrow–derived wild-type DCs (not Ag primed) suggests that the transferred DCs can respond directly to TNF-α in TNFR1−/− mice. Together, these results suggest that in vivo TNF-α also directly stimulates DC maturation through TNFR1 expressed on immature DCs.

Second, our results reveal an alternative T cell-dependent pathway by which TNF-α may stimulate DC maturation and CD8 T cell response in vivo. We found that in TNFR1−/− mice DC mobilization and maturation and CD8 T cell response to the attenuated MHV are also restored by adoptive transfer of TNFR1-expressing naive T cells. Because in these mice only the transferred T cells can respond to TNF-α, restoration of DC maturation and mobilization and response by endogenous CD8 T cells must go through the transferred T cells. Macrophages play a critical role in the clearance of MHV (39). It can be envisioned that TNF-α secreted by activated macrophages could stimulate the transferred naive T cells, resulting in expression of molecules that can in turn stimulate DC maturation and mobilization. For example, CD40L is expressed by activated T cells and can engage CD40 expressed on immature DCs to stimulate DC maturation (40). Nevertheless, the molecular mechanism mediating the T cell-dependent pathway of DC maturation and mobilization has yet to be elucidated.

Our results also provide a possible explanation for the apparently contradictory results of CD8 T cell responses to different infections in TNF-α−/−, TNFR1−/−, TNFR2−/−, and TNFR1−/− and TNFR2−/− double-knockout mice (17, 23, 29). The recombinant RA59/GOS was constructed by replacing a 283-bp fragment of ORF4 with eGFP–OV A–SIY. The resulting virus is attenuated in both 17C1-1 cells and mice when compared with the wild-type MHV-A59 virus (Fig. 1B, 1C). In addition, the duration of viruria was reduced to the first day postinfection with RA59/GOS, whereas viruria was detected for 3 d postinfection with RA59/WT (Fig. 1C). Consistent with the minimal viral replication, no serum ALT was detected following inoculation of RA59/GOS. However, virus infection and limited translation occur because CD8 T cell response to SIY can be detected in a dose-dependent manner (Fig. 2). Das Sarma et al. (32) have reported a recombinant MHV-A59 in which ORF4 was replaced with GFP. The resulting virus replicated similarly as wild-type A59 in vitro. However, Sperry et al. (41) reported that single amino acid substitutions in ORF1b–nsp14 and ORF2a of the MHV are attenuating in vivo. The difference between the two recombinant viruses is the addition of OVA–SIY sequences to the GFP sequences in our virus, resulting in an insert of 860-bp fragment in our virus versus an insert of 720 bp in Das Sarma’s virus. As the replaced fragment of ORF is only 283 bp, the increased insert size could have interfered with virus replication, resulting in an attenuated RA59/GOS.

It is notable that T cell response is impaired in TNF-α−/− mice following infection with a replication-defective rAd (17) and in TNFR1−/− mice following infection with an attenuated MHV. In contrast, T cell responses were all enhanced in TNF-α−/− or TNFR−/− mice following acute or chronic infection with replication-competent pathogens, such as LCMV, influenza virus, and Mycobacterium (17, 22–25). Studies have shown that DCs can be stimulated to mature independent of TNF-α (42), probably through direct interaction between microbial components and TLRs (6) or other pathogen recognition receptors (43, 44). The TNF-α–dependent pathway of DC maturation is more likely to occur when pathogen can replicate, whereas the replication-defective or highly attenuated pathogens may not produce sufficient amount of microbial components to directly engage TLRs and other pathogen recognition receptors to active DCs directly. Thus, T cell response is selectively impaired in TNF-α−/− or TNFR1−/− mice only in response to infection with replication-defective rAd or highly attenuated MHV. It is also possible that different pathogens, such as MHV, rAd, LCMV, influenza virus, and Mycobacterium, express different microbial components, infect different cell types in mice, and have different replication cycles; these differences may also contribute to the observed different outcomes of CD8 T cell responses in TNF-α−/− or TNFR-deficient mice.

In summary, our results show that in vivo TNFR1 mediates TNF-α–dependent DC maturation either through a direct binding of TNF-α to TNFR1 on immature DCs or through an indirect T cell-dependent pathway. The divergent CD8 T cell responses to different infections in TNF-α−/− and TNFR-deficient mice may also relate to the pathways by which DCs are activated.

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Disclosures
The authors have no financial conflicts of interest.

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