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## Role for TLR2 in NK Cell-Mediated Control of Murine Cytomegalovirus In Vivo

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**Natural killer (NK) cells are essential for the early control of murine cytomegalovirus (MCMV) infection. Here, we demonstrate that toll-like receptor 2 (TLR2) plays a role in the NK cell-mediated control of MCMV. TLR2 knockout (KO) mice had elevated levels of MCMV in the spleen and liver on day 4 postinfection compared to C57BL/6 mice. In vivo depletion of NK cells with anti-NK1.1 antibodies, however, eliminated the differences in viral titers between the two groups, suggesting that the effect of TLR2 on MCMV clearance on day 4 was NK cell mediated. The defect in early antiviral control was associated with a decreased NK cell population in the spleen and liver and reduced amounts of interleukin-18 and  $\alpha/\beta$  interferon secreted in the TLR2 KO mice. Our studies suggest that in addition to the reported involvement of TLR9 and TLR3, TLR2 is also involved in innate immune responses to MCMV infection.**

NK cells play a crucial role in the early phase of murine cytomegalovirus (MCMV) infection in mice. It was demonstrated decades ago that natural killer (NK) cell depletion decreased resistance to MCMV, and mouse mutants with impaired NK cell function (such as beige mice) had high susceptibility (48, 41). Moreover, young mice that lacked fully developed NK cell responses succumbed to MCMV infection, but the transfer of mature NK cells prevented a fatal outcome in these young hosts (10).

The activation, proliferation, trafficking, and effector functions of NK cells in response to MCMV infection are regulated by complex pathways. Multiple mechanisms are utilized to activate NK cells in response to the virus. In C57BL/6 mice, the viral gene product gp m157 serves as a ligand for the activating NK receptor Ly49H (4, 43), and this interaction contributes to the induction of NK cytotoxicity directed against virus-infected targets. Ly49H is now considered the product of the *Cmv-1* locus, a genetic determinant of MCMV resistance located in the NK gene complex on chromosome 6 (9, 16, 28, 29, 42, 43). Ly49H is the first confirmed example of a positively signaling mouse NK receptor that can specifically recognize a viral gene product. Cytokines secreted by dendritic cells (DC) and macrophages activated by the infection also make a large contribution to NK cell activation.  $\alpha/\beta$  interferon (IFN- $\alpha/\beta$ ), long known to be an NK cell activator (50), has recently been shown to be secreted by Ly6 G/C<sup>+</sup> plasmacytoid DC after MCMV infection (15). IFN- $\alpha/\beta$  and interleukin-15 (IL-15) promote NK cell proliferation and survival, whereas IL-12 and IL-18 augment the secretion of IFN- $\gamma$  by NK cells (33, 36). More recently, roles for IL-12 and IL-18 in the expansion of the NK cell population were demonstrated (3).

The kinetics of the expansion of the NK cell population after systemic MCMV infection is well characterized. In the spleens,

the number of NK cells shows a transient decrease on day 2 postinfection, followed by an increase that reaches peak numbers by day 6 (16). A recent study pointed out the essential role of the CD8 $\alpha^+$  CD11c<sup>+</sup> splenic DC in the expansion of the NK cell population, and a complex relationship between these DC and Ly49H<sup>+</sup> NK cells was suggested. The presence of Ly49H<sup>+</sup> NK cells was associated with low virus titers and promoted the survival of the CD8 $\alpha^+$  CD11c<sup>+</sup> DC population in the spleen. In turn, these DC seemed to be required for the subsequent expansion of Ly49H<sup>+</sup> NK cells, and this process was dependent on cytokines, mostly IL-18 (3, 16).

In spite of extensive research on the MCMV model, the mechanisms involved in the initial detection of MCMV infection by cells of the innate immune system (e.g., DC and macrophages) are not completely understood, and the possible role of innate receptors in this process has just recently started to be addressed. Toll-like receptors (TLRs) are transmembrane proteins that function as microbial pattern recognition molecules (1, 51). Common features of TLRs are the leucine-rich extracellular domain and the intracellular signaling motif called the Toll/IL-1 receptor domain (39). The activation of TLRs by pathogen-associated molecular patterns leads to a rapid innate immune response and also orchestrates the induction of the appropriate adaptive immune responses (1, 5, 35, 39, 45, 51). Initially, only molecules of bacterial and fungal origin were regarded as TLR-activating ligands. Recently, however, it has become clear that virus infections are also detected by TLRs. Intracellular molecular signatures of virus infection (such as double-stranded RNA, CpG DNA motifs in viral genomes, or single-stranded viral RNA sequences) function as TLR activators (2, 17, 19, 31), as some TLRs are situated inside cells, not on the cell membranes. Several examples of TLR-activating viral proteins have also been reported, although it is not known what pathogen-associated molecular patterns are displayed on viral proteins. Innate immune responses to the fusion protein of respiratory syncytial virus (RSV) were shown to be mediated by TLR4 (18, 26). Cytokine responses to human CMV (HCMV), herpes simplex virus type 1 (HSV-1), and mouse mammary tumor virus (MMTV) envelope

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proteins and measles virus hemagglutinin are mediated by TLR2 (6, 13, 27, 38). The viral proteins that have major roles in virus entry may initiate innate immune recognition at this early stage of the viral life cycle.

Recent studies have demonstrated that TLR9 is involved in host responses to MCMV (24, 44), similar to responses to some other herpesviruses, such as HSV-1 and HSV-2 (25, 27, 31). Mice deficient in TLR9 or in MyD88, an adapter molecule that mediates TLR9 signaling, have higher splenic MCMV titers than wild-type mice. The impaired resistance to MCMV in these mice is associated with lower levels of the cytokines IFN- $\alpha/\beta$  and IL-12 produced by plasmacytoid dendritic cells and decreased NK cell activity (25). The TLR3-Lps2 (TRIF) pathway is also activated by MCMV infection (20). Mice defective in TLR3 showed an increased susceptibility to MCMV infection and diminished secretion of the cytokines IFN- $\alpha/\beta$ , IL-12p40, and IFN- $\gamma$  (44).

Studies demonstrating TLR2 activation by HCMV *in vitro* (13) prompted us to ask whether TLR2 in the mouse would play a role in regulating MCMV infection. We show here that in addition to the recently reported roles of TLR9 and TLR3, TLR2 activation also makes a significant contribution to the control of MCMV infection in a manner dependent on NK cells.

#### MATERIALS AND METHODS

**Mice and virus infection.** Mice deficient in TLR2 or TLR4 were the gift of Shizuo Akira and were generated by gene targeting (46, 47). The TLR2 knockout (KO) mice were completely backcrossed (F<sub>11</sub>) onto the C57BL/6 background. C57BL/6 control mice were purchased from the Jackson Laboratories. All mice were housed in the Department of Animal Medicine of the University of Massachusetts Medical School under specific-pathogen-free conditions in micro-isolator cages. The age- and sex-matched groups of mice were used between 8 and 16 weeks of age. High-titer salivary gland stocks of the MCMV Smith strain (10<sup>7</sup> PFU/ml) were diluted in phosphate-buffered saline and were given intraperitoneally at  $2 \times 10^4$  to  $5 \times 10^5$  PFU/mouse.

**In vivo NK cell depletion.** One day before infection, mice were injected intraperitoneally with 100  $\mu$ l of a previously determined optimal dilution of a monoclonal antibody (MAb) specific for NK1.1. On day 4 postinfection, the percentage of NK1.1<sup>+</sup> cells in the spleens was below 0.08% in the anti-NK1.1-treated groups.

**Virus titration.** Organs (spleen, lung, and liver) harvested from infected mice were homogenized in DMEM-5% fetal calf serum, and the MCMV titers of the organ homogenates were determined by plaque assay on mouse embryo fibroblasts). Student's *t* test was used for the statistical analysis of the data.

**FACS analysis of spleen and liver leukocytes.** Leukocytes were isolated from the spleen by homogenizing the organ between frosted surfaces of glass microscope slides, followed by lysis of the erythrocytes with 0.84% NH<sub>4</sub>Cl. One million spleen cells per sample were stained with the appropriate surface antibodies, as described previously, and were analyzed by fluorescence-activated cell sorter (FACS) Cellquest Software (San Diego, CA). The antibodies used for analyses were specific for murine CD3 and NK1.1 and were purchased from BD Biosciences Pharmingen (San Diego, CA). Liver leukocytes were isolated by flushing the blood out of the organ by injecting medium through the portal vein and then homogenizing the liver tissue and digesting it with type II collagenase and DNase I (both from Sigma-Aldrich) for 35 min at 37°C (32). This step was followed by isolation of the liver leukocytes on a metrizamide density gradient.

**Cytokine measurements.** Bioassays for IFN- $\alpha/\beta$  were performed by culturing  $3 \times 10^4$  NCTC929 cells/well overnight in 96-well flat-bottom plates in the presence of twofold dilutions of the samples (in duplicate), followed by infection with  $2 \times 10^5$  PFU/well vesicular stomatitis virus (VSV) strain Indiana. The assay was evaluated 2 days postinfection, and the highest sample dilutions which protected the NCTC929 cells from VSV-induced cytopathic effects were noted. The experiment was also performed with twofold dilutions of recombinant human IFN- $\alpha$  ("universal type I interferon"; PBL Biomedical Laboratories, Piscataway, NJ), and the IFN- $\alpha/\beta$  titers in the samples were expressed as U/ml based on the standard curve obtained with this recombinant type I IFN. A mixture (1:1) of

neutralizing antibodies directed against both mouse IFN- $\alpha$  and IFN- $\beta$  (Calbiochem-Novabiochem, San Diego, CA) prevented the protective effect of the samples on VSV-infected cells, confirming that the assayed activity was due to IFN- $\alpha/\beta$  in the samples. IL-18, IL-12p40, and IL-12p70 enzyme-linked immunosorbent assays (ELISAs) were done using BD Pharmingen OptEIA (San Diego, CA) kits. Student's *t* test was used for statistical analysis.

**Histopathology.** Liver tissues harvested from infected mice were fixed in buffered formalin, embedded in paraffin, sectioned, stained in standard hematoxylin and eosin (HE), and evaluated for pathological changes. The inflammatory foci were counted under a microscope at low magnification.

#### RESULTS

**MCMV replicates to higher titers in the spleens and livers of TLR2-deficient mice.** To test whether TLR2 was involved in early antiviral responses *in vivo*, we compared viral titers in organs of MCMV-infected normal C57BL/6 mice and in their TLR2-deficient counterparts. The mice were infected with  $2 \times 10^4$  PFU MCMV intraperitoneally, and their spleens, livers, and lungs were harvested at different time points postinfection. The viral load was determined by plaque assay of the organ homogenates and expressed as log<sub>10</sub> PFU/organ. We consistently found that on day 4 postinfection, spleen and liver titers of TLR2 KO mice were significantly (approximately 10-fold) higher than those of wild-type C57BL/6 mice, whereas lung titers were not different. Results from one representative of three experiments are shown in Fig. 1A.

Depletion of NK cells *in vivo* with the MAb specific for NK1.1 resulted in a dramatic increase in virus titers in the spleens and livers of C57BL/6 mice. The NK cell depletion of TLR2 KO mice, however, did not lead to a statistically significant increase in virus titers, and both NK cell-depleted and untreated TLR2 KO mice had spleen and liver MCMV titers as high as those of NK cell-depleted C57BL/6 mice (Fig. 1B). These results suggested that the effect of TLR2 activation on MCMV clearance may be NK cell mediated. Unlike TLR2 KO mice, TLR4 KO mice did not have elevated virus titers in the spleens and livers compared to their wild-type counterparts (Fig. 1C).

Both C57BL/6 and TLR2 KO mice had small foci of inflammatory infiltrates in the liver, characteristic of MCMV infection. TLR2 KO mice, however, had a much higher number of these infiltrates than C57BL/6 mice (Fig. 2). Thus, more severe pathological changes were associated with the higher virus loads in the livers of TLR2 KO mice.

**MCMV-infected TLR2 KO mice have fewer NK cells in the spleen and liver than normal mice.** We asked whether the 10-fold-higher MCMV load in the spleens of TLR2-deficient mice was due to a diminished NK cell population in the organ. In uninfected mice, and in mice at the onset of infection, when the number of NK cells transiently decreases in the spleen (day 1.5), there were no significant differences between wild-type C57BL/6 and TLR2 KO mice in the size of the splenic NK cell population (Fig. 3 A). On day 4 postinfection, however, we found a significantly lower number of NK1.1<sup>+</sup> CD3<sup>-</sup> cells in TLR2 KO mice than in normal C57BL/6 mice (Fig. 3B and C). Leukocytes isolated on day 4 postinfection from livers of four C57BL/6 and four TLR2 KO mice were pooled and analyzed by FACS. We found a lower percentage of NK1.1<sup>+</sup> CD3<sup>-</sup> NK cells in the livers of TLR2 KO mice than in their wild-type C57BL/6 counterparts (data from one representative of three experiments are shown in Fig. 3C). By day 8, although the

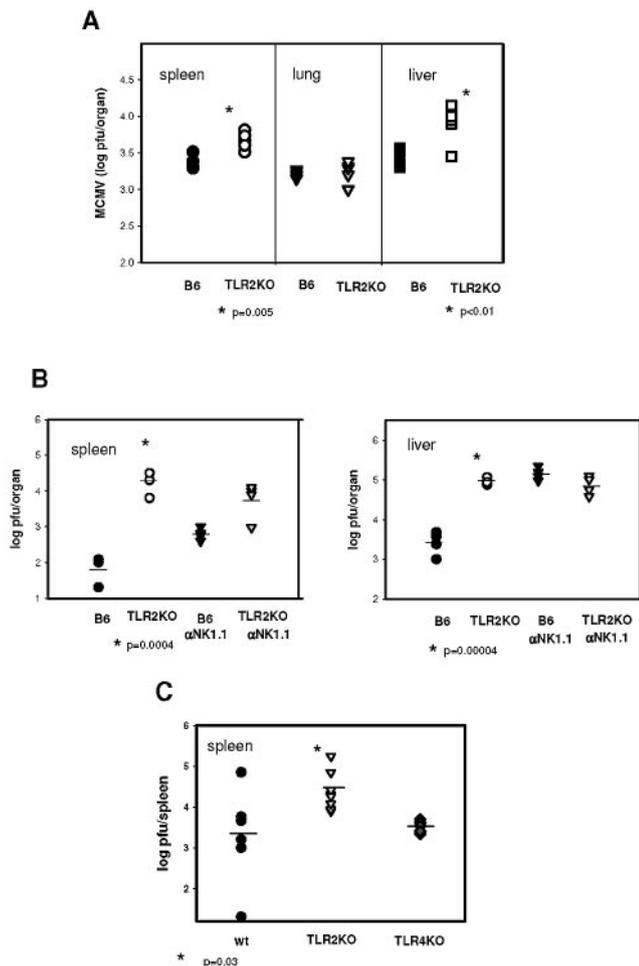


FIG. 1. MCMV titers in organs of wild-type C57BL/6 and TLR2 KO mice. (A) Viral titers in spleens, lungs, and livers of C57BL/6 and TLR2 KO mice on day 4 postinfection, determined by plaque assay. Four or five mice per group were infected with  $2 \times 10^4$  PFU MCMV. The asterisks indicate statistically significant differences. The *P* values were determined by Student's *t* test. (B) Viral titers in wild-type and TLR2 KO mice after in vivo NK cell depletion. Mice were infected with  $5 \times 10^5$  PFU MCMV intraperitoneally. NK cell depletions were performed on the day before infection in the indicated groups. The plaque assays were performed with homogenates of organs harvested on day 4 postinfection. The limit of detection of the plaque assays was 20 plaques per organ (log 1.3) in the spleens and 200 plaques per organ (log 2.3) in the livers. Three or four mice per group were tested. (C) Comparison of spleen titers of MCMV in wild-type, TLR2 KO, and TLR4 KO mice (on identical B6-backbred backgrounds). The mice were infected with  $2 \times 10^4$  PFU MCMV intraperitoneally, and six mice per group were tested. The asterisk indicates statistically significant differences between wild-type and TLR2 KO mice. The horizontal lines indicate the mean values.

percentage of NK cells in the liver remained lower in the TLR2 KO mice, no statistically significant difference in the NK cell numbers was seen in the spleens (Fig. 3D).

The antiviral effects of NK cells are primarily mediated by two distinct mechanisms, the secretion of IFN- $\gamma$  and direct granule-mediated cytotoxicity, as indicated by perforin dependence (49). We examined whether NK cells in the spleens of TLR2 KO mice differ in these functions from those of C57BL/6 mice by using intracellular staining of CD3<sup>-</sup> NK1.1<sup>+</sup> spleen

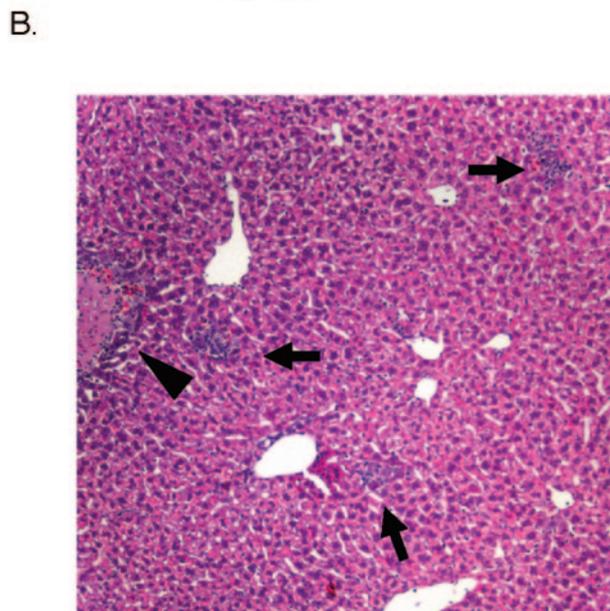
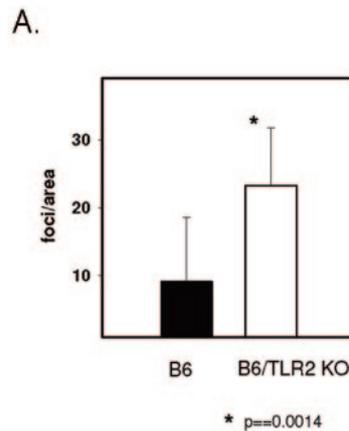


FIG. 2. MCMV-induced liver pathology in C57BL/6 and TLR2 KO mice. (A) Increased numbers of inflammatory foci in HE-stained liver sections of MCMV-infected TLR2 KO mice on day 3 postinfection. The foci were counted under a microscope using low magnification ( $\times 40$ ) by evaluating two fields per liver sample (five or six mice per group). The error bars indicate standard deviations. (B) Liver pathology on day 4 following MCMV infection in TLR2 KO mice. An HE-stained tissue section is shown at  $\times 100$  magnification; the arrows point to the inflammatory foci, and the arrowhead indicates a necrotic area.

cells with a MAb specific for IFN- $\gamma$ , and in another experiment with granzyme B, but we found no differences in the numbers of positively staining NK cells in the spleens of the two groups (data not shown).

**MCMV-infected TLR2 KO mice have reduced levels of IL-18 and IFN- $\alpha/\beta$ .** Cytokines, such as IL-18 and IFN- $\alpha/\beta$ , have crucial roles in the activation of NK cell responses (7, 14) and in the regulation of the size of the activated NK cell population following MCMV infection. The production of IL-18 and IFN- $\alpha/\beta$  exhibits a sharp peak on day 1.5 postinfection (37, 34). Thus, we asked whether there were major differences in the levels of these cytokines at this time point in the spleens and livers of MCMV-infected TLR2 KO mice and wild-type C57BL/6

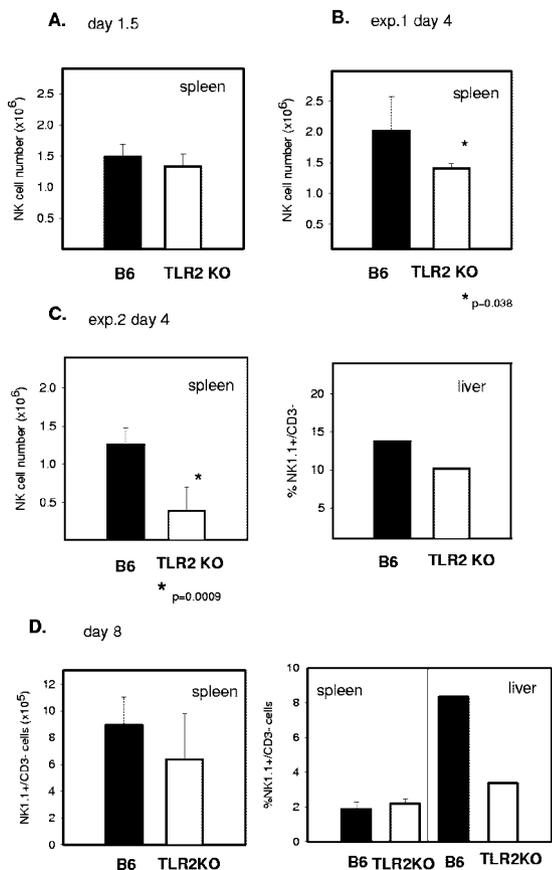


FIG. 3. NK cell populations in the spleens and livers of MCMV-infected C57BL/6 mice and TLR2 KO mice. (A) Numbers of NK1.1<sup>+</sup> CD3<sup>-</sup> NK cells in the spleen leukocyte populations of wild-type and TLR2 KO mice on day 1.5 postinfection. Four mice per group were infected with  $2 \times 10^4$  PFU MCMV. The error bars indicate standard deviations. (B) Experiment 1: numbers of NK1.1<sup>+</sup> CD3<sup>-</sup> NK cells in the spleens of wild-type and TLR2 KO mice on day 4 postinfection. The asterisk indicates a statistically significant difference. (C) Experiment 2: numbers and percentages of NK1.1<sup>+</sup> CD3<sup>-</sup> NK cells in the spleens and livers on day 4. The percentage in the liver was determined using pooled cells obtained from four livers per group. (D) Numbers and percentages of NK1.1<sup>+</sup> CD3<sup>-</sup> NK cells in the spleens and livers on day 8.

mice. ELISAs demonstrated a significantly lower concentration of IL-18 in TLR2 KO mice in the spleen, as well as in the liver (Fig. 4A). A crucial role for IL-18 in the expansion of the NK cell population controlling MCMV in the spleen was demonstrated previously (3). The levels of IFN- $\alpha/\beta$  in the spleen, determined by bioassays in several independent experiments, were consistently found to be decreased by approximately 50% in TLR2 KO mice compared to wild-type mice on day 1.5 postinfection (Fig. 4B).

**DISCUSSION**

The major finding of this study was that in TLR2 KO mice the early control of MCMV replication is impaired, as elevated virus titers were found on day 4 in the spleen and in the liver. The early control of this virus is, at least in part, due to NK cells. It was shown previously that in vivo, NK cell depletion led to a dramatic increase in virus titers in the spleens and livers

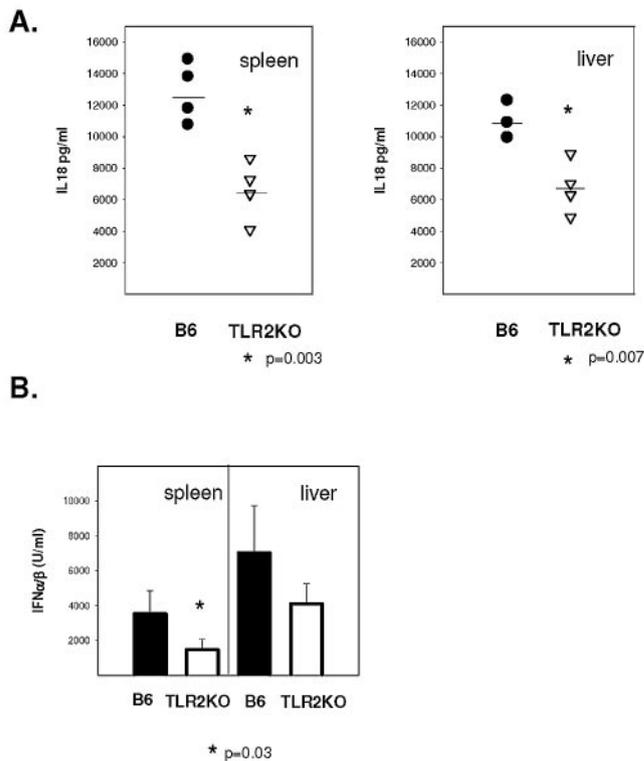


FIG. 4. Cytokine levels in the spleens of MCMV-infected wild-type and TLR2-deficient mice. (A) IL-18 concentrations in organ extracts harvested on day 1.5 postinfection and determined by ELISAs. Four mice per group were tested. (B) IFN- $\alpha/\beta$  concentrations determined by bioassays of organ extracts harvested on day 1.5 postinfection ( $n = 4$ /group). Student's *t* test was used to calculate *P* values. The asterisk indicates statistical significance. The error bars indicate standard deviations. The horizontal bars indicate the mean values.

but had little effect on day 3 lung virus titers in C7BL/6 mice infected intraperitoneally (11). Our findings that spleen and liver, but not lung, virus titers were elevated in TLR2 KO mice and that NK cell depletion eliminated the differences in virus clearance between wild-type and TLR2 KO mice suggest that the effect of TLR2 on MCMV clearance is NK cell mediated. Consistent with this notion, on day 4 postinfection, the spleens and livers of TLR2 KO mice had largely diminished NK cell populations in comparison to infected C57BL/6 controls. It is known that after MCMV infection, the number of splenic NK cells decreases on day 2, due to apoptotic death (16) and migration to the liver (40). By days 4 and 5, however, the NK cell numbers recover in C57BL/6 mice and exceed the numbers seen in uninfected mice. This is particularly true for Ly49H<sup>+</sup> NK cells (16). The rapid expansion of the NK cell population in the spleen is governed by cytokines. Our results suggest that in TLR2 KO mice, this recovery phase may be impaired after the initial decrease in the NK cell population on day 2. This may be caused by lack of proliferation of the NK cell population in the spleen, which could occur because of reduced levels of cytokines in the TLR2 KO mice. Alternatively, NK cell migration to the spleen could be diminished or the NK cells may be more sensitive to apoptotic death.

Recently, CD11c<sup>hi</sup> CD8 $\alpha^+$  DC were shown to be important in the expansion of the NK cell population in the spleens of

MCMV-infected mice (3). The survival of these CD11c<sup>hi</sup> CD8 $\alpha$ <sup>+</sup> DC, in turn, was dependent on the presence of Ly49H<sup>+</sup> NK cells. It is not clear how Ly49H<sup>+</sup> NK cells contribute to the maintenance of the DC, but the low virus titer in mice with Ly49H NK cells may be an important factor. The effect of the CD11c<sup>hi</sup> CD8 $\alpha$ <sup>+</sup> DC on the increase in Ly49H<sup>+</sup> NK cell numbers, however, was shown to be mediated mostly by IL-18. In IL-18 KO mice, there was no increase in Ly49H<sup>+</sup> NK cells in the spleen on day 6 of MCMV infection (3). Our finding that spleen and liver extracts of MCMV-infected TLR2 KO mice have lower IL-18 concentrations than those of wild-type mice suggests that TLR2 activation by MCMV may be involved in the induction of IL-18 *in vivo*. Thus, a decrease in IL-18 and, subsequently, the lack of increase in NK cell numbers may be key factors in the early defect of MCMV clearance in the spleens of MCMV-infected TLR2 KO mice.

The fact that in TLR2 KO mice there was no decrease in the percentage of IFN- $\gamma$ -secreting NK cells in response to MCMV despite diminished IL-18 levels in the spleens and livers of these mice seems to contradict previous reports of defective IFN- $\gamma$  responses in MCMV-infected IL-18 KO mice. In our studies, however, TLR2 KO mice had low, but still detectable, IL-18 concentrations; they did not completely lack IL-18, and we speculate that the IL-18 levels in these mice may have been sufficient to allow the induction of normal IFN- $\gamma$  production. Indeed, a potent synergism of low levels of IL-18 with IL-12 in IFN- $\gamma$  induction is suggested by a report by Kanakaraj et al. (23). IL-12 levels were not significantly different in MCMV-infected B6 and TLR2 KO mice (data not shown).

IFN- $\alpha/\beta$  has long been known to enhance NK cell cytotoxicity and, by induction of IL-15, NK cell proliferation in virus-infected mice (8, 50). Thus, the decreased IFN- $\alpha/\beta$  levels in TLR2 KO mice may also contribute to the difference in NK cell numbers between TLR2 KO and wild-type mice. The induction of IFN- $\alpha/\beta$  is regulated by several TLRs using diverse mechanisms. TLR3 induces type I IFN via TRIF- and IRF3-mediated pathways; TLR 7, 8, and 9 uses MyD88 and IRF7; and TLR4 can use both of these pathways (21). The decreased IFN- $\alpha/\beta$  secretion in TLR2 KO mice following MCMV infection, and also a previous report of TLR2-dependent induction of type I IFN in LCMV-infected mice (52), suggests the existence of a still-uncharacterized pathway linking TLR2 and IFN- $\alpha/\beta$  induction.

In this report, TLR2-deficient mice completely backcrossed onto the C57BL/6 background had elevated MCMV titers in both their spleens and livers. We have also extensively studied the impact of TLR2 deficiency in TLR2 KO mice partially backcrossed onto the C57BL/6 background. Mice were screened for NK1.1 and Ly49H expression and compared to similarly backcrossed 129  $\times$  C57BL/6 wild-type mice. In these mice with mixed backgrounds, TLR2 deficiency was associated with enhanced MCMV titers in the spleen, but not in the liver, and decreased granzyme B, but normal IFN- $\gamma$  expression in the NK cells. Previous studies performed with partially backcrossed (129  $\times$  C57BL/6) perforin KO or IFN- $\gamma$ -deficient mice indicated that, in the control of MCMV, IFN- $\gamma$  is of greater significance in the liver and perforin is of greater significance in the spleen (49). Subsequently, however, a profound impact of perforin deficiency on both spleen and liver MCMV titers was reported in mice with a C57BL/6 background (30), and these results were

also confirmed in our laboratory. Taken together, these observations suggest the presence of an undefined additional genetic factor contributing to organ-specific distinctions in antiviral clearance between 129 and C57BL/6 mice.

Recent publications (33–35) have reported a role for TLR9 and TLR3 in the early control of MCMV. TLR9 and the TLR3 defects were both manifested in decreased serum concentrations of IFN- $\alpha/\beta$ , low IFN- $\gamma$  production by spleen NK cells, elevated MCMV titers in the spleen, and increased mortality. TLR3 signaling is thought to be mediated by TRIF, whereas TLR9 signals go through the MyD88 adapter molecule. The virus titers, however, were higher in mice deficient in MyD88 than in TLR9 KO mice in studies using both moderate-dose (25) and high-dose (44) infection, suggesting that an additional TLR(s) signaling through MyD88 may be involved in the control of MCMV. TLR2, shown here to participate in the early control of MCMV, also signals through MyD88, and we also found that MyD88 KO mice had higher virus titers than TLR2 KO mice (data not shown). Taken together, these data suggest that MyD88 KO mice exhibit the combined effects of deficiencies in several TLRs on host responses to MCMV infection.

Recently, a number of viral proteins have been reported to activate TLRs. For example, the RSV F protein and MMTV Env activate TLR4 (12, 18, 26), and measles virus H protein, HCMV, and HSV-1 virions activate TLR2 (6, 13, 27). In addition to viral proteins, sequences of the HSV-1 genome have also been reported to act as ligands for TLR9 (31). Double-stranded RNA, generated by many virus infections, is recognized by TLR3 (2); single-stranded RNA oligonucleotides of human immunodeficiency virus were found to activate TLR7 and TLR8 (19); and the single-stranded influenza virus RNA genome was found to be recognized by TLR7. The effect of TLR signaling on viral pathogenesis *in vivo* has been addressed in a few studies. In RSV infection, TLR4 deficiency caused delayed virus clearance, associated with a smaller population of NK cells, macrophages, and monocytes in the lungs; decreased IL-12 secretion; and impaired NK cell-mediated cytotoxicity (18). In HSV-1 infection, the lack of TLR2 enhanced survival due to diminished inflammatory-cytokine secretion in the central nervous system (27). TLR4 signaling in MMTV infection was also disadvantageous for the host by causing IL-10 secretion, which suppressed cytotoxic-T-lymphocyte responses to virus-induced tumors (22). Thus, in different virus infections, completely different scenarios emerge as to how TLR signaling affects viral clearance and responses causing protection and/or immunopathology. Infection with MCMV (as well as some other virus infections) may activate several TLRs that detect a variety of molecular signatures characteristic of the infection. The simultaneous activation of a particular combination of TLRs may coordinate responses that are most appropriate for the infecting pathogen.

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