Poly(I:C)-Induced Protection of Neonatal Mice Against Intestinal Cryptosporidium parvum Infection Requires an Additional TLR5 Signal Provided by the Gut Flora

Louis Lantier,1,2 Françoise Drouet,1,2 William Guesdon,1,2 Roselyne Mancassola,1,2 Coralie Metton,1,2 Richard Lo-Man,3 Catherine Werts,4 Fabrice Laurent,1,2,a and Sonia Lacroix-Lamandé1,2,a

1INRA Val de Loire, UMR1282 Infectiologie et Santé Publique, Nouzilly; 2Université François Rabelais, UMR1282 Infectiologie et Santé Publique, Tours; 3Institut Pasteur, Unité de Régulation Immunitaire et Vaccinologie, Paris; and 4Institut Pasteur, Unité de Biologie et Génétique de la Paroi Bactérienne, Paris, France

The neonatal intestinal immune system is still undergoing development at birth, leading to a higher susceptibility to mucosal infections. In this study, we investigated the effect of poly(I:C) on controlling enteric infection by the protozoan Cryptosporidium parvum in neonatal mice. After poly(I:C) administration, a rapid reduction in parasite burden was observed and proved to be dependent on CD11c+ cells and TLR3/TRIF signaling. Protection against C. parvum required additional signals provided by the gut flora through TLR5 and MyD88 signaling. This cooperation gave rise to higher levels of expression of critical mutually dependent cytokines such as interleukin 12p40 and type 1 and type 2 interferons, the last 2 being known to play a key role in the elimination of infected enterocytes. Our findings demonstrate in neonatal mice how gut flora synergizes with poly(I:C) to elicit protective intestinal immunity against an intracellular pathogen.

Keywords. poly(I:C); TLR3; neonatal mice; Cryptosporidium parvum; intestine; microbiota; dendritic cells.

At the time of birth, the immune system of neonates is still undergoing development. The neonatal immune response has been shown to be quantitatively and qualitatively distinct from that of adults, rendering neonates more susceptible to infections [1, 2]. Lacking a fully developed adaptive immune system, newborns must rely on innate immune responses and maternally transmitted immunity for early protection against pathogens [3]. The development of effective strategies to protect against neonatal diseases is challenging.

After delivery, the intestine of neonates is rapidly colonized with a vast diversity of microbes. Bacterial recognition of the intestinal microbial flora by pathogen recognition receptor has been shown to contribute to developmental programming of epithelial barrier function and gut homeostasis, as well as the innate and adaptive host immune function [4]. In the gut mucosa, commensal-related bacteria are constantly in contact with epithelial cells and mononuclear phagocytes that extend dendrites across the epithelial barrier [5]. Moreover, the loss of gut epithelial integrity when mice are infected or after chemical exposure favors interaction between bacteria and lamina propria resident dendritic cells (DCs).

Cryptosporidiosis is a zoonotic disease caused by the protozoan Cryptosporidium parvum. This intracellular parasite infects intestinal epithelial cells and causes debilitating diarrhea [6]. The severity of the disease is related to the immune status of its host, with young and immunocompromised individuals being the primary targets of this infection. In the neonatal mouse model of infection, cytokines such as interleukin 12 (IL-12) and interferon (IFN) γ have been shown to be involved in controlling the infection within 2–3
weeks [7, 8], whereas CD4+ T cells are dispensable [9]. Several in vitro studies have shown that type 2 and type 2 IFNs can inhibit C. parvum multiplication in intestinal epithelial cells [10, 11]. We have hypothesized that enhancement of innate immunity using strong immunostimulants such as pattern recognition receptor ligands leading to the production of these key cytokines could afford protection against this enteric pathogen.

In this study, we investigated the capacity of poly(I:C) that binds to multiple double-stranded RNA (dsRNA) sensors, including RIG-I, MDA5, and TLR3 [12], to stimulate intestinal immune responses in neonatal mice and increase their resistance to intestinal infection by C. parvum. We found that poly (I:C) reduced parasite load in the intestine of neonatal mice by a TLR3- and TRIF-dependent mechanism that required the presence of CD11c+ cells. Protection depended on the concomitant presence of IL-12 and type 1 and type 2 IFNs that cross-regulate each other to efficiently control infection. Of note, we observed that the protection induced by poly(I:C) was dependent on the presence of gut flora, whose influence was mediated through TLR5 and MyD88 signaling. The increase in intestinal permeability during C. parvum development in neonatal enterocytes favored CD11c+ cell activation by both poly(I:C) and enteric flora for optimal production of IL-12p40 and type 1 IFNs.

METHODS

Mouse Models
IFN-γ−/−, IL-12p40−/−, Toll-like receptor (TLR) 2/4−/−, Nod1/2−/−, IFN-αR−/−, MyD88−/−, TLR5−/−, Lps2−/− (TRIF), TLR9−/−, and CD11c-DTR mice, all with a C57BL/6 genetic background, were raised and maintained in animal facilities of the Plateforme d’Infectiologie Expérimentale (INRA-Tours) or Institut Pasteur in accordance with European guidelines.

Parasite Preparation and Mouse Infections
Oocysts of the C. parvum CpiINRA isolate were initially purified from the feces of an infected child and were maintained by regular passages in newborn calves. Purification of C. parvum oocysts was performed as previously described [7]. Three-day-old neonatal mice were infected orally with 5 × 105 C. parvum oocysts. The level of infection in individual neonatal mice was assessed by counting the number of oocysts in the intestinal content, as previously described [13].

Ethics Statement
All experimental protocols were conducted in compliance with French legislation (Décret: 2001-464 29/05/01) and European Economic Community (86/609/CEE) governing the care and use of laboratory animals after validation by the local ethics committee for animal experimentation (CEEA VdL; No. 2011-09-11 and 2011-10-2).

RESULTS

Collection of Mesenteric Lymph Node Cells, Flow Cytometry, and Cell Sorting
After dissociation, cells of mesenteric lymph nodes (MLNs) were first incubated with anti-CD16/CD32 antibody and then with the antibodies against cell surface molecules for 30 minutes. All antibodies were purchased from BD Pharmingen: APC-anti-CD11, PE-anti-CD40, PE-anti-CD86, PE-anti-CD103, APC-H7-anti-CD8α and FITC-anti-I-A/I-E antibodies. Cells were analyzed on a Becton-Dickinson fluorescence-activated cell sorter with FSC Express3 software. To isolate CD103+ CD8α+ CD11c+ and CD103+ CD8α+ CD11c+ subsets from the MLNs, pools were constituted from 10–40 infected neonatal mice. Cells were first gated for CD11c+ MHCI+ and further analyzed for CD8α expression among the CD103+ population. Sorting was performed on a MoFlo highspeed cell sorter.

Analysis of mRNA Levels by Reverse-Transcription Polymerase Chain Reaction (RT-PCR) Array and Quantitative RT-PCR
RNA was extracted from purified cells and tissue using PicoPure RNA isolation kit and TRIZOL solution, respectively. Gene expression for inflammatory cytokines, chemokines, and their receptors was measured by real-time RT-PCR using commercially available reagents (RT2 profiler system), according to the manufacturer’s protocol. Raw data were acquired and processed with the Chromo4 software to calculate the threshold cycle (Ct) value and relative gene expression values, subsequently determined according to the standard ΔΔCt method. For quantitative RT-PCR, samples were normalized internally using the average cycle quantification (Cq) of the 3 most suitable reference genes—hypoxanthine phosphoribosyltransferase (HPRT), TATAA-box binding protein (TBP), and peptidylprolyl isomerase A (PPIa)—selected using geNorm application among 5 commonly used genes. Primers used for mRNA quantification were described in Supplementary Table 1. Expression data are expressed as relative values after Genex macro analysis.

Permeability Study
Intestinal permeability was determined by measuring the appearance in blood of FITC-dextran MW4000 orally administered 3 hours earlier (60 mg/100 g FITC-dextran). The concentration of fluorescein in the sera was determined by spectrophotofluorometry.

Statistics
Nonparametric analyses were performed using the Mann–Whitney test. P < .05 was considered significant.
cytokines and chemokines in the intestine of neonatal mice. We observed that intraperitoneal administration of poly(I:C) elicited a chemokine response in the intestine of neonatal mice that was higher than in adult mice (Supplementary Figure 1A). These results are in line with the data previously observed in the systemic compartment after poly(I:C) injection [14].

We therefore evaluated whether administration of poly(I:C) could protect neonatal mice against C. parvum infection. To this end, neonatal mice were inoculated with Cryptosporidium parvum and received poly(I:C) by intraperitoneal or oral route 5 days later. As shown in Figure 1A, intraperitoneal administration of poly(I:C) significantly reduced the parasite load after 24 hours, whereas oral administration of poly(I:C) was without measurable effect. Because a strong intestinal chemokine response was only observed when poly(I:C) was administered by intraperitoneal route (Supplementary Figure 1B), we hypothesized that poly(I:C) may have been degraded when given orally. The protection induced by intraperitoneal administration of poly(I:C) was dose-dependent (Supplementary Figure 2A) and was significant for the next 48 hours (Supplementary Figure 2B). Multiple repetitions were required to reduce the level of parasite load during the window of infection (Supplementary Figure 2C).

**Both TLR3 Dependent TRIF Signaling and CD11c+ Cells are Required for the Protection Induced by Poly(I:C)**

Poly(I:C) can act through different dsRNA sensors, including TLR3, MDA5, and RIG-I [12]. We first investigated the contribution of TLR3 in the poly(I:C)-mediated mechanism of protection against cryptosporidiosis. As shown in Figure 1B, TLR3-deficient neonatal mice that received poly(I:C) exhibited the same parasite load 24 hours after treatment as untreated neonatal mice. We confirmed that the protective effect of poly(I:C) is mediated through TLR3 by using TRIF-deficient neonatal mice that are defective for TLR3 signaling and that are not protected against the infection after poly(I:C) administration. McCartney et al showed that DCs and all other radio-sensitive hematopoietic cells respond to poly(I:C) through the TLR3 receptor, whereas radio-resistant stroma cells respond through the MDA5 receptor [15]. We observed that CD11c+ cells from the MLNs of poly(I:C)-treated neonatal mice were more highly activated 24 hours after intraperitoneal injection, as revealed by the higher expression of MHCII and costimulation markers CD40 and CD86 (Figure 1C). We next investigated the role of CD11c+ cells in the protection of neonatal mice induced by poly(I:C) using the CD11c-DTR mouse model, which allows transient depletion of CD11c+ cells after injection of diphtheria toxin (DT) [16]. Four days after infection, CD11c-DTR neonatal mice received DT by the intraperitoneal route. Twenty-four hours later, the efficiency of CD11c+ cell depletion was about 85% in the intestine of neonatal mice (data not shown). When neonates received poly(I:C) 24 hours after DT treatment, the decrease in parasite load was no longer detectable in neonatal mice depleted of CD11c+ cells (Figure 1D). In addition, TLR3 mRNA expression in the infected mucosa was strongly reduced after DT administration (Figure 1E). Taken together, these data suggest that CD11c+ cells are essential for the protection induced by poly(I:C) and represent the majority of TLR3+ cells recruited in the infected mucosa.

**IL-12p40 and IFN-β Are Required for the Protection Induced by Poly(I:C)**

After poly(I:C) stimulation, TLR3 of hematopoietic cells is required for production of IL-12p40 and, to a lesser extent, for
IFN-β [15]. We observed that expression of both IL-12p40 and IFN-β mRNA was strongly increased as soon as 4 hours after poly(I:C) stimulation in the ileum of infected neonatal mice (Figure 2A). The increased expression of IL-12p40 induced by poly(I:C) was 3- to 4-fold higher in infected neonatal mice, in which TLR3⁺ cells were recruited, than in uninfected neonates (Supplementary Figure 3). We did not observe any significant change in mRNA expression for interleukin 12p35 or interleukin 23p19 after poly(I:C) stimulation (data not shown). The contribution of IL-12p40 and IFN-β to the protection induced...
by poly(I:C) was investigated with neonatal mice deficient for IL-12p40 or the type 1 IFN receptor (IFN-αR). In both strains of mice, poly(I:C) administration did not induce significant reduction in the parasite load (Figure 2B and 2C). We observed that after depletion of CD11c+ cells, overexpressions of IL-12p40 and IFN-β mRNA observed after poly(I:C) administration were significantly reduced (Figure 2D and 2E). All together, these data suggest that production of IL-12p40 and IFN-β by CD11c+ cells contributes to the protective immune response induced by poly(I:C).

Interdependence Between IFN-β, IFN-γ, and IL-12p40 for Poly(I:C)-Induced Protection

During cryptosporidiosis, IFN-γ is a key cytokine for the elimination of the parasite [10, 17]. After administration of poly(I:C), IFN-γ-deficient neonatal mice displayed a parasite load similar to nontreated neonatal mice, suggesting that this cytokine was critical for the protection induced by poly(I:C) (Figure 3A). IL-12p40 and type 1 and 2 IFNs are all required for the protection induced by poly(I:C). We therefore investigated by quantitative RT-PCR the expression and cross-regulation that could occur in vivo between these cytokines in the ilea of neonatal mice. We observed that the increased expression of IL-12p40 mRNA after poly(I:C) injection was type 2 and type 2 IFN dependent (Figure 3B) and the IFN-β overexpression was dependent on both IL-12p40 and IFN-γ production (Figure 3C). Moreover, the increased intestinal expression of IFN-γ mRNA was significantly reduced in the absence of functional type 1 IFN receptor (Figure 3D). Collectively, these data suggest that IL-12p40 and IFN-β produced by poly(I:C)-activated CD11c+ cells can cooperate with IFN-γ-producing cells, leading to a synergistic production of both type 1 and type 2 IFNs involved in the control of intracellular parasite development.

Microbiota Is Necessary for Poly(I:C)-Induced Protection Against C. parvum Infection in Neonatal Mice

C. parvum has been shown to decrease transepithelial resistance of intestinal epithelial cell monolayers in vitro [18]. We therefore hypothesized that in the context of higher intestinal permeability in infected neonatal mice, gut flora might synergize with poly(I:C) to increase CD11c+ cell activation. We first measured the intestinal permeability to FITC-dextran in neonatal mice 6 days after infection. Three hours after oral administration of FITC-dextran, a higher level of FITC-dextran was measured in the sera of infected neonatal mice (Figure 4A), demonstrating that C. parvum infection had indeed increased intestinal permeability. We next used an experimental model in which pregnant mice received a mixture of broad-spectrum antibiotics in their drinking water 2 days before the expected day of delivery and throughout the experiment to reduce their gut intestinal bacterial burden and that of their pups. The antibiotic treatment did not affect parasite development 6 days after infection in neonatal mice that did not receive poly(I:C) (Figure 4B). However, upon administration of poly(I:C) to neonatal mice born from antibiotic-treated dams, parasite load was not diminished (Figure 4B). This was specific for poly(I:C) because for CpG-ODN, a TLR9 agonist that we have previously shown to be efficacious to control C. parvum development [19], the antibiotic treatment was without influence on the level of protection measured. After poly(I:C) injection and antibiotic treatment, the increase in the frequency of CD11c+ cells expressing costimulatory molecules was no longer detectable (Figure 4C), and the over-expression of IL-12p40 and IFN-β was reduced by >2- to 3-fold (Figure 4D). Therefore, microbiota plays a key role in the induction of the cytokines involved in the protection induced by poly(I:C).

We next investigated the receptors triggered by the microbiota that synergizes with the TRIF signaling induced by poly(I:C) stimulation. We first analyzed the protection induced by poly(I:C) in Nod1/2-deficient neonatal mice because Nod1 and Nod2 agonists that mimic the peptidoglycan of gut bacteria were known to synergize with TLR3 agonists to stimulate IL-12 production by DCs in vitro [20]. The absence of these cytoplasmic nod-like receptors (NLRs) did not impair the reduction in parasite load induced by poly(I:C) (Figure 5A). We performed additional studies to investigate the role of MyD88, an adaptor molecule essential for the induction of inflammatory cytokines triggered by all TLRs except TLR3. In MyD88-deficient neonatal mice, the protection induced by poly(I:C) was no longer detectable (Figure 5A), suggesting that the microbiota contributes to protection by an interaction with 1 or several TLRs requiring MyD88 for signal transduction. By using different TLR-deficient neonatal mice, we observed that the absence of TLR9 or TLR2/4 was without detectable effect but that the presence of TLR5 was necessary for the protection induced by poly(I:C) treatment (Figure 5A). Furthermore, the increased mRNA expressions of IL-12p40 and IFN-β were lower in TLR5-deficient neonatal mice than in wild-type neonatal mice (Figure 5B). Analysis of TLR3 and TLR5 mRNA expression in CD103+ CD8α− and CD103+ CD8α+ CD11c+ DC subsets in the MLNs of infected neonatal mice revealed that CD103+ CD8α+ DCs express both TLR3 and TLR5, whereas CD103+ CD8α− DCs express TLR3 but low levels of TLR5 (Figure 5C). Taken together, these results show that in C. parvum-infected neonatal mice, microbiota can synergize with a TLR3 ligand through TLR5 to activate CD103+ CD8α+ DCs in a preferential manner.

DISCUSSION

The neonatal period is characterized by a high susceptibility to respiratory and intestinal infections. However, we and others have observed that neonates can exhibit stronger innate cytokine responses than adults to TLR agonist stimulation, which
makes these molecules attractive for immunostimulation strategies [14, 21-23]. Poly(I:C) has often been used to control mucosal viral diseases (eg, herpes simplex virus 2) and occasionally to control enteric bacteria such as Yersinia enterocolitica or Salmonella typhimurium, but to our knowledge its utility for controlling intestinal protozoan infection has never

Figure 2. Poly(I:C)-induced protection is interleukin 12p40 (IL-12p40) and type 1 interferon (IFN) receptor dependent. A, Poly(I:C) was administered by the intraperitoneal route to neonatal mice at 5 days postinfection, and ilea were removed for RNA extraction 4 and 24 hours later. Analyses of IL-12p40 and IFN-β mRNA expression were performed by quantitative reverse-transcription polymerase chain reaction (n = 4 neonates in each group, 2 experiments). B and C, Three-day-old IL-12p40−/− (B), IFN-αR−/− (C), and wild-type C57BL/6J neonates were orally infected with Cryptosporidium parvum and received poly(I:C) as previously described. Twenty-four hours after poly(I:C) injection, parasite load in the intestine of neonates was evaluated (n ≥ 6 in each group) (B and C). D and E, Four hours after poly(I:C) injection, ilea from CD11c-DTR neonatal mice were sampled for RNA extraction and reverse-transcription polymerase chain reaction analysis of IL-12p40 (D) and IFN-β (E) (n ≥ 6 in each group). Data represent mean ± standard deviation. Results are representative of 2 or 3 individual experiments. Abbreviations: IFN, interferon; IL, interleukin; PBS, phosphate-buffered saline.
been investigated [24–26]. We previously showed that an oral administration of CpG-ODN was effective in protecting neonatal mice against severe intestinal infection by *Cryptosporidium parvum* [19]. In this work, we demonstrated for the first time that poly(I:C), a mimetic of viral dsRNA, can also induce protection against intestinal parasite development by a mechanism strictly dependent on the presence of the microbiota.

Poly(I:C) activates various immune cell types through 2 major dsRNA sensors, MDA5 and TLR3. After intraperitoneal administration of poly(I:C), MDA5 has been shown to promote IFN-α and IFN-β secretion by stroma cells, whereas TLR3 was essential for IL-12p40 and IFN-β production by hematopoietic cells in the spleen [15]. We demonstrated that the protection induced by poly(I:C) in neonatal mice was critically dependent on the TLR3 signaling molecule TRIF and the cytokines IL-12p40 and type I IFN. TLR3 is expressed in mouse intestinal epithelial cells; however, only a low level of TLR3 expression is detected the first 10 days of life [27]. In response to poly(I:C), human intestinal epithelial cells produce IFN-β in a RIG-I/MDA5/MAVS–dependent manner [28, 29]. All together these data suggest that in response to poly(I:C), epithelial cell contribution to IFN-β production is not essential for parasite elimination.

We have previously shown that CD11c+ cells are recruited in the ileum of *C. parvum*–infected neonatal mice [30] and hypothesized that these cells could be activated by poly(I:C) through TLR3 to participate in the induction of a protective immune response. With neonatal mice transiently depleted of CD11c+ cells, we observed a loss of the protection induced by poly(I:C) and a reduced expression of IFN-β and IL-12p40. Moreover, mRNA expression of TLR3 in the ileum of these neonatal mice was severely reduced to a level close to that
observed in uninfected neonates. This reflects the depletion of CD11c+ cells that are recruited by the infection and that express TLR3 in the intestine. Recently, a new subset of CD11c+ CD103+ CD8α+ DCs that expresses TLR3 was described in the lamina propria of adult mice [31]. In the MLNs of infected neonatal mice, we found that TLR3 was expressed at a similar level in both CD11c+ CD103+ CD8α+ cells and CD11c+ CD103+ CD8α− cells. These two subsets of CD11c+ could therefore both play a role in the protective mechanism induced by poly(I:C).

After infection with C. parvum, we showed that intestinal permeability was increased in the intestine of neonatal mice, as revealed by experiments using FITC-dextran. Because the gut mucosa is constantly exposed to commensal bacteria, we hypothesized that pathogen recognition receptor ligands derived from these bacteria may provide an additional signal to DCs. Synergy between members of the TLR family using different adaptor molecules or between TLRs and NLRs have been shown to favor DC maturation [32, 33]. When the microbiota of neonatal mice was altered with a cocktail of antibiotics, the upregulation of MHCII, CD40, and CD86 on CD11c+ cells was reduced after poly(I:C) administration. Accordingly, in the presence of such altered commensal flora, IL-12 overexpression and the protection induced by poly(I:C) were also diminished. In contrast, when CpG-ODN, which signals by TLR9 and MyD88, was administered to neonatal mice, the protection was conserved after antibiotic treatment. The importance of MyD88 in the mechanism of protection was confirmed with MyD88-deficient mice. We further investigated which TLR signaling through MyD88 was involved and identified TLR5 as a
key molecule for the synergy with poly(I:C) mediated by the commensal flora. MyD88 and other molecules such as IRAK and TRAF6 are involved in TLR5 signaling [34, 35]. We can assume that the loss of protection induced by poly(I:C) in MyD88−/− mice is simply due to the absence of signaling initiated by TLR5 ligands derived from the commensal flora. Bacterial flagellins are the ligands of TLR5 and are present in both Gram-negative (Escherichia coli, Salmonella typhimurium, and Pseudomonas aeruginosa) and Gram-positive bacteria (Eubacterium rectale and Butyrivibrio fibrisolvens) of the intestine [36]. Enteric infections, such as infections by Rotavirus leading to diarrhea-associated disturbance of host intestinal homeostasis, are known to modify the composition of the different phyla of bacteria [37, 38]. For example, rotavirus infections have the capacity to adversely affect the host’s gut Bacteroides community [38].

In the MLNs of infected neonatal mice, we observed a dichotomy in TLR5 expression in the different subsets of CD103+ CD11c+ with TLR5 much more expressed in CD103+ CD8α− CD11c+ than in CD103+ CD8α+ CD11c+. This is in agreement with previous observations on adult intestinal DCs [31]. Neonatal CD103+ CD8α+ CD11c− and CD103+ CD8α− CD11c+ cells express both TLR3 and TLR5 and synergy could therefore occur directly in response to poly(I:C) and to the signals provided by the commensal flora. In adults, CD103+ CD8α− CD11c+ subsets produced similar amounts of IL-12p40 in response to flagellin and poly(I:C), respectively [31]. In neonatal mice born from antibiotic-treated mothers, we observed that the level of IL-12p40 was reduced by >2-fold. One can therefore speculate that under our experimental conditions both subsets cooperate in IL-12p40 production.

Figure 5. MyD88 and TLR5 signaling are required for the poly(I:C) induced protection of Cryptosporidium parvum–infected neonatal mice. A, Three-day-old MyD88−/−, TLR9−/−, TLR5−/−, TLR2/4−/−, NOD1/2−/−, and C57BL/6J wild-type neonatal mice were orally infected by C. parvum and received poly(I:C) as previously described. The level of infection in different strains of mice cannot be compared because the strains were often considered in separate sets of experiments. Parasite load in the intestine of neonates was evaluated 24 hours later (n ≥ 5 in each group). B, Four hours after poly(I:C) injection, expression of interleukin 12p40 and interferon β mRNA was measured in the ileum of TLR5−/− neonatal mice (n ≥ 5 in each group). C, CD11c+ MHCII+ CD103− CD8α− cells (n = 3) and CD11c+ MHCII+ CD103+ CD8α+ cells (n = 4) were sorted from mesenteric lymph nodes of 6-day–infected neonates, and TLR3 and TLR5 mRNA expression was analyzed by real-time reverse-transcription polymerase chain reaction on both sorted cell subsets. Abbreviations: IFN, interferon; IL, interleukin; PBS, phosphate-buffered saline; TLR, Toll-like receptor; WT, wild-type.
Both type 1 and type 2 IFNs can inhibit C. parvum multiplication in intestinal epithelial cells [10, 11]. We observed that poly(I:C) is also a strong inducer of type 1 IFN that participates in the control of C. parvum infection because in IFN-αR−/− neonates, poly(I:C) injection did not reduce the intestinal parasite burden. In addition, we report that type 1 IFNs participate in the reduction of the intestinal parasite load by favoring IL-12p40 and IFN-γ mRNA expression, which participate in the reduction of infection induced by poly(I:C). All together these data suggest that these cytokines cooperate together to reduce intestinal parasite load in neonatal mice after poly(I:C) administration.

In conclusion, our results show that a single poly(I:C) administration to neonatal mice during C. parvum infection led to a reduction of the parasite load within 24 hours. To this end, cooperation between TLR3–TRIF activation by poly(I:C) and TLR5–MyD88 signals provided by the commensal flora was required for optimal IL-12p40, IFN-β, and IFN-γ production which is necessary for the mechanism of protection. Poly(I:C) is therefore an adjuvant to be considered for controlling neonatal intestinal diseases such as cryptosporidiosis.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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