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UNIVERISTY OF CALIFORNIA, SAN DIEGO

Poly(I:C)-Induced Small-Intestinal Injury as a Model for Viral Infection

A Thesis submitted in partial satisfaction of the requirements for the degree

Master of Science

in

Biological Sciences

by

Daniel Thomas Dempsey

Committee in charge:

Professor Martin Kagnoff, Chair
Professor Michael David, Co-Chair
Professor Elina Zuniga

The Thesis of Daniel Thomas Dempsey is approved, and it is acceptable in quality and form for publication in microfilm and electronically:

Co-Chair

Chair

University of California, San Diego

2011

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ABSTRACT OF THE THESIS

Poly(I:C)-Induced Small Intestinal Injury as a Model for Viral Infection

by

Daniel Thomas Dempsey

Master of Science

University of California, San Diego, 2011

Professor Martin Kagnoff, Chair

Professor Michael David, Co-Chair

It has been shown that viruses and their ability to activate innate immunity may play a role in the pathogenesis of Celiac Disease. Toll-like receptor 3 is an endosomal receptor that recognizes dsRNA as well as its synthetic analog, polyinosinic-polycytidilic acid. It is shown here that intrapertioneal injection of poly(I:C) resulted in atrophy of small-intestinal villi and induction of inflammatory cytokines in a time and dosage-dependent

manner. Small intestinal injury was observed in mice that were null for interleukin-15 (IL-15) and no induction of IL-15 was observed in wild-type mice in response to poly(I:C). Matrix-Metalloproteinase 13 (MMP-13) showed induction that correlated with the poly(I:C)-induced small intestinal injury. Furthermore, it was shown that the induction of inflammatory cytokines is much higher in the lamina propria cells than in the intestinal epithelial cells of the small intestine.

Introduction

Celiac Disease and Connection to Innate Immunity

Celiac Disease (CD) is an autoimmune disease of the small-intestinal mucosa that is activated in genetically susceptible individuals of all ages. It is a chronic inflammatory disease that is characterized by injury to the mucosal layer of the small intestine and inability to absorb nutrients properly. The disease is activated by proteins collectively termed "gluten" which are comprised of the gliadins and glutenins in wheat and the secalins and hordeins in rye and barley. CD has been predicted by screening studies to afflict approximately 1% of the population of the United States (circa 3 million people). Despite the prevalence of this disease, only about 10-15% of affected individuals are diagnosed and treated. The reason for this is that CD patients will often exhibit a broad range of intestinal and/or extraintestinal symptoms or they can remain relatively asymptomatic [1].

Intestinal symptoms associated with CD include weight loss, malnutrition, and steatorrhea. A closer look at the small intestine reveals that patients with CD can show complete loss of villi (Figure 1) as well as increased intraepithelial lymphocytes (IELs), and lymphocytes in the lamina propria. This is in sharp contrast to a healthy small intestine which is marked by relatively long and abundant villi that resemble a microscopic forest that harbors its

own population of flora and fauna! These villi have normal numbers of IELs, lymphocytes, and plasma cells in the lamina propria.

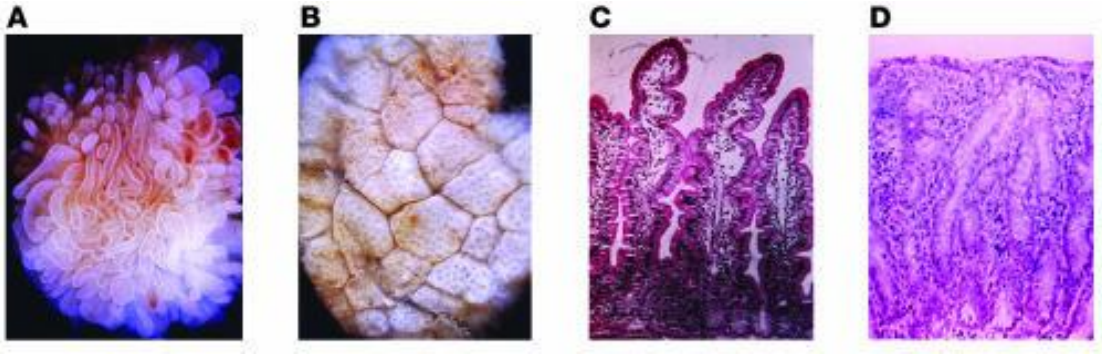


Figure 1. Small-intestinal mucosal biopsy of a normal individual (**A**) and an individual with celiac disease (**B**) as viewed through a dissecting microscope. H&E stained section of a small-intestinal mucosal biopsy from a normal individual (**C**) and an individual with celiac disease (**D**). *Kagnoff, 2007

There is a large genetic factor involved in the pathogenesis of CD. Almost all affected individuals express specific MHC class II alleles that encode for either HLA-DQ2 or HLA-DQ8 heterodimers on antigen-presenting cells (APCs). Once a genetically susceptible patient ingests "gluten", it passes through the epithelial layer into the lamina propria where tissue TGase deamidates the glutamine converting it to negatively charged glutamic acid which has a higher binding affinity for the HLA-DQ2 and HLA-DQ8 heterodimers on APCs[2, 3]. The APCs can then present the peptides to HLA-DQ2- or HLA-DQ8-restricted CD4+ T cell populations that release mediators that precipitate tissue damage.

There is also much evidence to portray the innate immune system as a prominent player in CD. Not only are there more IELs in the mucosal layer of a

patient with CD, but these IELs are also transformed from antigen-specific T cells to NK-like cells that mediate epithelial cell damage[4]. A cytokine known for causing proliferation of natural killer cells, interleukin-15 (IL-15) has gathered attention for its role in the pathogenesis of this disease. It is typical to see upregulation of IL-15 in the epithelial cells and DCs of the lamina propria in CD and this seems to alter the signaling of the $CD8^+$ IELs. IL-15 also increases expression of epithelial cell surface ligands that are bound by the increased number of cytotoxic NK-like cells[5-8]. These findings have made IL-15 a prime candidate for study here to further elucidate the mechanisms of inflammatory disease.

Viral Infection and Celiac Disease

The pathogenesis of celiac disease, like many diseases, is marked by both genetic and environmental factors. It has been shown that infections by viruses such as rotavirus or human adenovirus[9] may play a role in the development of celiac by genetically susceptible individuals. One study showed that rotavirus infection predicted a higher risk of CD autoimmunity[10]. This has been supported by epidemiological observations on the seasonal pattern of CD in which the incidence of the disease is correlated with rotavirus infection[11]. The viruses, with their ability to activate innate immunity, may be setting the stage for the induction of the adaptive gluten-specific immune response seen in celiac patients. The hypothesis is that contact with gluten at a time where there is preexisting intestinal

inflammation, altered intestinal permeability, upregulation of HLA-DQ2 and HLA-DQ8 on dendritic cells, and type I and type II interferon production will increase the risk of developing CD in genetically susceptible individuals. The main focus of this thesis is to investigate the changes that take place in innate immunity upon viral infection that may facilitate the development of CD.

Toll-like Receptor 3 and Microbial Recognition

Toll-like receptors (TLRs) have been shown to be major receptors that can signal innate immunity. The first connections between TLRs and the immune system were drawn in 1996 when Hoffmann and colleagues noticed that *Drosophila* flies that were mutant for Toll receptors were particularly susceptible to fungal infection[12]. Shortly thereafter, it was found that Toll-like receptors also exist in mammalian systems and they recognize a broad range of pathogen-associated molecular patterns (PAMPs). For this reason, they have been classified as pattern recognition receptors (PRRs). The binding of microbial components to TLRs triggers the activation of signaling cascades which culminate with the induction of genes responsible for the host's antimicrobial defense. Upon binding of ligand, the TLRs dimerize and recruit adaptor molecules to the TIR domain of the TLR. The four adaptor proteins are called MyD88, TIR-associated protein (TIRAP), TIR-domain-containing adaptor protein-inducing IFN-b (TRIF)/TIR-domain-containing molecule 1 (TICAM1), and

TRIF-related adaptor molecule (TRAM). Each of the TLRs generates different responses due to their selective usage of these adaptor molecules.

Toll-like receptor 3 is of particular interest here due to its established role in recognizing dsRNA and its synthetic analog polyinosine-deoxycytidylic acid (poly I:C). Once activated, the TIR domain of TLR3 recruits the adaptor protein TRIF (TICAM-1) (Fig. 2). TRIF then interacts with receptor-interacting protein 1 (RIP-1) which serves to activate the NF-κB promoter[13]. TRIF can also activate TRAF-family-member-associated NF-κB activator binding kinase 1(TBK1) through TRAF3 (Hacker, 2006). Once activated, TBK1 along with inducible IκB kinase (IKK-i/IKK-ε) directly phosphorylate IRF-3 and IRF-7[14]. Phosphorylated IRF-3 and IRF-7 form homodimers and translocate into the nucleus where they bind to ISREs and express the IFN-inducible genes. It has been shown that poly (I:C) is a potent inducer of type-1 interferons[15] and is also responsible for NK cell activation[16, 17].

Toll-like receptor 3 activation by poly(I:C) in wild-type mice produces an intestinal phenotype that is similar to that seen in celiac disease: shortened villi and loss of epithelial cells. Weight loss is also conserved. Therefore, poly(I:C)-induced small intestinal injury can be a useful model for some aspects of the tissue-damage seen in CD.

TLR3 is specifically expressed in conventional dendritic cells (cDCs) that actively phagocytose apoptotic or infected cells, but not in plasmacytoid dendritic cells (pDCs). TLR3 is also expressed in a variety of epithelial cells

including intestinal epithelial cells that are found in the lumen of the small bowel (Akira 2006). TLR3 expression is upregulated by viral infection and exogenous addition of poly(I:C) or type I IFN[18-20]. However, these epithelial cells are not known to express TLR3 on the cell surface which raises the question: how is poly(I:C) recognized by the innate immune system? The mechanisms of the TLR3-dependent poly(I:C)-induced intestinal injury are still largely unknown and are the focus of this investigation.

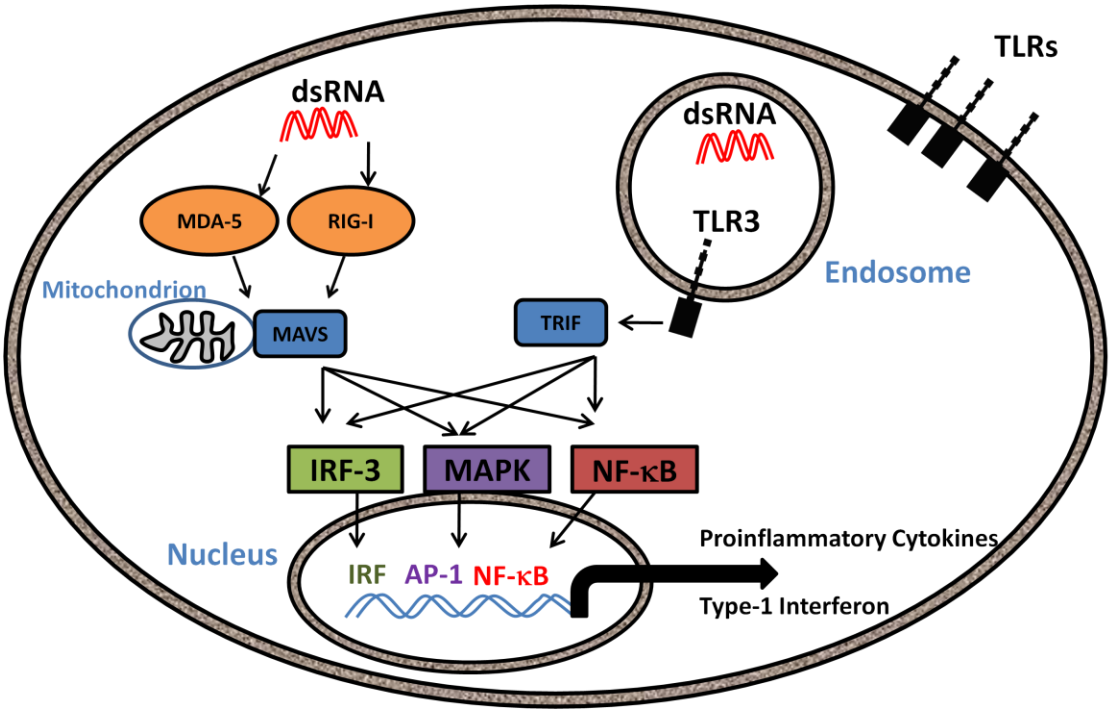


Figure 2. Simplified diagram of the endosomal and cytoplasmic RNA recognition receptors and their downstream signaling cascades.

Materials and Methods

Mice

Wild type (WT) C57BL/6J (B6) mice were obtained from The Jackson Laboratory. TLR3$^{-/-}$ (B6 background) mice and IFN type I-R$^{--/--}$ were provided by Dr. E. Raz (University of California, San Diego [UCSD], La Jolla, CA). TRIF$^{-/-}$ (B6 background) mice were provided by Dr. B. Beutler (The Scripps Research Institute, La Jolla, CA). MAVS$^{-/-}$ mice (C57BL/6J/129 mixed background) [21] and the corresponding WT mice were provided by Dr. Chen (University of Texas Southwestern Medical Center, Dallas, TX). All mice handling and experimental procedures were approved by and conducted in accordance with the regulations and policies put forth by the Institutional Animal Care and Use Committee (IACUC) and the Animal Care Program (ACP) at the University of California San Diego.

Poly (I:C) Injection Protocol

Polyinosinic-polycitidylic acid sodium salt (Poly(I:C)) obtained from Sigma-Aldrich and Invitrogen was dissolved in sterile PBS so that the total dose was 5-30 μg/g body weight of the mouse. Concentrations of poly(I:C) were created so that the injection volume was 100-200μl per mouse. All injections were administered intraperitoneally (IP) and followed with a gentle massage to ensure uniform distribution of the PBS (control) or poly(I:C) in the peritoneum.

Treated and untreated mice were housed in separate cages following injection to prevent cross contamination through consumption of fecal matter. Water was provided to the mice but the food was removed.

Histology

Tissues were collected in processing cassettes and fixed overnight with 10% neutral buffered formalin. After dehydrating with ethanol and clearing with clearing agent (Citrisolv, Fisher), tissues were infused with paraffin. They where then sectioned with a microtome at 5 micrometers and placed on a slide. After 24 hours, the slides were deparaffinized with xylene and rehydrated in 100%, 95%, and 70% alcohol baths followed by deionized MilliQ water. The slides were then stained with hematoxylin (Surgipath) and then eosin (Surgipath). Coverslips were mounted using xylene bases Cytoseal XYL(Richard-Allan Scientific).

RT-PCR

Whole tissue or IECs were homogenized and total RNA was extracted using TRIzol reagent (Invitrogen), according to the protocol provided by the manufacturer. A total of 1mg of RNA was reverse transcribed using the Improm II- Reverse Transcription System (Promega) and the included oligo (dT)

primers. These were incubated together for 5 minutes at 25C, 1 hour at 42C for extension, and then 15 mintutes at 70C for thermal inactivation of the reverse transcriptase. The primer sequences used were as follows:

GAPDH sense, 5'-CGATGCCCCCATGTTTGTGAT-3', and

GAPDH antisense, 5'-GGTCATGAGCCCTTCCACAATGC-3';

IL-6 sense, 5'-ACAACCACGGCCTTCCCTAC-3', and

IL-6 antisense, 5'-ACAATCAGAATTGCCATTGCAC-3';

IFN-β sense, 5'-GGAGATGACGGAGAAGATGC-3', and

IFN-β antisense, 5'-CCCAGTGCTGGAGAAATTGT-3';

MMP-13 sense, 5'-CCGGGAATCCTGAAGAAGTCT-3', and

MMP-13 antisense, 5'-CTCTAAGCCAAAGAAAGATTGCATT-3';

IL-15 sense, 5'- CATCCATCTCGTGCTACTTGTGTT-3', and

IL-15 antisense, 5'- CATCTATCCAGTTGGCCTCTGT-3';

To verify results, an additional set of IL-15 primers were used with the following sequences:

IL-15 sense, 5'-TCTTGATTGTTCCGTTGCTT-3', and

IL-15 antisense, 5'-GGCTTTCAATTTTCTCCAGG-3'.

Quantitative PCR

cDNA from each of the samples was combined with Applied Biosystems Sybr-green PCR reagents and primers. Each sample was run in triplicate in order to obtain an accurate final C_t value. The ddCt averages were taken prior to adding the relative quantification exponent and performing statistical analysis. All induction was calculated relative to the GAPDH housekeeping gene.

Isolation of IECs

Proximal sections of small intestine were cut open longitudinally and then cut into small pieces(~1cm). Add 5 ml PBS-minus CaCl/MgCl. Wash in metal strainer with PBS. Add 5 ml cold RPMI 1640/1 mM DTT/2 mM EDTA. Shake 30 minutes @ 37^0C 200 rpm. Filter 100 um into 50 ml conical tube. Aliquot flow through into 3 microfuge tubes. Spin 1000 x g 5 min 4^0C. Aspirate supernatant and visualize pellet. Add 500 ml Trizol reagent to one tube.

Results

Intraperitoneal injection of poly(I:C) induces severe destruction of small intestinal tissue

Administration of poly(I:C) produced an acute response in mice that resulted in observable injury to the small intestine. Initial inspection of poly(I:C) treated mice reveals distension of the small intestine. This is characterized by thinning of the mucosal layers and swelling of the intestine from intraluminal fluid collection. (Fig. 2). Cross sections of the small intestine show that there is pronounced villus atrophy in poly(I:C) treated mice (Fig. 3).

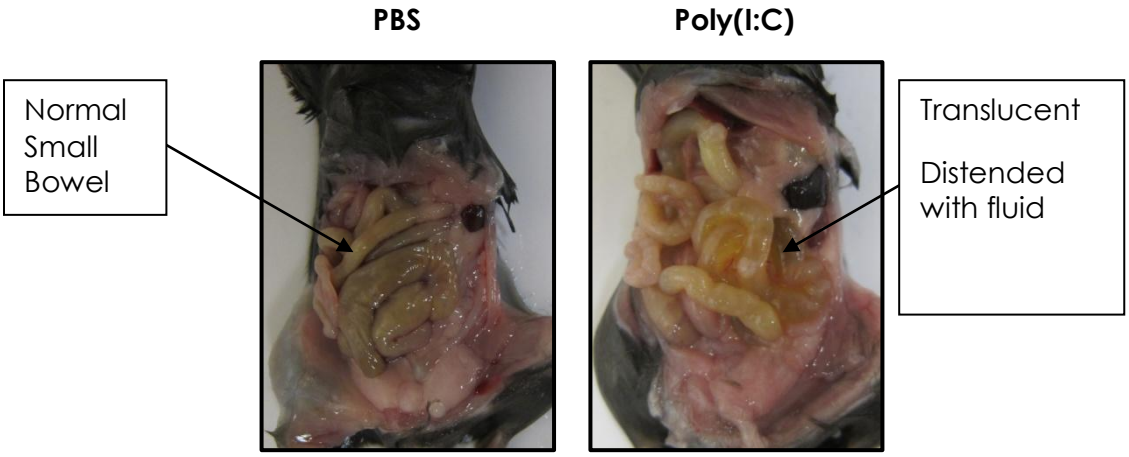


Figure 3. Comparison of the gross dissections of wild-type mice given intraperitoneal injections (IP) of phosphate buffered saline (PBS) or poly (I:C) in PBS at a dosage of 15 μg/g and a time-point of 3 hours.

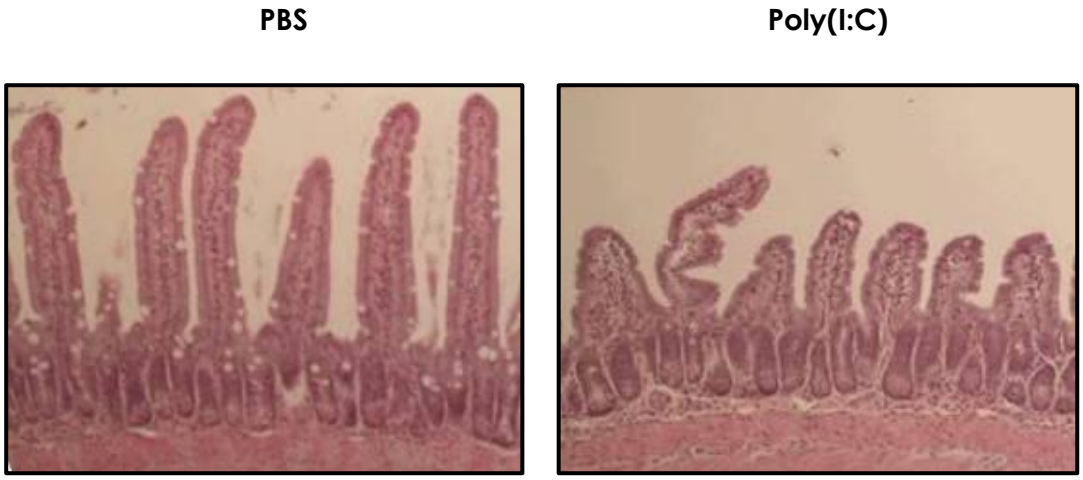


Figure 4. Cross sections of the proximal small intestine in mice given intraperitoneal (IP) injections of phosphate buffered saline (PBS) or poly (I:C) in PBS at a dosage of 15 μg/g and a time-point of 3 hours.

Villi throughout the small bowel were decreased in length with a more pronounced effect seen in the longer villi of the proximal intestine (Supp. Fig. 1). The damage was dose-dependent (Fig. 4) where an increased dosage of poly(I:C) exacerbated villus atrophy. Since the 15 μg/g and 30 μg/g dosages of poly(I:C) yielded similar injury, the 30 μg/g dosage was chosen for subsequent experiments in order to detect subtleties in the expression of genes associated with the inflammatory response. The villus damage was also time-dependent and becomes progressively more severe in each at each later time-point in the 6-hour timeframe investigated(Fig. 5). Previous experiments performed by our lab show that the villus length almost completely recovered by 48 hours (Supp. Fig. 2).

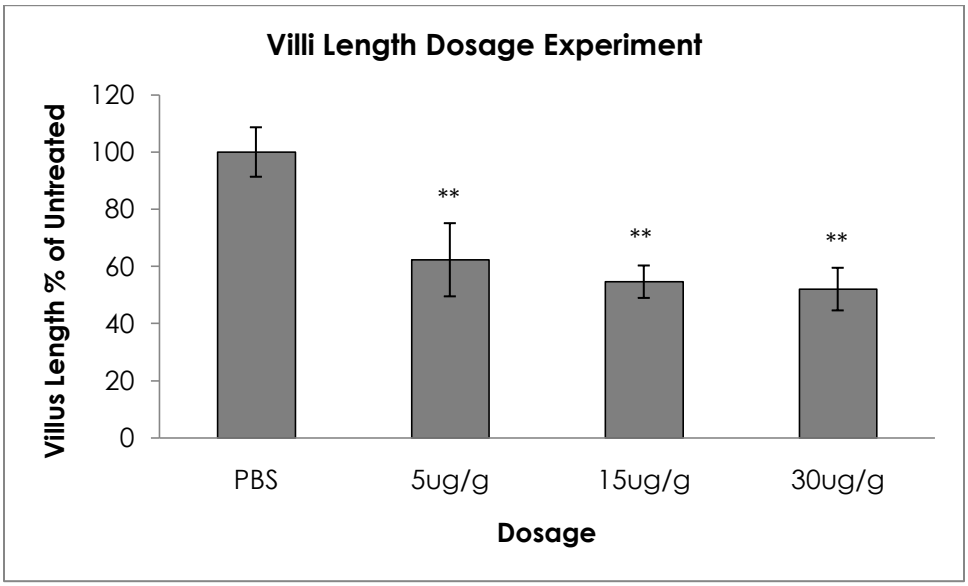


Figure 5. Villus length in wild-type mice given various dosages of poly(I:C) intraperitoneally and calculated relative to untreated mice (PBS). Significance is calculated relative to untreated mice (PBS) with ** $p<0.01$. N=3 mice per dosage.

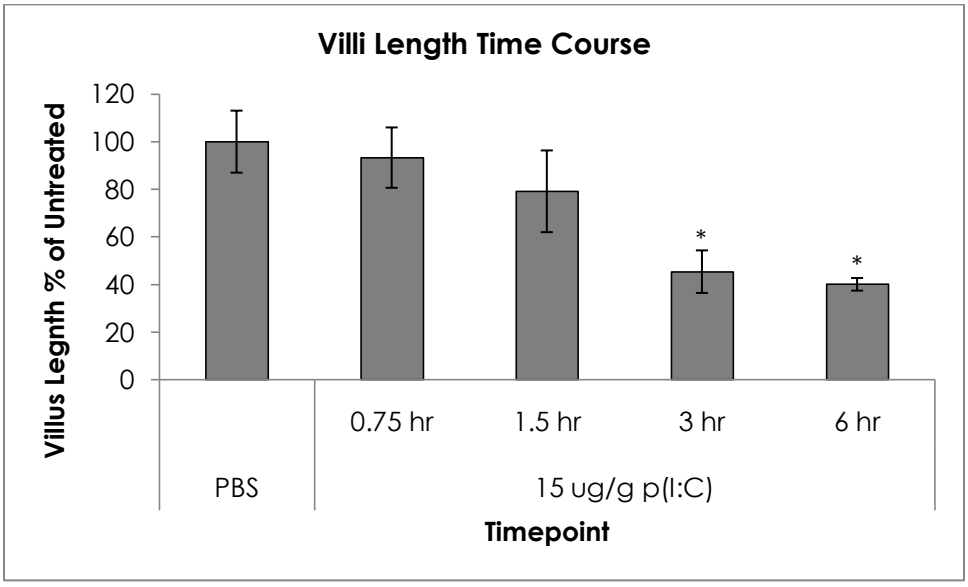


Figure 6. Villi length in wild-type mice at multiple time-points following intraperitoneal poly(I:C) injection and calculated relative to untreated mice (PBS). Significance is calculated relative to untreated mice (PBS) with * $p<0.05$. N=2-5 mice per time-point.

IL-15 Is not required for villus atrophy to occur in the small intestine following poly(I:C) treatment

In order to investigate the role of IL-15 in poly(I:C)-induced intestinal damage, poly(I:C) was administered to IL-15 knockout mice. These mice displayed the same phenotype as wild-type mice after poly(I:C) injection (Fig. 6). In addition to the inflamed appearance of the small bowel, the degree of villus atrophy was found to be comparable to wild-type mice (Fig. 7). Together these results indicate that poly(I:C) induced small intestinal injury occurs in the absence of IL-15 which suggests that it is not an absolute requirement for the response. Since mice without IL-15 have no NK cells and markedly decreased numbers of CD8 T cells, NK T cells, and T cells, and DCs [22, 23] , this draws the focus away from these cell types as mediators of the response that is observed in these mice.

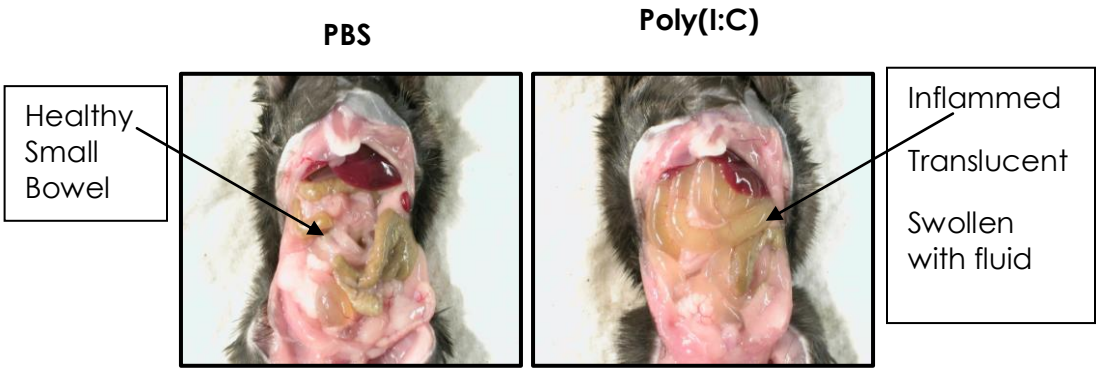


Figure 7. Comparison of the gross dissections of IL-15 knockout mice given intraperitoneal injections (IP) of phosphate buffered saline (PBS) and poly (I:C) at a dosage of 30 μg/g and a time-point of 3 hours.

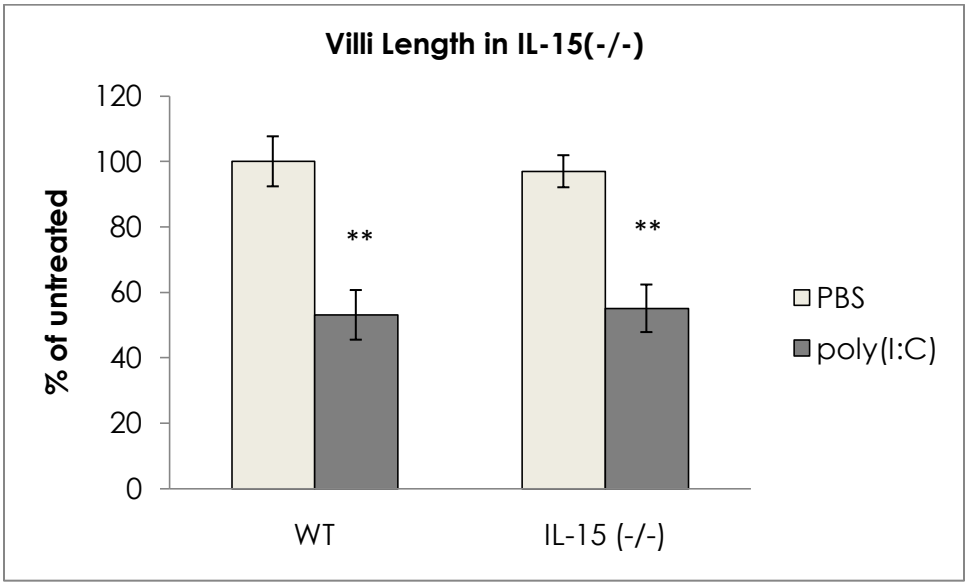


Figure 8. Villus lengths of wild-type and IL-15 knockout mice given intraperitoneal injections (IP) of phosphate buffered saline (PBS) and poly (I:C) at a dosage of 30 μg/g and a time-point of 3 hours. ** $p<0.01$.

Wild-Type mice at various time points and dosages were examined for expression of IL-15 mRNA following intraperitoneal injection of poly(I:C) (Fig. 8). The time points and dosages examined here were the same ranges that have previously been shown to produce significant injury in the mice. Normally, downstream effectors such as cytokines that are involved in the immune response would show upgregulation from their basal levels. No upregulation in IL-15 mRNA was seen in wild-type mice at any of the time points and dosages of interest. It is important to note that these results are contrary to those published in two recent studies[24, 25].

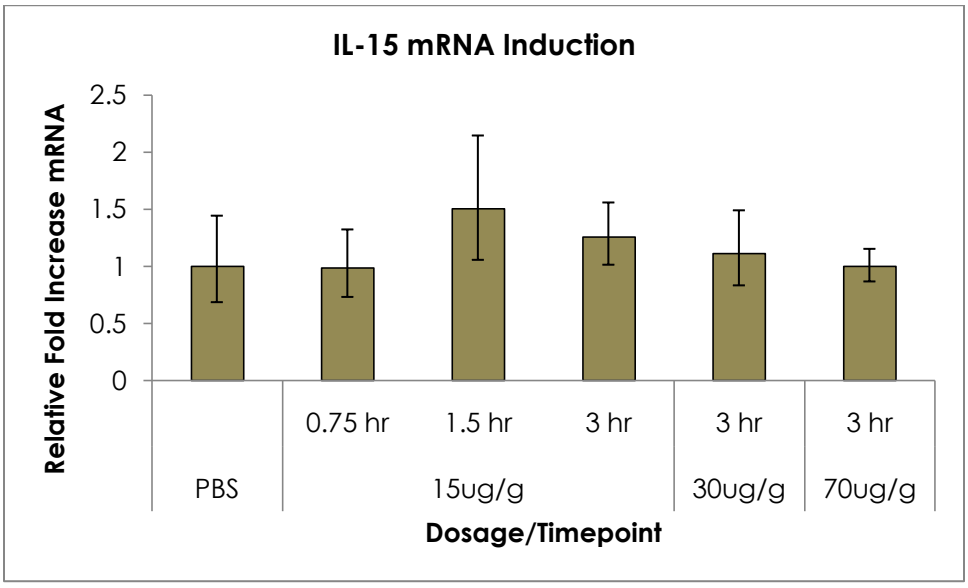


Figure 9. Induction of IL-15 mRNA in wild-type mice given intraperitoneal (IP) injections of poly(I:C) at various dosages and time points and calculated relative to mice given PBS. N=2-4 per group.

In addition to wild-type mice, poly(I:C) was also administered to mice that were knockout for Toll-like receptor 3, the TRIF adapter protein, and the IPS-1 adapter protein (MAVS). TLR3 and TRIF are relevant in the endosomal pathway activated by poly(I:C) whereas IPS-1 is a part of the cytosolic pathway. Previous studies in this laboratory have shown that TLR3, TRIF, and to a lesser extent, MAVS mice display less villus shortening than wild-type mice after poly(I:C) injection. No increased expression of IL-15 was observed in these mice following treatment (Fig. 9). Since the disruption of each of these signaling pathways failed to either upregulate or downregulate IL-15 mRNA expression, this suggests that the absence of induction observed in wild-type is not the net effect of these pathways operating in opposition.

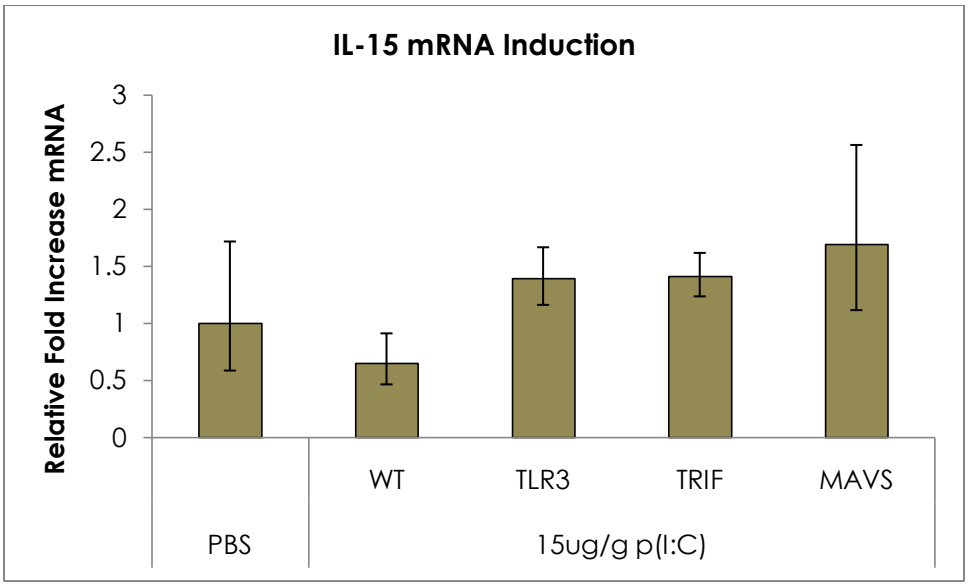


Figure 10. Induction of IL-15 in WT, TLR3, TRIF, and MAVS knockout mice given intraperitoneal (IP) injections of poly(I:C) at a dosage of 15ug/g and a time point of 3 hours and calculated relative to mice given PBS. N=2-4 per group.

Poly(I:C)-induced upregulation of inflammatory cytokines is time and dose dependent

To further investigate the mechanism behind the poly(I:C)-induced intestinal injury, the activity of three proteins were chosen for their established inflammatory [interleukin-6 (IL-6), interferon-beta (IFN-β)] and protease activity [matrix metalloproteinase-13 (MMP-13)]. IL-6 is a general marker of inflammation so it was important to investigate its activity in the whole tissue of the small intestine following poly(I:C) injection. IFN-β is one of the type I interferons that displays antiviral activity and can serve as an indicator of IRF-3 activation. Lastly, MMP-13 is a member of a family of collagenases that have been shown to be induced by activation of toll-like receptors[26]. Other MMPs such as MMP-1, -3, -9, and TIMP-1 were considered and no poly(I:C)-induced mRNA expression was observed (Supp. Fig. 3).

A time course experiment was performed to determine the optimal time at which cytokine activity could be observed in future experiments. The results showed that IFN-β was induced very quickly (0.75 hrs) in response to poly(I:C) whereas IL-6 and MMP-13 showed induction at later time-points (Fig. 10A,B,C). Each of the proteins of interest showed significant induction at 3 hours which is also a time-point that was previously shown to display significant villus damage. Thus, all future studies involving mRNA and histology were conducted at a 3 hour time-point.

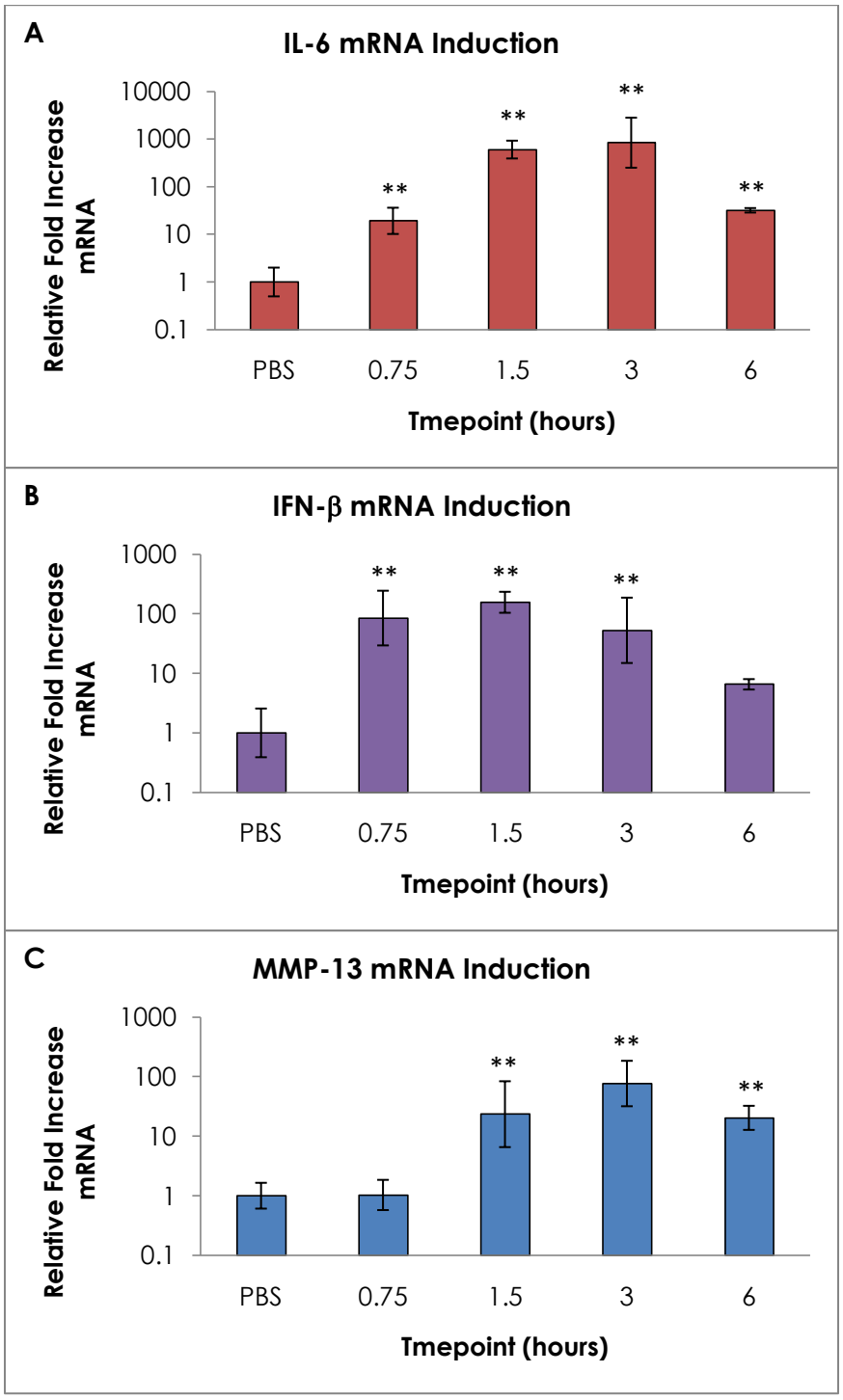


Figure 11 (A, B, C). Induction of IL-6 (A), IFN-β (B), and MMP-13 (C)at various timepoints in mice given 30μg/g IP injections of poly(I:C) or PBS as a control. Significance is calculated relative to untreated mice (PBS) with ** $p<0.01$. N=15,5,5,5,2 for each graph.

It was also important to consider the effect of poly(I:C) dosage on the induction of these proteins. Only slight differences were observed in the induction as a result of increased dosage (Fig. 11 A,B,C). The smallest dose (5 μg/g) was sufficient to upregulate each of the proteins and any subsequent increases did not result in greater mRNA production. However, since the medium dosage (15 μg/g) was previously shown to cause maximal villus atrophy, it was the dosage chosen for future studies.

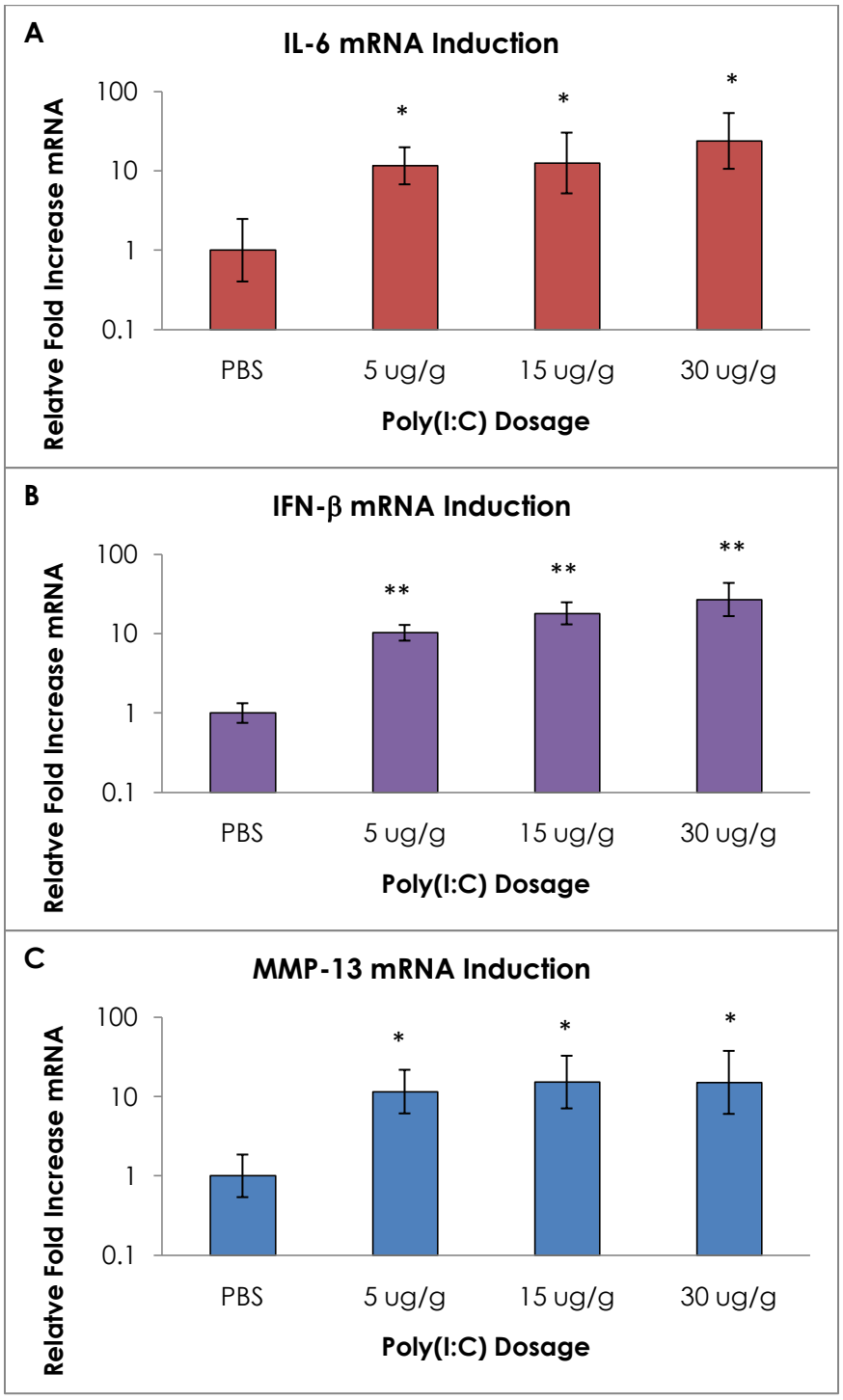


Figure 12 (A, B, C). Induction of IL-6 (A), IFN-b (B), and MMP-13 (C) in mice given intraperitoneal injections of poly(I:C)at various dosages or PBS as a control. Significance is calculated relative to untreated mice (PBS) with * $p<0.05$, ** $p<0.01$. N=2,4,4,4 for each graph.

Reduced inflammatory cytokine induction is observed in endosomal and cytosolic pathway knockouts

In order to determine the mechanism of the poly(I:C)-induced cytokine and proteinase mRNA induction, mice were obtained that were null for TLR3, TRIF, and MAVS (IPS-1). The TLR3 and TRIF knockouts showed significant decrease in IL-6, IFN-β, and MMP-13 mRNA expression when compared with wild-type mice (Fig. 12 A,B,C). However, the TLR3 knockout mice showed significantly more induction of IL-6 and MMP-13 than untreated mice and TRIF knockout mice. This suggests that poly(I:C) is still being recognized in the TLR3 mice. The TLR3 mice were genotyped and verified to be, in fact, knockout for the gene. Although there was no statistically significant difference between induction in WT and induction in $MAVS^{-/-}$, the possibility of poly(I:C) signaling through the cytoplasmic pathway remains due to the persistent trend that the average induction in MAVS was lower than wild-type. Another explanantion, which would account for the discrepancy between the induction seen in TLR3 and TRIF, could be that the poly(I:C) is not 100% pure. If the poly(I:C) were contaminated with endotoxin, it would still be able to induce inflammatory cytokines through TLR4 in the TLR3 knockout. However, in the TRIF knockout, only signaling through the MyD88-independent pathway would be available.

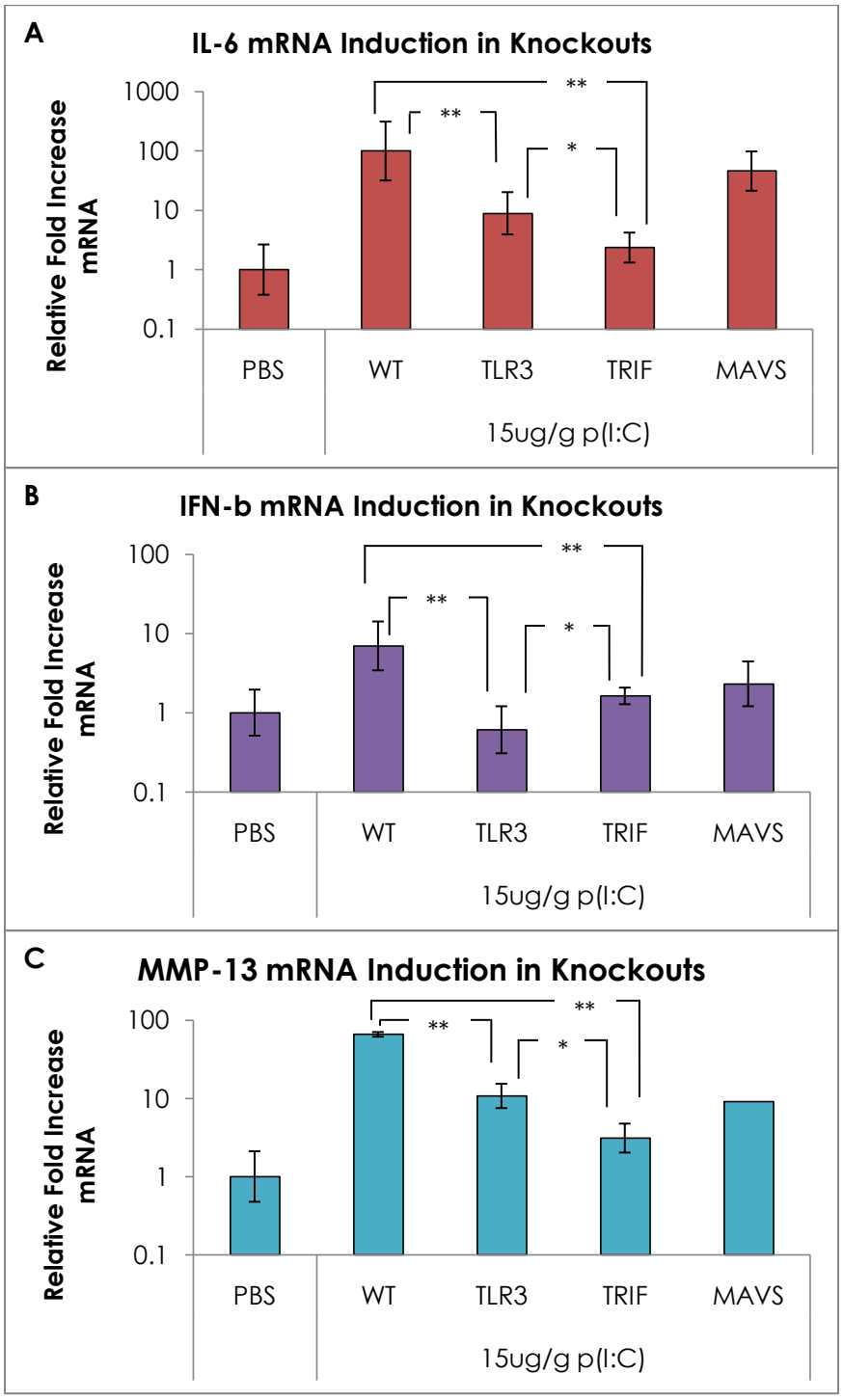


Figure 13 (A, B, C). Induction of IL-6 (A), IFN-b (B), and MMP-13 (C) in wild-type, TLR-3(-/-), TRIF(-/-), and MAVS(-/-) mice given intraperitoneal injections of poly(I:C) and calculated relative to mice given control (PBS). Significance is calculated relative to untreated mice (PBS) with * $p<0.05$, ** $p<0.01$. N=3-6.

Evaluation of cytokine induction in intestinal epithelial cells (IECs)

After establishing that inflammatory cytokines are induced in small intestinal whole tissue in response to poly(I:C), one next step was to determine which cell types were showing the induction. Intestinal epithelial cells are the first cells of the small intestine to come into contact with a luminal invading pathogen so it was hypothesized that they may be involved in eliciting an inflammatory response. In this experiment, the wild-type and TLR3$^{-/-}$ mice showed typical levels of IL-6, IFN-β, and MMP-13 induction in whole tissue. The IECs, however, showed significantly less induction of each of these cytokines when compared to whole tissue (Fig. 13 A,B,C).

Evaluation of cytokine induction in lamina propria cells (LP cells)

Since the IECs were not significantly contributing to the induction of IL-6, IFN-β, and MMP-13 our attention was drawn to the cells of the lamina propria. In wild-type mice, typical IL-6, IFN-β, and MMP-13 mRNA expression was observed for whole tissue whereas the IECs showed significantly less induction than whole tissue. The lamina propria cells were shown to have significantly higher levels of IL-6 and IFN-β mRNA expression than both the IECs and the whole tissue (Fig. 14 A,B,C) which suggests that the lymphocytes in the lamina propria are a major site for expression of these cytokines and protease.

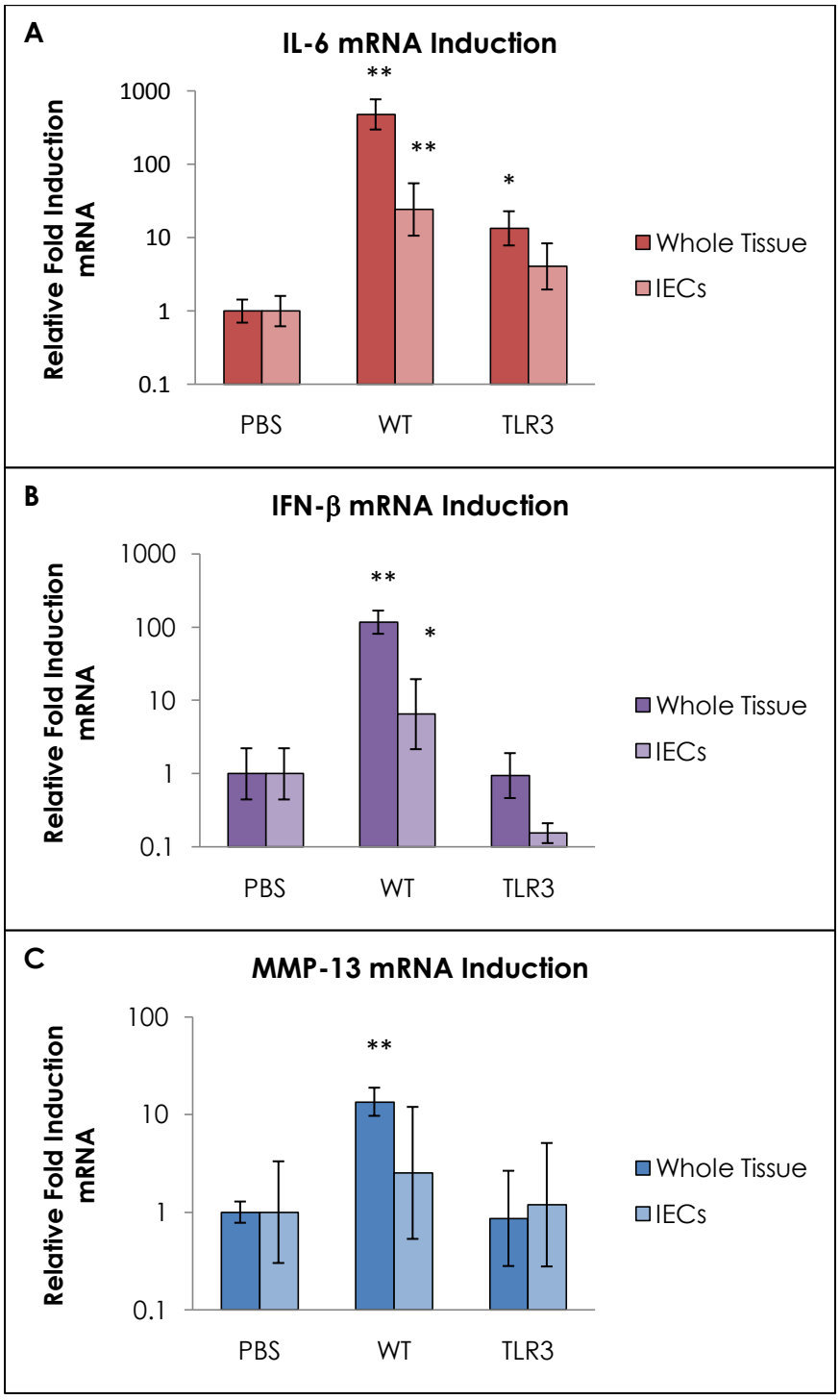


Figure 14 A,B,C. Induction of IL-6 and IFN-β in WT and TLR3 knockout mice given 30μg/g intraperitoneal injections of poly(I:C) and calculated relative to mice given control (PBS). Significance is calculated relative to untreated mice (PBS) with * $p<0.05$, ** $p<0.01$. n=4.

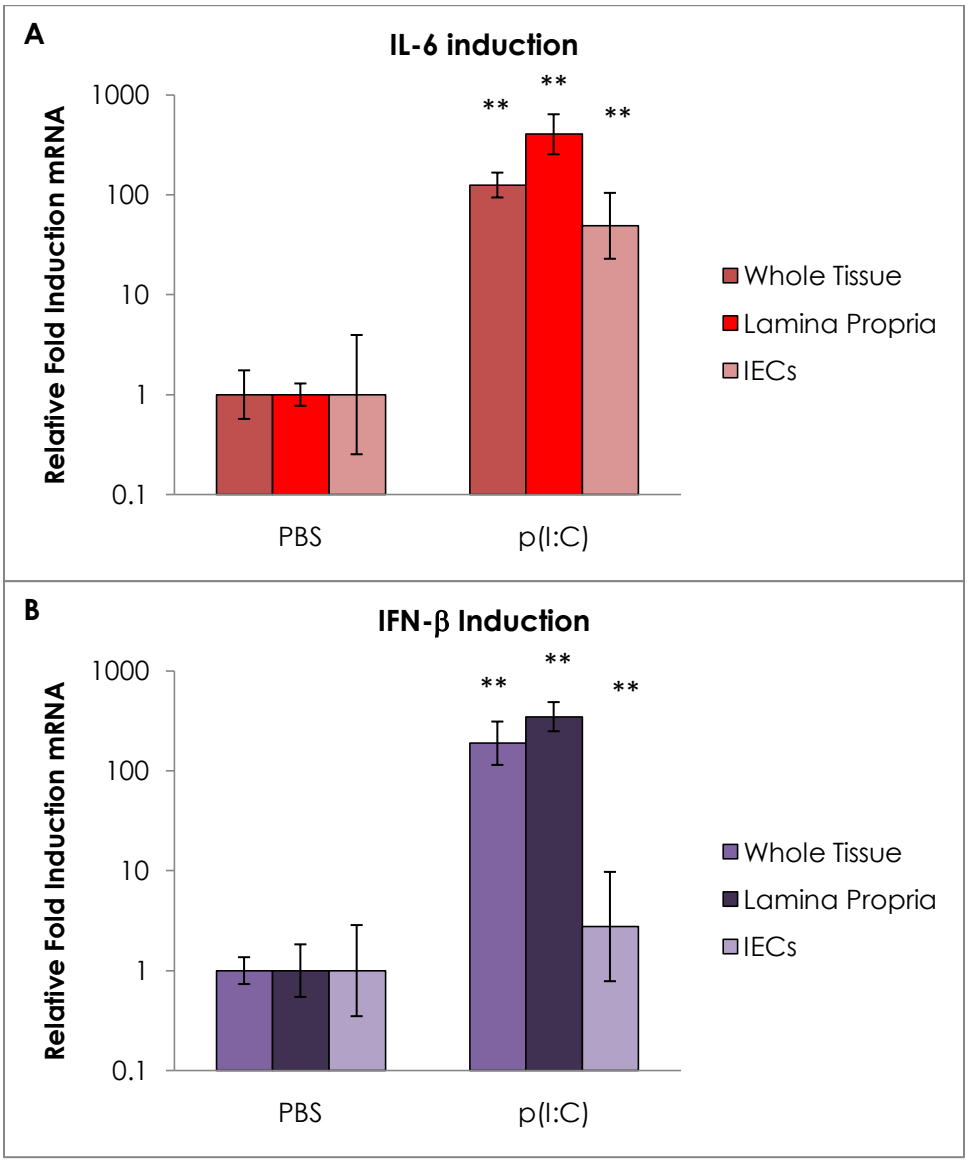


Figure 15 A & B. Induction of IL-6 and IFN-β in WT and Interferon α/β receptor knockout mice given 30μg/g intraperitoneal injections of poly(I:C) and calculated relative to mice given control (PBS). Significance is calculated relative to untreated mice (PBS) with ** $p<0.01$. n=4.

Conclusions

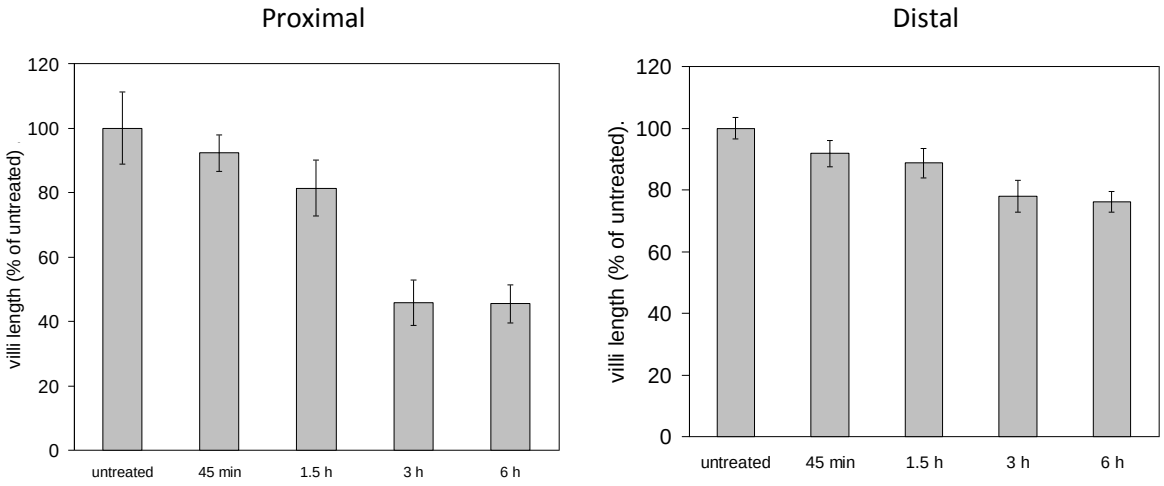
The data shows that poly(I:C) induced expression of IL-6 and IFN-β mRNA in the small intestine were increased with an increased dosage and time after poly(I:C) injection. This correlates with the increases in the destruction of mucosal tissue in the small bowel. The first significant reduction in villi length was observed at 3 hours post-injection at which point IL-6 and IFN-β were already induced. This correlation suggests that if these increases in mRNA are paralleled by increased protein translation then each of these cytokines could be a candidate to be important in the mediation of poly(I:C) induced small-intestinal injury. Furthermore, the induction of these cytokines could contribute to the inflammatory environment that sets the stage for the adaptive response to gluten in celiac disease.

The IL-15 knockout mice were shown to display damage to the small intestine that was comparable to that see in wild-type mice after IP injection of poly(I:C). They showed an overall distension of the small intestine as well as similar reduction in villi length. In addition to this, wild-type mice that showed damage in respose to poly(I:C) did not show IL-15 induction in the whole tissue of the small intestine. These results indicate that small intestinal damage is occurring without the requirement of IL-15 and that it is therefore not a major player in the underlying mechanism.

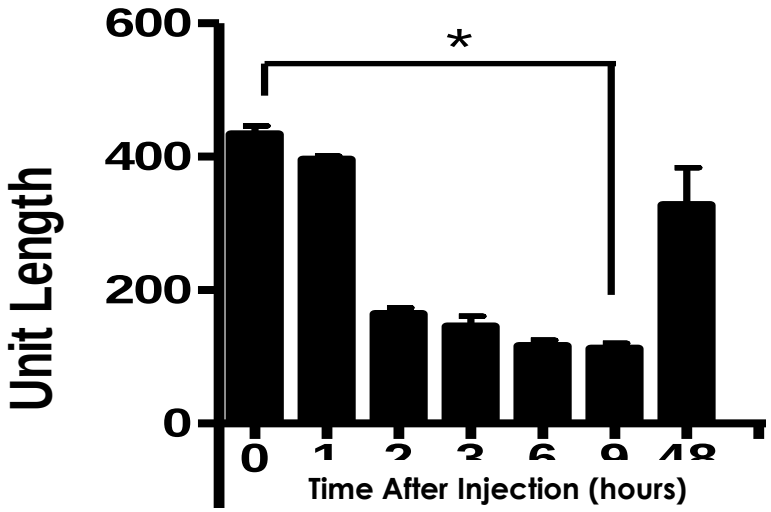
The matrix-metalloproteinases were thought to be involved in the destruction of small intestinal tissue when exposed to virus or poly(I:C). The results showed that MMP-1, -3, -9 and TIMP-1A did not have significant induction in response to poly(I:C). MMP-13 mRNA, however, did show significant induction at 3 hours post-injection which is the exact time point that the villus damage begins. These results suggest that MMP-13 could be involved in the destruction of the collagen in the small intestinal tissue eventually leading to atrophy of the villus and leakage of fluid and possibly IECs into the lumen.

After determining that there was upregulation of IL-6, IFN-b, and MMP-13 in whole tissue, it was shown that the intestinal epithelial cells (IECs) had little to no upregulation of these cytokines and proteases which draws the focus away from them as the creators of an inflamed environment. More importantly, it was shown that the lamina propria (LP) cells had greater levels of expression of each of these cytokines and proteases which suggests that they may play a role in the ultimate generation of small intestinal injury. Furture studies using flow cytometry and FACS should reveal which subset of lamina propria lymphocytes are leading this response.

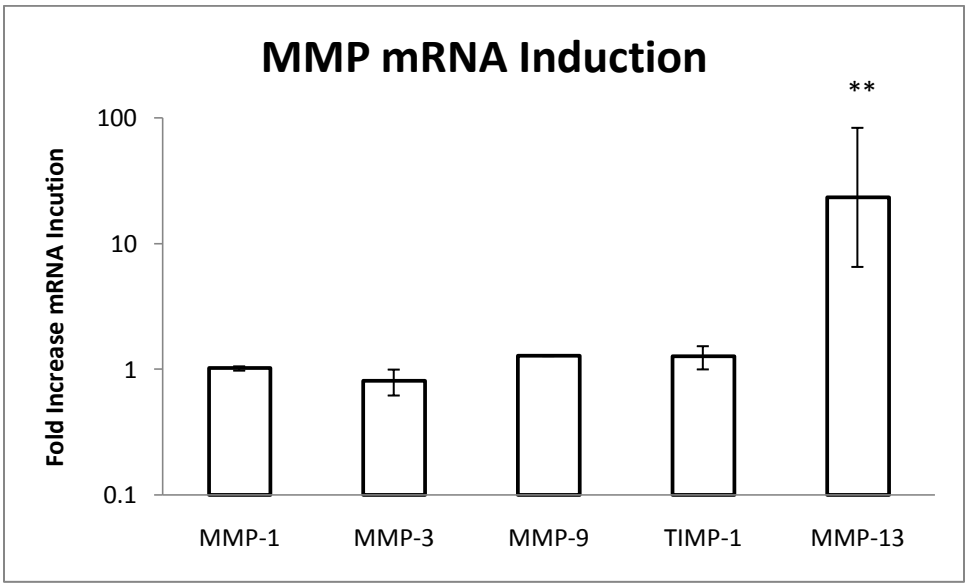
Supplementary Figures



Supplementary Figure 1. Villus lengths in proximal and distal sections of small intestine in wild-type mice at multiple time-points following intraperitoneal poly(I:C) injection at a dosage of 30 μg/g. * p<0.05.



Supplementary Figure 2. Villi length in wild-type mice at multiple time-points following intraperitoneal poly(I:C) injection at a dosage of 30 μg/g. * p<0.05.



Supplementary Figure 3. Induction of various matrix metalloproteinases following intraperitoneal poly(I:C) injection at a dosage of 30 μg/g and a time-point of 3 hours. ** $p<0.01$.

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