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*J Immunol* 2007; 178:5124-5131; doi: 10.4049/jimmunol.178.8.5124

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MyD88 Is Required for the Formation of Long-Term Humoral Immunity to Virus Infection

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Development of long-term humoral immunity is a major goal of vaccination, but the mechanisms involved in the formation of long-term Ab responses are still being determined. In this study, we identify a previously unknown requirement for MyD88, an adaptor molecule that mediates signals at most TLRs, for the generation of long-term humoral immunity during live virus infection. Polyoma virus-infected MyD88 knockout mice generated strong acute T cell-dependent antiviral IgM and IgG responses and developed germinal centers. Activation-induced cytidine deaminase, an enzyme required for isotype switching and somatic hypermutation, was also induced in germinal center B cells, similar to wild-type mice. However, MyD88 knockout mice failed to develop bone marrow plasma cells and did not maintain long-term serum antiviral Ab responses. The isotype distribution of antiviral IgG responses was also altered; serum IgG2a and IgG2b levels were diminished, whereas IgG1 responses were not affected. The requirement for MyD88 for the formation of long-term humoral immunity to polyoma virus was intrinsic to B cells and was independent of IL-1R and IL-18R, cytokine receptors that also signal through MyD88. Our findings show that MyD88-dependent signaling pathways in B cells are essential for effectively generating long-term Ab responses and implicate a role for TLR in the formation of long-term humoral immunity.


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Abbreviations used in this paper: AID, activation-induced cytidine deaminase; ASC, Ab-secreting cell; BM, bone marrow; FDC, follicular dendritic cell; GC, germinal center; KO, knockout; PyV, polyoma virus; TD, T cell dependent; TI, T cell independent; PNA, peanut agglutinin.

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Received for publication November 13, 2006. Accepted for publication January 30, 2007.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

This study was supported by National Institutes of Health Grants CA66644, AI49309, AI17672, and AR5506 and Training Grant AB07272.

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are necessary for effectively generating long-term Ab responses to PyV infection.

Materials and Methods

Mice and infections

MyD88 KO mice were provided by Dr. K. Alugupalli (Thomas Jefferson University, Philadelphia, PA) with the permission of Dr. S. Akira (Osaka University, Osaka, Japan). IL-1R KO were provided by Drs. R. Finberg and E. Kurt-Jones (University of Massachusetts Medical School, Worcester, MA), and μMT KO mice were provided by Dr. K. Rock (University of Massachusetts Medical School). IL-18R KO, TCR-β KO, and SCID mice were purchased from The Jackson Laboratory. All of the mice were on a C57BL/6 background and were bred and housed in the animal facility of the University of Massachusetts Medical School. Age- and sex-matched C57BL/6 mice were purchased from The Jackson Laboratory and housed in our animal facility with KO mice for several weeks before use. Mice were infected i.p. with 2 × 10⁷ PFU of PyV strain A2. The mice were used according to protocols approved by the University of Massachusetts Medical School Animal Care and Use Committee.

VP1-specific ELISA

VP1-specific ELISAs were conducted as previously described (20). Briefly, purified recombinant VP1 protein (0.1 μg/ml in carbonate buffer) was used as an immunoadsorbant. Bound Ab was detected using biotin-conjugated goat Abs specific for either mouse IgM, total IgG, IgG2a, IgG2b, or IgG3; and streptavidin-conjugated HRP (Vector Laboratories). ELISA plates were developed using 3,3',5,5'-tetramethylbenzidine (Sigma-Aldrich), and reactions were stopped with 2 N sulfuric acid. Optical densities were read at 450 nm using a microplate reader and Softmax software. Serum Ab titers are expressed as the reciprocal of the serum dilution necessary to give an OD₄₅₀ equal to that of 3× background.

VP1-specific ELISPOT

To determine the number of VP1-specific Ab-secreting cells (ASC), we modified a previously described method for detecting influenza virus-specific ASC by ELISPOT (21). Multiscreen HA plates (Millipore) were coated with 100 μl of purified VP1 protein (0.1 μg/ml) overnight at 4°C and blocked the next day for 30 min at 37°C with RPMI plus 10% FBS. Cells were plated in duplicate in 0.2 ml of 1 × 10⁶, 2.5 × 10⁶, 6.25 × 10⁶, and 1.25 × 10⁷ cells/well and incubated for 4 h at 37°C. Bound Ab was detected using 100 μl of biotin-conjugated goat Ab specific for either mouse IgM or IgG (diluted to 1 μg/ml in PBS containing 1% FBS and 0.1% Tween 20; Vector Laboratories) and streptavidin-conjugated HRP (diluted to 1 μg/ml in PBS containing 1% FBS and 0.1% Tween 20; Vector Laboratories). Plates were developed with 3-amino-9-ethylcarbazole (Sigma-Aldrich) according to the manufacturer’s protocol. Spots were counted using a dissection microscope.

Flow cytometry

To examine the expression of cell surface markers by FACS, splenocytes were treated with 0.84% NH₄Cl to lyse RBC and then washed with FACS buffer (PBS plus 1% FCS, 2 mM EDTA, and 0.02% Na₃H₂BO₃). Cells were treated with purified anti-CD16/32 (Fc block; clone 2.4G2; BD Pharmingen) and then stained with Abs diluted in FACS buffer for 30 min at 4°C. Abs used in these studies included purified rat anti-mouse T and B cell-activating Ag GL7 (clone GL7; ebioscience), FITC-anti-mouse I-A² (clone AF6-120.1; BD Pharmingen), PE-anti-rat IgM (clone HIS40; ebioscience), PerCP-anti-mouse CD3ε (clone 145-2C11; BD Pharmingen), PE-Cy7-anti-mouse IgM (clone R6-60.2; BD Pharmingen), allophycocyanin-Cy7-anti-mouse CD19 (clone 1D3; BD Pharmingen), and Pacific blue-anti-mouse CD45R/B220 (clone RA3-6B2; BD Pharmingen). Purified rat anti-mouse GL7 was detected using PE-anti-rat IgM Ab (clone HIS40; ebioscience). At least 100,000 events were counted using a LSRII (BD Biosciences) and analyzed using FlowJo software (Tree Star).

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In several experiments, T cell-depleted splenocytes were adaptively transferred into SCID mice, and purified splenic B cell populations were transferred into (B cell-deficient) μMT KO mice. To obtain these cell populations, splenocytes were treated with 0.84% NH₄Cl to lyse RBC. T cell-depleted splenocyte populations were obtained by negative selection using anti-Thyl.2 MACS beads (Miltenyi Biotech). Purified splenic B cells were obtained using the MACS (Miltenyi Biotech) negative selection B cell isolation kit. MACS depletions were done according to Miltenyi’s protocols. Depletion of T cells and purification of B cells was confirmed by FACS using Abs to CD3ε and CD19, respectively. On average, T cell-depleted populations contained <1% CD3⁺ cells, and enriched B cell populations contained >98% CD19⁺ cells.

RT-PCR

RT-PCR of activation-induced cytidine deaminase (AID) mRNA was conducted using a Qiagen RNeasy Mini Kit and a SuperScript First-Strand Synthesis System (Invitrogen Life Technologies) for RT-PCR. AID cDNA intron-spanning primers were used to avoid genomic DNA amplification: sense 5'-ATATGGACGTCTCTGTAAGC-3’; antisense 5’-TGTCTCAGAAATCCCAACATAGC-3’. These AID primers were provided by Dr. J. Stavnezer (University of Massachusetts Medical School).

Immunohistochemistry

Frozen sections of spleen were stained with peanut agglutinin (PNA), Abs to mouse CD4 and follicular dendritic cells (FDC; FDC-M1), and counterstained with methyl green as previously described (22). PNA and Abs were detected using VIP kit (Vector Laboratories) and diaminobenzidine (Sigma-Aldrich).

Real-time PCR

Quantification of viral load was done by measuring PyV DNA copies by real time PCR as previously described (23). Briefly, DNA was purified from spleen, lung, and kidney tissues harvested from PyV-infected mice. Viral and cellular genomic DNA was amplified using primer pairs specific for the PyV VP1 coding region (PyV forward primer CCCCCCCGTACAGGTTCAGGCATCCATCAT; PyV reverse primer GCGCAACAAGCTGACCCCGCTCGACAG) and for β-actin (β-actin forward primer CGAGGCCCAGAGCAAGAGAG; β-actin reverse primer CGGTTGGCCCTAGGGTGTCAG) using Bio-Rad iCycler. Serial dilutions of uninfected mouse DNA and recombinant plasmid DNA encoding VP1 sequences, respectively, were used to generate standard calibration curves. Samples were run in duplicate. The PyV cop cop number was normalized for the amount of genomic DNA determined by the β-actin-specific reaction. Results were first expressed as PyV copies/μg of genomic DNA and then converted to PyV copies/10⁷ cells assuming that 2 × 10⁷ mammalian cells contain 1 μg of DNA. The limit of detection of this assay is 1 copy of PyV DNA per 2 × 10⁷ cells.

Statistical analysis

Statistical significance was determined by a two-tailed Student t test. A value of p < 0.05 was considered statistically significant.

Results

Effective initiation of antiviral Ab responses in MyD88 KO mice

To determine whether MyD88-dependent signaling pathways are required for the generation of Ab responses to viral infections, we examined the ability of MyD88 KO and wild-type mice to generate IgM and IgG Ab responses to the major capsid protein of PyV. VP1, at weekly time points after PyV infection. ELISPOT assays showed that VP1-specific IgM ASC numbers in the spleen peaked in wild-type mice ~7 days after infection (3628 ± 1462 ASC/spleen) and then slowly decreased over time (Fig. 1A). VP1-specific IgG ASCs were also detectable in wild-type spleens 7 days after infection (4464 ± 1276 ASC/spleen) and in roughly similar numbers to IgM ASC. Unlike the declining IgM responses, IgG ASC responses in wild-type mice increased in magnitude after day 7, peaking 14–21 days after infection, and persisting over the course of a month (Fig. 1B). MyD88 KO mice by day 7 after infection had strong VP1-specific IgM and IgG responses, with spleen ASC numbers (6760 ± 5986 IgM and 7601 ± 4672 IgG) slightly elevated compared with those in wild-type mice (Fig. 1, A and B). Thus, antiviral Ab responses to a live virus infection were effectively initiated in the absence of MyD88-mediated signaling.

Defective progression of antiviral IgG responses in MyD88 KO mice

Despite the effective initiation of antiviral Ab responses, VP1-specific IgM- and IgG-secreting ASC numbers were ~10- and 4-fold lower (p < 0.05 for both responses), respectively, in MyD88 KO
spleens at 14 days after infection, and they remained lower over the course of a month (Fig. 1, A and B). ELISAs measuring serum Ab levels also showed that MyD88 KO and wild-type mice contained similar levels of VP1-specific IgM and IgG serum Abs 7 days after infection (Fig. 1, C and D), but that MyD88 KO mice had significantly (p < 0.05) lower levels of VP1-specific IgG Abs than wild-type mice 14 (Fig. 1E) and 21 (data not shown) days after infection.

The formation of long-term humoral immunity is impaired in MyD88 KO mice

The impairment of antiviral Ab responses in the late stages of the acute response led us to question the requirement for MyD88 signaling in long-term antiviral immunity. A hallmark of B cell memory formation after virus infections is the migration to the bone marrow (BM) of long-lived IgG ASCs (24). These ASCs account for the long-term production of serum Abs, which protect against subsequent infections upon re-exposure to viruses (24). VP1-specific IgG ASCs were detected in the BM of wild-type mice several weeks after infection with PyV (Fig. 2A), and their frequency greatly increased by 1.5 years postinfection (197 ± 181 ASCs/10⁵ lymphocytes; Fig. 2B), likely due to the persistence of PyV in mice after infection. In contrast, VP1-specific IgG ASCs were not detected in MyD88 KO BM until 4 mo postinfection (Fig. 2A) and were present at lower frequencies than in wild-type at this time point (3 ± 3 vs 9 ± 1 ASC/10⁵ lymphocytes; p < 0.05; Fig. 2A). This deficiency continued into very long-term memory, with no increase in VP1-specific ASC in the BM of MyD88 mice by 1.5 years postinfection (3 ± 1 ASC/10⁵ lymphocytes; p < 0.05; Fig. 2B), resulting in a 25-fold difference in ASC frequencies between MyD88 KO and wild-type mice at this time point. Consistent with these findings of BM ASC, MyD88 KO mice also had ~25-fold lower levels of VP1-specific IgG serum Ab than wild-type mice 1.5 years postinfection (p < 0.05; Fig. 2C).

Initiation of acute TD responses to PyV in the absence of MyD88-mediated signaling

Some virus infections, including that of PyV, can induce TI Ab responses, and this may have explained our findings of normal early but defective long-term Ab responses to PyV in MyD88 KO mice. TI responses occur in the absence of CD4 T cell help, and they differ from TD responses in that they are limited to certain IgG isotypes and fail to induce GC formation (4). It was important, therefore, to test whether the acute B cell responses observed in MyD88 KO mice exhibit characteristics of TI or TD responses. In normal TD responses, several days after viral infection, GC are formed, and GC B cells can be readily identified by flow cytometry based on their expression of Fas, GL7, and reactivity with PNA (FashighGL7highPNA⁺; Refs. 25 and 26). Flow cytometric analyses of MyD88 KO and wild-type splenocytes showed similar numbers of GC B cells (B220⁺CD19⁺FashighGL7highPNA⁺ cells) in wild-type and MyD88 KO mice 7 and 14 days after PyV infection (which were rare in naive mice), indicating that MyD88 KO mice normally initiated the GC reaction (Fig. 3, A–C). Moreover, these cells similarly up-regulated MHC class II (Fig. 3B), and induced
the expression of mRNA encoding AID (Fig. 3E), a protein required for isotype switching and somatic hypermutation (27). These findings are consistent with normal development of GC B cells in MyD88 KO mice. Frozen sections of spleens from both wild-type and MyD88 KO mice taken on day 8 postinfection also showed the appearance of PNA⁻/H11001 GC and CD19⁻ B220⁺ GL7⁺/Fas⁻/H11001 non-GC B cells, 7 and 14 days after infection. In some experiments, lymphocytes were also gated on CD4⁺ cells to reduce contaminating autofluorescent cells.

To directly examine the role of MyD88 in the generation of TI vs TD Ab responses, we used an adoptive transfer system. We previously showed that SCID mice reconstituted with T cell-depleted wild-type and MyD88 KO mice generated VP1-specific IgM and IgG Ab responses after infection with PyV (20). We therefore reconstituted SCID mice with either unfractionated or T cell-depleted wild-type or MyD88 KO splenocytes and measured VP1-specific IgM and IgG Ab responses after PyV infection. SCID mice reconstituted with unfractionated (T cell-containing) wild-type or MyD88 KO splenocytes generated VP1-specific IgM and IgG serum Ab responses of similar relative magnitude to that measured in PyV-infected wild-type and MyD88 KO mice by 14 days after infection (Figs. 1 and 4). SCID mice reconstituted with T cell-depleted wild-type splenocytes also produced VP1-specific IgM and IgG serum Abs, although at lower levels than mice that received unfractionated splenocytes (Fig. 4). SCID mice reconstituted with T cell-depleted MyD88 KO splenocytes, however, generated VP1-specific IgM responses, but not IgG responses (Fig. 4). Thus, MyD88 was required for generating isotype-switched TI Ab responses to PyV, consistent with other in vitro and in vivo studies showing that induction of IgG in response to TI Ags is MyD88 dependent (11, 28). These findings, taken together with the
formation of GC in PyV-infected MyD88 KO mice, indicate that MyD88-mediated signals are not required for the initiation of acute TD Ab responses to PyV.

**Formation of long-term humoral immunity to PyV is IL-1R and IL-18R independent**

In addition to TLRs, MyD88 is also required for mediating signals at IL-1R and IL-18R (18). Immune responses of MyD88 KO mice can be skewed toward predominantly Th2 responses as a result of defective IL-18R signaling (18). Therefore, we tested whether the Ab responses of MyD88 KO mice to PyV had altered IgG profiles. On day 14 of the infection, MyD88 KO mice had normal levels of VP1-specific IgG1 and IgG3 Abs, whereas IgG2b levels were 10% of the wild type and IgG2a levels were barely detectable (Fig. 5). Interestingly, a similar IgG profile was found 1.5 years after infection, in which IgG1 levels were comparable with those of wild-type mice, but IgG2a (3% of wild type; \( p < 0.05 \)) and IgG2b levels (33% of wild type; \( p = 0.1 \)) were lower (Fig. 5), indicating a defect in Th1 humoral responses and suggesting a possible role for IL-18R-mediated signals in humoral immunity to PyV.

To determine whether the requirement for MyD88 reflected a need for IL-18R signaling during immune responses, we examined the ability of IL-18R KO mice to generate VP1-specific IgG serum Ab responses and BM ASC 1 mo after PyV infection. Because MyD88 is also required for IL-1R signaling, we tested long-term Ab responses to PyV in IL-1R KO mice. IL-1R KO mice have previously been shown to generate normal Ab responses to both noninfectious and infectious Ags (29, 30). However, immunization with killed intact *S. pneumoniae* elicited diminished PsPA surface protein-specific IgG responses in IL-1R KO mice, due to impaired CD4 T cell function (31), consistent with a previously shown role for IL-1 in enhancing Th cell costimulatory molecule expression (32). We found that IL-1R KO mice contained similar levels of VP1-specific IgG serum Abs and BM ASC as wild-type mice 1 mo after infection (Fig. 6A). In addition, neither the maintenance of VP1-specific serum IgG levels nor the formation of VP1-specific IgG BM plasma cells was impaired in IL-18R KO mice (Fig. 6B), indicating that IL-1R KO or IL-18R KO mice were not impaired in their ability to form long-term humoral immunity to PyV. Moreover, the IgG profiles of these mice were similar to that of wild-type mice (data not shown). Thus, the impaired long-term humoral immunity to PyV and IgG isotype skewing observed in MyD88 KO mice was not due to defects in IL-1R and IL-18R signaling, suggesting that activation of MyD88-dependent TLR was required to induce the formation of normal long-term humoral immunity to PyV.

**FIGURE 4.** Ab responses of SCID mice reconstituted with MyD88 KO or wild-type spleen cells. SCID mice were reconstituted with either 1.5 × 10⁷ unfractionated (\( n = 2 \); A) or 1 × 10⁷ Thy1.1-depleted (\( n = 3 \)) MyD88 KO or wild-type splenocytes (B) and then infected with PyV 1 day later. VP1-specific IgM serum Abs were measured 14 days after infection and IgG serum Abs were measured 21 days after infection by ELISA. Error bars, SD. ●, Wild-type mice; □, MyD88 KO mice. Data are representative of three experiments.

**FIGURE 5.** IgG subtype responses to PyV. Sera of MyD88 KO (\( n = 6 \)) and wild-type (\( n = 6 \)) mice were examined for VP1-specific IgG1, IgG2a, IgG2b, and IgG3 Abs 14 days and 1.5 years after infection by ELISA. Error bars, SD. ●, Wild-type mice; □, MyD88 KO mice.
MyD88 is required in B cells for the generation of long-term Ab responses to PyV

We next wanted to determine whether MyD88 was required in B or T cells for the generation of long-term Ab responses to PyV. To examine the role of MyD88 in these cell types, we reconstituted B cell-deficient μMT KO mice (33) with 1.5 × 10⁷ MyD88 KO or wild-type B cells, or, alternatively, we reconstituted T cell-deficient TCRβ × δ KO mice (34) with 1.0 × 10⁷ MyD88 KO or wild-type (Thy1.2⁺) T cells. The mice were inoculated with PyV the next day and then tested for VP1-specific IgG serum Ab levels 1 mo after infection. μMT KO mice reconstituted with wild-type B cells contained significant levels of VP1-specific IgG serum Ab 1 mo after infection, whereas VP1-specific IgG was barely detectable in the serum of μMT KO mice reconstituted with MyD88 KO B cells (Fig. 7). In contrast, TCRβ × δ KO mice reconstituted with either MyD88 KO or wild-type T cells had similar levels of VP1-specific IgG serum Ab, present at 25-fold higher levels than in TCRβ × δ KO mice, which received no cells (Fig. 7). Thus, MyD88 was required in B cells, but not T cells, to maintain IgG serum Ab levels to PyV, indicating that activation of MyD88-dependent signaling pathways within B cells is essential for the generation of long-term humoral immunity to PyV.

Discussion

This study identifies a critical requirement for MyD88 for the formation of long-term humoral immunity to a viral infection. MyD88 KO mice generated antiviral IgM and IgG ASC in the spleen and serum IgM and IgG levels similar to those in wild-type mice early after virus infection. The development of GC was also unimpaired as judged by GC B cell phenotypic markers and GC morphology. However, antiviral Ab responses then rapidly decreased in mice deficient in MyD88-dependent signaling, and these mice failed to generate normal long-term humoral immunity. The early IgG responses in MyD88 KO mice showed hallmarks of TD responses, such as GC formation and the secretion of Abs of the IgG1 isotype. Adoptive transfer studies showed that acute TI IgG responses did not occur in the absence of MyD88, suggesting that the acute IgG responses observed in MyD88 KO were TD responses. Adoptive transfer studies also showed that the requirement for MyD88 for the generation of long-term Ab responses to
PyV was intrinsic to B cells. Therefore, these studies demonstrate an essential role for MyD88-dependent signaling in B cells for normal long-term antiviral humoral immunity. A trivial explanation for the lack of normal long-term Ab responses might be clonal exhaustion of B cells by high Ag load, similar to that which occurs to T cells in lymphocytic choriomeningitis virus-infected mice lacking IFN-signaling pathways. This clearly is not the case here, because PyV-infected MyD88 KO mice had no significant increase in virus load compared with wild-type when tested on day 35 by real-time PCR (Fig. 8).

Although much has been learned about events involved in the activation of B cells, the transition of activated B cells into cell types maintaining long-term Ab responses, including long-lived PC and memory B cells, is still not well understood. The formation of long-term humoral immunity is thought to be closely associated with the GC, a specialized microenvironment facilitating the interaction of B cells, Th cells, and FDC loaded with Ag (1–3). Mice deficient in the adaptor molecule, SAP, that mediates signaling through a family of leucocyte receptors, have also been shown to generate acute TD Ab responses, but not long-term humoral immunity to virus infection (38). These mice had diminished GC formation, and SAP was found to be required within CD4+ Th cells for the generation of long-term humoral immunity (38). In MyD88 KO mice, however, the number and size of GC are not diminished after virus infection, suggesting that MyD88-dependent signals are required at a later step than SAP, during or after the GC reaction. In contrast to the CD4 T cell defect in SAP-deficient mice, our adoptive transfers of wild-type or MyD88 KO B cells into B cell-deficient mice indicate that the MyD88 defect is intrinsic to B cells.

The diminished IgG responses to PyV detected in MyD88 KO mice could reflect a defect in affinity maturation in these mice, but it is unlikely that the lower VP1-specific IgG titers would be solely due to the lower affinity of the Abs. The ASC numbers in the spleen of the MyD88 KO mice decreased by day 14, whereas they increased in wild-type mice, and eventually BM ASC accumulated in wild-type, but not in MyD88 KO mice. These changes, which are consistent with the observed decrease in serum IgG responses, cannot be accounted for by mere affinity differences. The diminished antiviral IgG responses in MyD88 KO mice after day 14 postinfection exhibited an altered isotype profile; IgG2a and IgG2b were reduced, whereas IgG1 levels, which are normally a minor component of the response, were unaffected in comparison with wild-type mice. This finding raises the possibility that a selective deficiency in long-lived BM-residing plasma cells that switched to IgG2a and IgG2b isotypes may occur in MyD88 KO mice, and this deficiency is not compensated for by plasma cells that switched to other IgG subclasses. If this is the case, then MyD88-mediated signals in B cells would be required for the formation of long-term type I humoral immunity.

The requirement for MyD88 may also define a unique differentiation step in the transition of GC B cells into long-lived plasma cells, or MyD88 might be required for the expansion, survival, or migration of long-lived plasma cells. Indeed, previous studies showed that human B cells bearing a GC or memory phenotype could be induced to proliferate in vitro when stimulated with CpG DNA, an activator of TLR9 (16).

Which MyD88-dependent signaling pathways are required for the generation of long-term humoral immunity? MyD88 mediates signals at all known TLRs (except TLR3) and the cytokine receptors IL-1R and IL-18R. It is possible that several MyD88-dependent receptors are involved in the generation of long-term antiviral Ab responses, and a defect in only one will not impair long-term humoral immunity. In fact, many viruses can activate multiple TLRs, and this may make the identification of MyD88-dependent TLR signal(s) involved in the generation of immune responses difficult (39–42). IL-1R signaling was not required for normal serum Ab responses to L. major (30), but according to a recent publication IL-1R signaling mediated by MyD88 in CD4 Th cells was needed for normal Ab responses to heat-killed S. pneumoniae. In this case, the secretion of all IgG isotypes specific for the surface protein PspA and capsular polysaccharide were affected from the very onset of the Ab responses (31). We showed here using IL-1R KO and IL-18R KO mice that neither receptor was required for generating long-term humoral immunity to PyV after infection. Thus, it seems likely that activation of TLRs initiates the MyD88-dependent signaling pathways in B cells that are essential for the development of long-term humoral immunity.

TLR activation was shown to contribute to the in vitro activation of human B cells (15, 16) and to the induction of humoral autoimmunity in some mouse models (10, 43–45). However, studies examining the role of TLR in Ab responses to exogenous Ags yielded contradicting results. Reduced IgM and IgG1 and a lack of IgG2 responses and GC formation were found in MyD88 KO mice in response to OVA given with LPS and IFA (11). Using an adoptive transfer strategy, this group showed that MyD88-dependent TLR signaling was required in B cells themselves for the initiation of these TD responses, leading to the conclusion that activation of TLR on B cells is a necessary costimulatory signal for the initiation of acute TD responses. However, IgM or IgG responses to LPS-free trinitrophenyl-hemocyanin given in alum to MyD88/ TRIF double KO mice, which lack all TLR pathways, were reported to be normal, indicating that adjuvants that do not activate TLR are also sufficient for the initiation of acute TD Ab responses (46). Ab responses to some nonviral pathogens, such as L. major, B. burgdorferi, and S. pneumoniae were shown to be shifted to Th2 isotypes in MyD88 KO mice, in some cases without an overall decrease in IgG (12–14). The longevity of Ab responses and the development of BM-residing ASC have not been tested in these models. Our study, addressing the role of TLR in B cell responses to a natural virus infection, shows that MyD88-dependent TLR are not needed for the induction of early IgM and IgG production and GC formation but are essential for the generation of long-term humoral immunity. A recent report identified a pediatric patient who has had severe recurring infections since birth and is deficient in IL-1R-associated kinase 4, a downstream mediator of MyD88 signaling. In vaccination studies, this individual demonstrated defective B cell responses similar to our findings in MyD88 KO mice; this patient generated acute TD Ab responses but was unable to generate long-term humoral immunity (47). This finding suggests that MyD88-dependent pathways may be essential for generating long-term Ab responses not only in mice but also in humans.

Acknowledgments
We thank Evan Jellison and Dr. Janet Stavnezer for thoughtful discussions and Thuvan Dinh, Xueya Liang, Chethana Gowda, and Dr. Susan Stepp for their assistance. We also thank Dr. Janet Stavnezer for primers and control cell lysates for AID RT-PCR, Drs. Robert Finberg and Evelyn Kurt-Jones for IL-1R KO mice, Dr. Ken Rock for µMT KO mice, Dr. Kishore Alugupalli for MyD88 KO breeder mice, and Dr. Shizuo Akira for permission to use MyD88 KO mice.

Disclosures
The authors have no financial conflict of interest.