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14. ABSTRACT

This proposal addresses a significant medical problem, namely, infection triggered inflammation in the intestines (technically called post-infectious inflammatory bowel disease) in military personnel. Compromised gut barrier integrity is an important risk factor that contributes to the onset of IBD, especially post-infection. The environmental cues and its molecular controls regulating intestinal barrier function are poorly understood in homeostatic and pathophysiologic states like infection-induced IBD. Our studies show a novel direct link between intestinal microbial metabolism (i.e. specific microbial metabolites) and regulation of intestinal permeability via a pathway regulated by an orphan nuclear receptor, PXR, and TLR4. We demonstrate that in the small intestines (which mirrors what happens in large intestines), where PXR is expressed in intestinal epithelial cells in a crypt-villus gradient, in homeostasis, dietary tryptophan-derived bacterial metabolites (i.e. indoles and indole metabolites in particular indole 3 propionic acid or IPA) tonically activate PXR and induce a down-regulation of the Toll-like Receptors, in particular TLR4, and its downstream signaling pathway. This results in modulating the abundance of TNF-α, which in turn modulates intestinal barrier function (i.e. permeability). In the context of an inappropriate increase in inflammatory signals (e.g., infection), suppression of PXR, and/or excess loss of dietary modulators (e.g., tryptophan), and/or specific indole metabolizing bacteria (e.g., antibiotics) results in increased permeability, thus exacerbating underlying disease predisposition and pathology. In this model, restitution of signaling homeostasis, either by reconstituting

15. SUBJECT TERMS
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INTRODUCTION:

This proposal addresses a significant medical problem, namely, infection triggered inflammation in the intestines (technically called post-infectious inflammatory bowel disease) in military personnel. Compromised gut barrier integrity is an important risk factor that contributes to the onset of IBD, especially post-infection. The environmental cues and its molecular controls regulating intestinal barrier function are poorly understood in homeostatic and pathophysiologic states like infection-induced IBD. Our studies show a novel direct link between intestinal microbial metabolism (i.e. specific microbial metabolites) and regulation of intestinal permeability via a pathway regulated by an orphan nuclear receptor, PXR, and TLR4.

We demonstrate that in the intestines where PXR is expressed in intestinal epithelial cells in a crypt-villus gradient, in homeostasis, dietary tryptophan-derived bacterial metabolites (i.e. indoles and indole metabolites in particular indole 3 propionic acid or IPA) tonically activate PXR and induce a down-regulation of the Toll-like Receptors, in particular TLR4, and its downstream signaling pathway. This results in modulating the abundance of TNF-α, which in turn modulates intestinal barrier function (i.e. permeability). In the context of an inappropriate increase in inflammatory signals (e.g., infection), suppression of PXR, and/or excess loss of dietary modulators (e.g., tryptophan), and/or specific indole metabolizing bacteria (e.g., antibiotics) results in increased permeability, thus exacerbating underlying disease predisposition and pathology. In this model, restitution of signaling homeostasis, either by reconstituting intestinal loss of indole-metabolite producing bacteria and/or PXR activating bacterial metabolites (i.e. IPA), could result in abrogating pro-inflammatory signals and loss of barrier permeability in the context of intestinal inflammation. Our proposal will address the role of these metabolites and of PXR in maintaining barrier function in infection induced colitis in mice. The immunologic implications of PXR in the pathogenesis of intestinal injury during infection, is unknown. Furthermore, the role of PXR in the pathogenesis of infection-induced colitis is unknown. Furthermore, the therapeutic mining for metabolite mimics is unexplored. Thus, we hypothesize that PXR is critical for regulating (abrogating) the inflammatory response in both epithelial and innate immune cells such as intestinal macrophages, and thus important for limiting pathology following enteric infection. We further hypothesize that the combinatorial binding of indole(s) to the human PXR LBD (ligand binding domain) can be chemically mimicked (bacterial metabolite mimicry) towards discovery of more potent new chemical entities and drugs that activate PXR and repress inflammation. The main goal of our lab (Khanna Lab) is to investigate the role of PXR, in the maintenance of intestinal immunological homeostasis in vivo under steady state conditions or after an enteric infection or other inflammatory cues.

For our studies we will use several different mouse models (including the PXR^{flx} –Villin-cre, LysM-cre, CD169-cre mice and wild-type littermates) to analyze differences under steady state and following infection with intestinal pathogens such as *C. Rodentium-stx* bacterial load, spread, translocation and mucosal immune responses before and after infection. These studies will uncover whether IEC intrinsic PXR regulates inflammation and through which key cytokines/inflammogens. The ultimate goal of understanding fundamental biology is to develop novel and unconventional approaches to curing disease (e.g., metabolite mimics for IBD therapy). The short-term goal is to further the knowledge of a novel cellular pathway dictated by PXR in post-infectious IBD.

The proposed studies to be carried out by Khanna lab specifically deals with the role of colonic macrophages in regulating infection induced inflammation and gut disease. To this end, the role of CD169+ macrophages in mediating infection induced colitis and inflammatory disease will be

investigated. Here, we propose that mechanistically PXR and other xenobiotic receptors regulate macrophage function in the gut. As we have shown previously with intestinal epithelial cells, PXR regulates immune regulatory mechanisms such as IL10 production and TLR4 expression and sensing, and likely other immune molecules.

KEYWORDS: *Intestinal epithelial cells, Inflammatory bowel disease, macrophages, innate immune cells, Pregnane X receptor, colitis*

ACCOMPLISHMENTS:

Although, the last year of the grant was very challenging we still accomplished a lot in the three years of the grant funding. The accomplishments are outlined below and the data are included in this section. Unfortunately, with respect to the mouse models and mechanistic studies we acquired a large amount of negative data, which nevertheless are important results and will serve to guide us in future studies in this area.

Our major goal of the project:

Aim 1. To investigate the role of PXR, in the maintenance of intestinal immunological homeostasis in vivo under steady state conditions or after an enteric infection:

What was accomplished under these goals?

To this end, we first began by determining the status of the macrophage subsets and other myeloid cells in the intestines of mice that were PXR deficient or normal. Since, the PXR-floxed mice are still being crossed to villin-cre, CD169-cre or LysM-Cre, we began our studies using global PXR deficient mice.

First, we imaged the colonic tissue from WT and PXR deficient mice. The colon was PLP fixed and frozen in OCT. 20um thick sections were cut and imaged using antibodies against CD169, CD11c, F480, EpCAM and CD11b. A Zeiss 880 confocal microscope was used to acquire the images. Imaris software was used to analyze and quantify imaging data.

As shown in Fig.1, the localization and number of CD11c+ cells which likely represents dendritic cells (DCs) in the colon of WT and PXR deficient mice appeared to be dissimilar. Majority of the CD11c cells were on the top of the villus structure in epithelial layer in WT and PXR KO mice, however, the number of CD11c+ cells was reduced in PXR KO mice. Furthermore, the DCs appeared to be in an organized network in WT mice, while in PXR KO mice the network structure was not as organized.

We also imaged the CD169+ macrophages in colon of WT and PXR deficient mice (Fig. 2). Interestingly, the number and concentration of CD169+ macrophages were dramatically increased in the colon of PXR KO mice when compared to WT mice. The networks of CD169+ macrophages in the PXR KO colonic tissue was more organized and the clusters of these cells were more pronounced in the KO mice compared to WT mice.

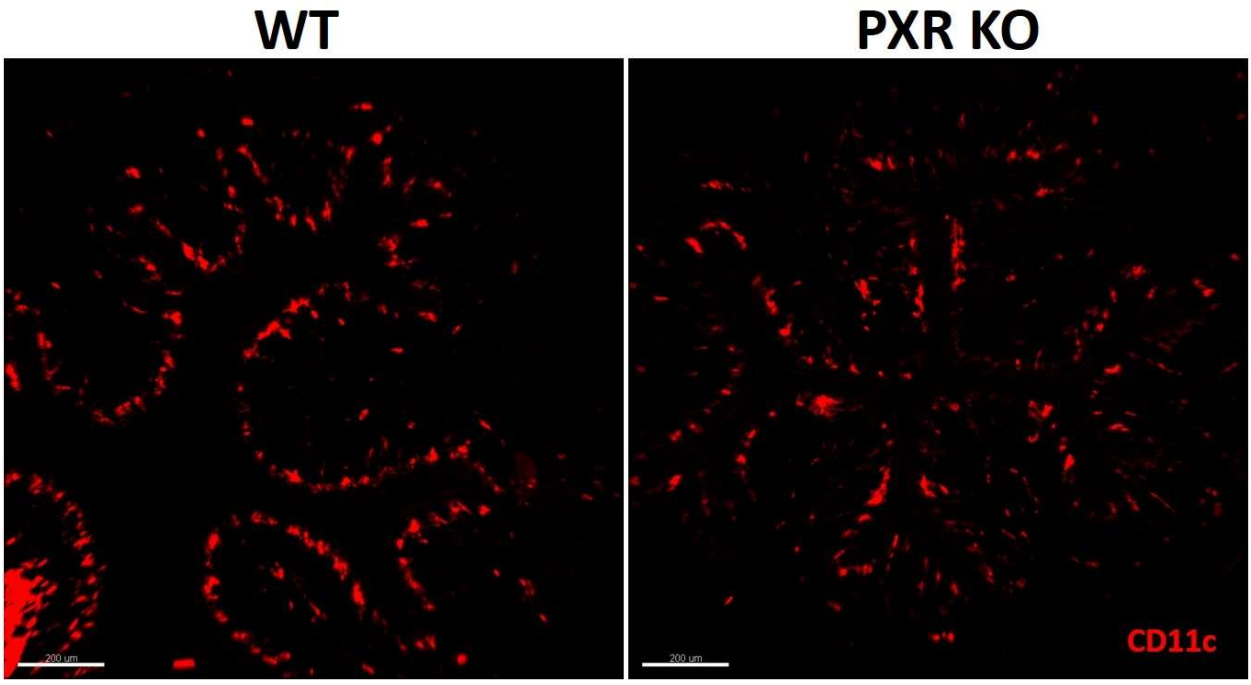


Fig.1. Colonic tissue from WT and PXR KO mice stained for CD11c.

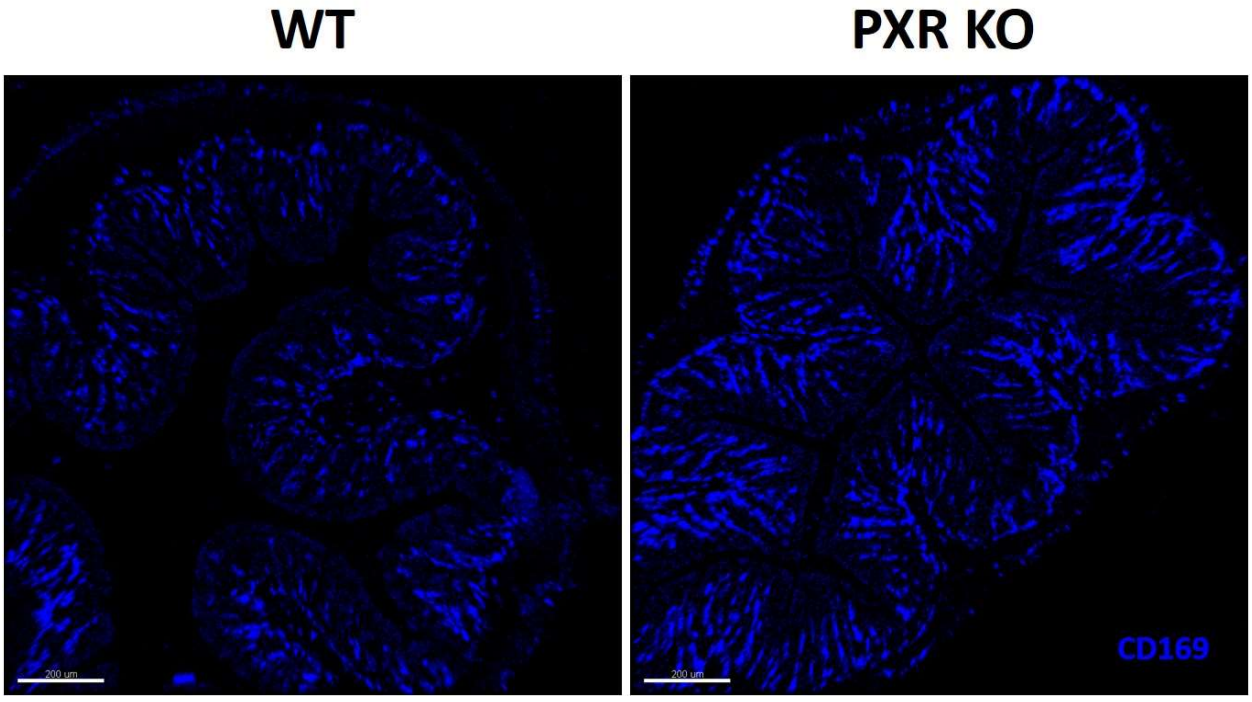


Fig.2. Colonic tissue from WT and PXR KO mice stained for CD169.

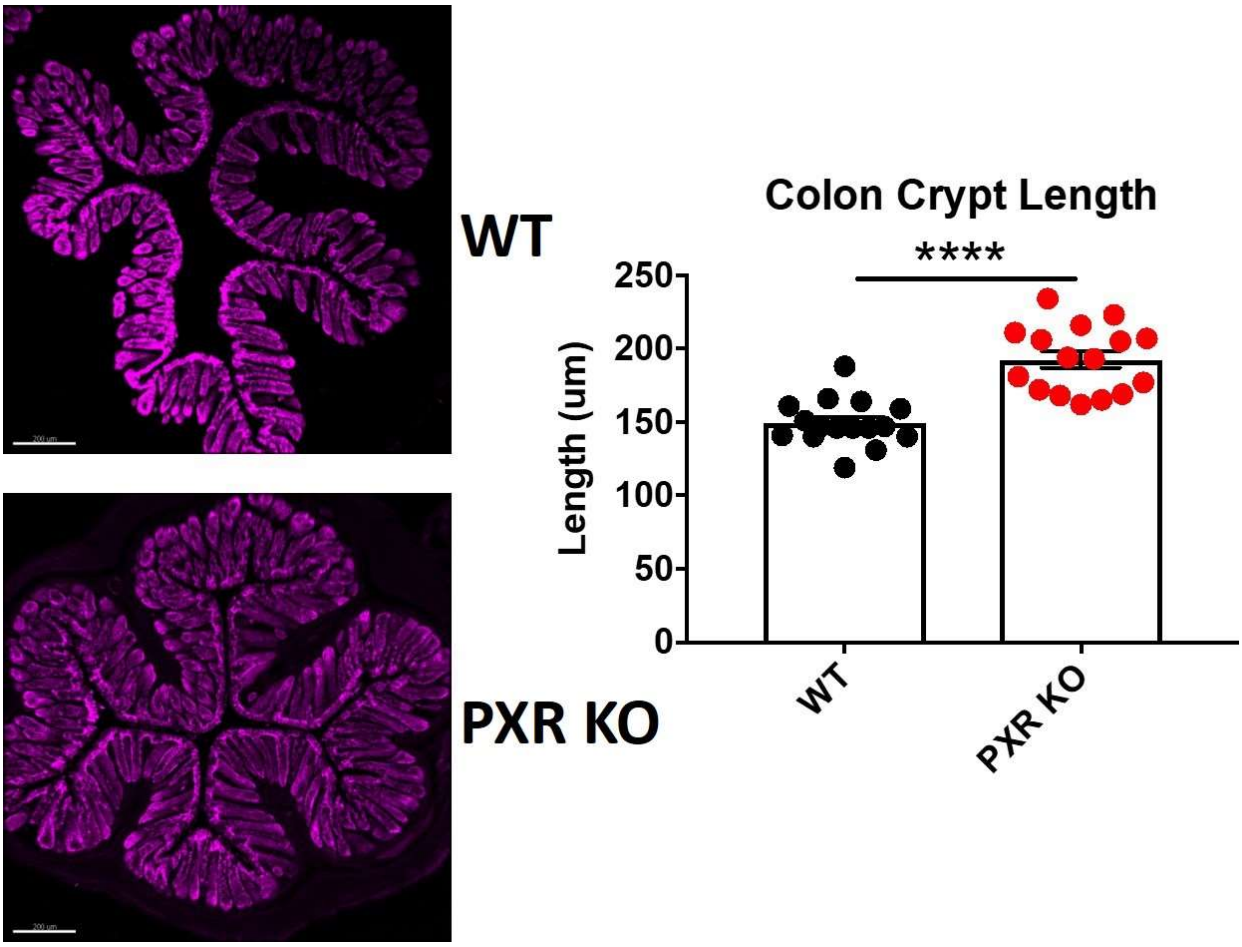


Fig.3. Colonic tissue from WT and PXR KO mice stained for EpCAM (purple) and the colonic crypt length was quantified using Imaris software. ****p<0.00005. Student t-test was performed on several different colonic tissue samples from 4 different mice.

The data with respect to the CD169+ macrophages in PXR KO mice suggested increased inflammation in PXR KO colons even at steady state. One important geographical result of elevated inflammation in the gut tissue is increase in colonic crypt length. Thus, we stained the colonic sections of WT and PXR KO mice with the epithelial marker EpCAM and used the Imaris software to quantify crypt length. Indeed, we observed a significant increase in crypt length in PXR KO mice.

Next we undertook an extensive flow cytometric analysis of myeloid cell infiltration in the colonic and small intestinal tissue of WT and PXR KO mice at steady state. 18 week old male mice in both groups were sacrificed and the colon and small intestines were enzymatically treated to acquire single cell suspension.

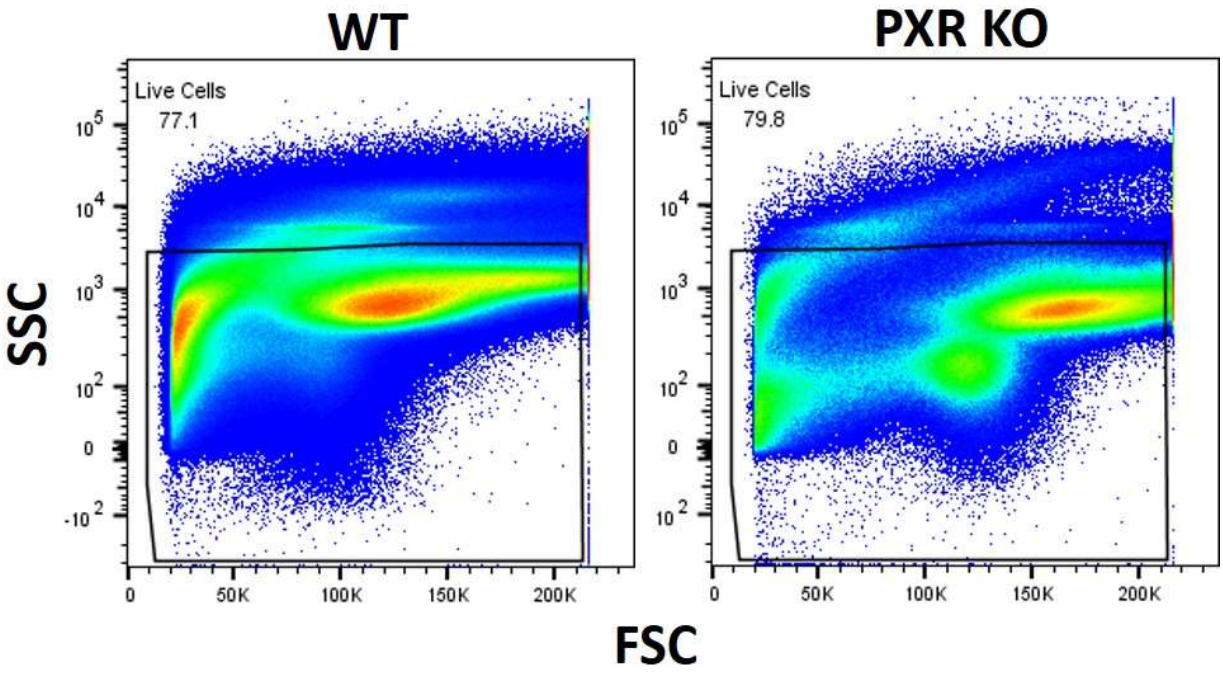


Fig.4. Forward and side scatter dot plot of a representative WT and PXR KO mouse. The cell population represents colon single cell suspension. Percoll gradient was used to enrich for hematopoietic cells. Representative data from 4 different mice are shown. Note the dramatic increase in FSC in cells isolated from PXR KO mice compared to WT mice.

As shown in Fig4, the increase in FSC (an indication of cell size) among the PXR KO colonic cells (compared to WT) again suggested an increased activation state of hematopoietic cells in PXR KO mice. It should be noted this increased inflammatory phenotype in PXR KO colons exists even though the mice were housed in pathogen free environment and thus, were under steady state conditions. Even though the data thus far clearly showed an increase in inflammation in PXR KO colons the percentage of live cells obtained from WT or PXR KO colonic tissues were equal (Fig.5)

Next we concentrated on determining if the imaging data could be validated using flow cytometric analysis. Indeed, the frequency of total number of CD169+ macrophages (that were also CD11b+ F480+) was significantly increased in the colons of PXR KO mice when compared to WT mice (Fig.6). Thus, in line with the imaging data, the frequency of CD169+ macrophages was also increased in PXR KO mice when compared to WT mice. Furthermore, among the CD169+ macrophages the subset that expressed the CX3CR1 chemokine receptor was particularly increased in PXR KO colons vs. WT colons (Fig.7)

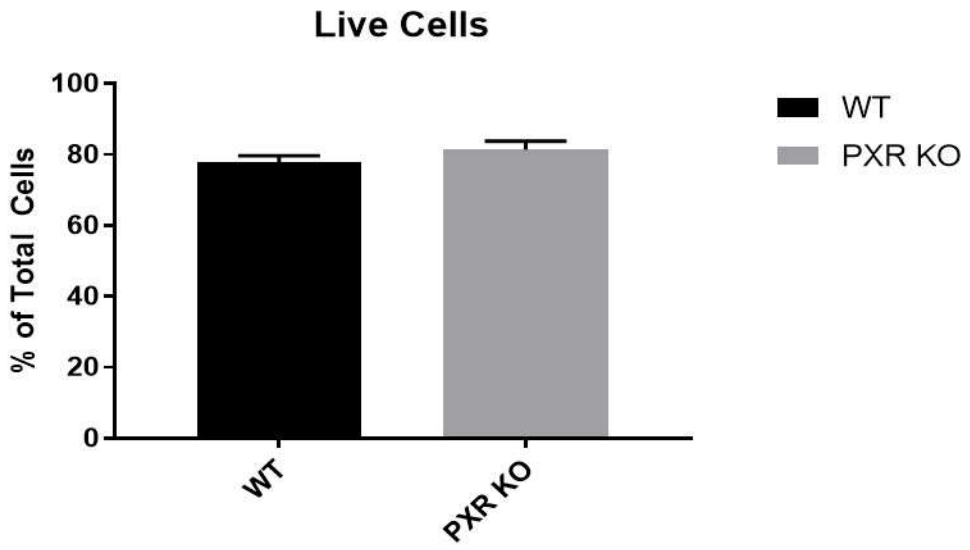


Fig. 5. Live cells recovered from the colon of each group of mice as indicated by live/dead stain

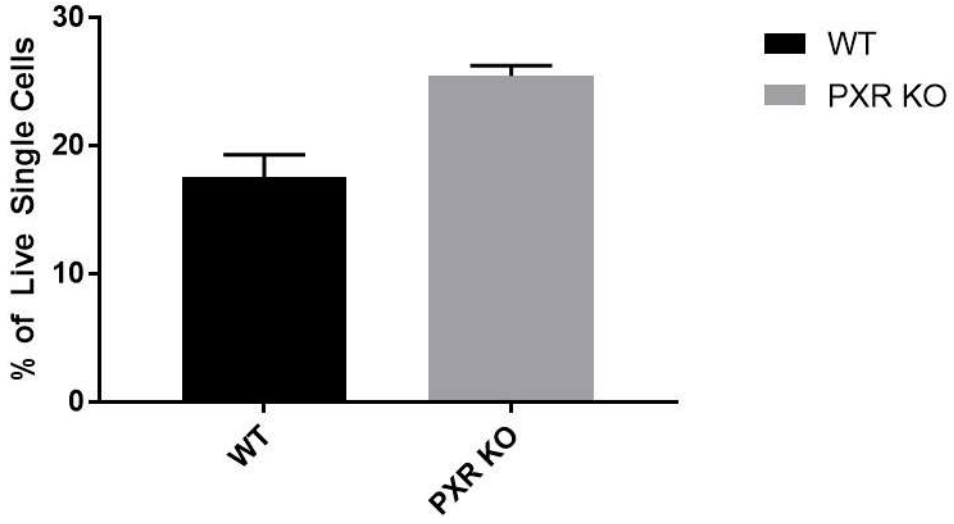


Fig.6. Frequency of CD169+ macrophages in the colon of the indicated strains of mice.

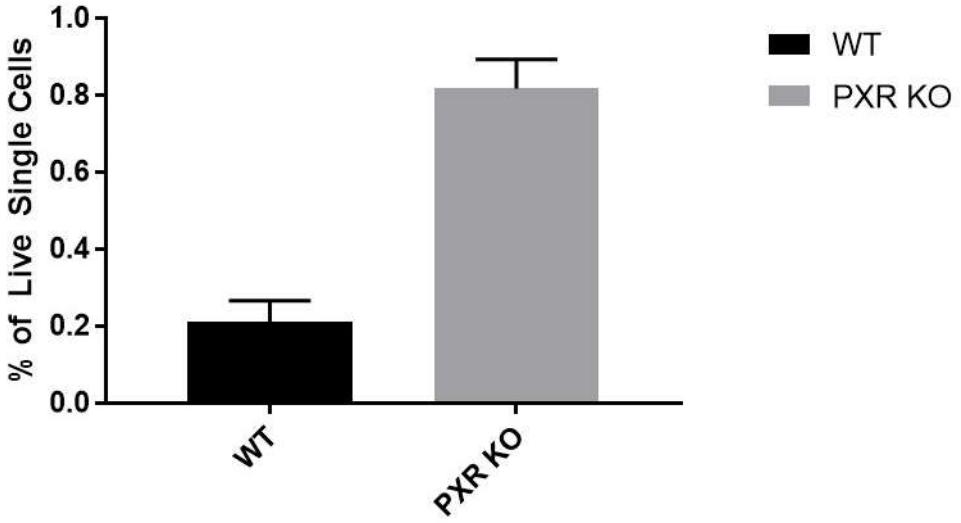


Fig.7. Frequency of CD169+ CX3CR1+ macrophages in the colon of the indicated strains of mice.

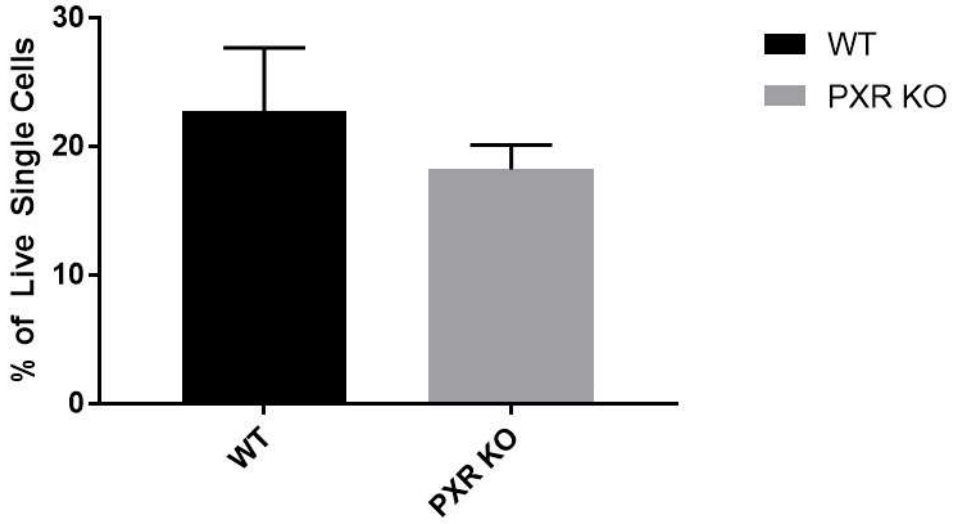


Fig.8. Frequency of CD11+ dendritic cells that fail to express any macrophage specific markers such as CD11b and F480 in the colon of the indicated strains of mice.

The flow cytometric data agreed with the imaging data where we observed a decrease in the number of CD11c+ DCs.

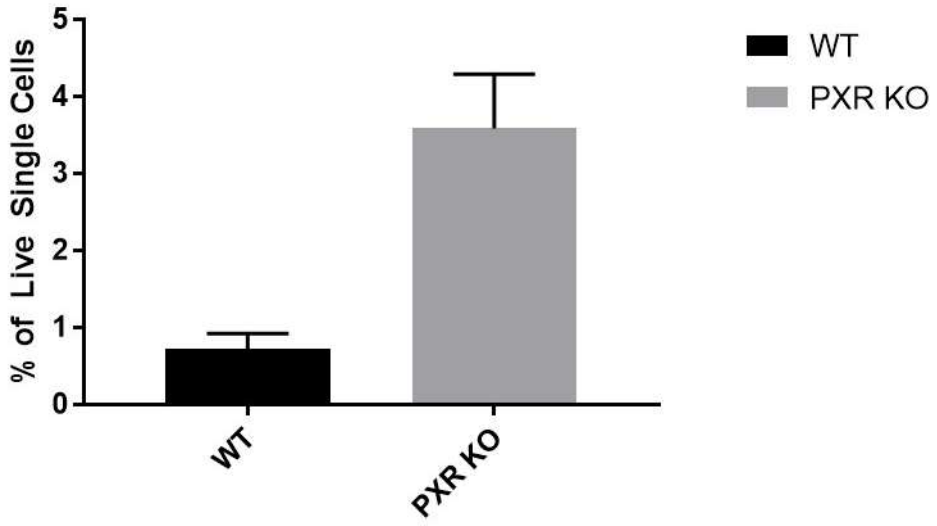


Fig.9. Frequency of LY6G+ neutrophils in the colon of the indicated strains of mice.

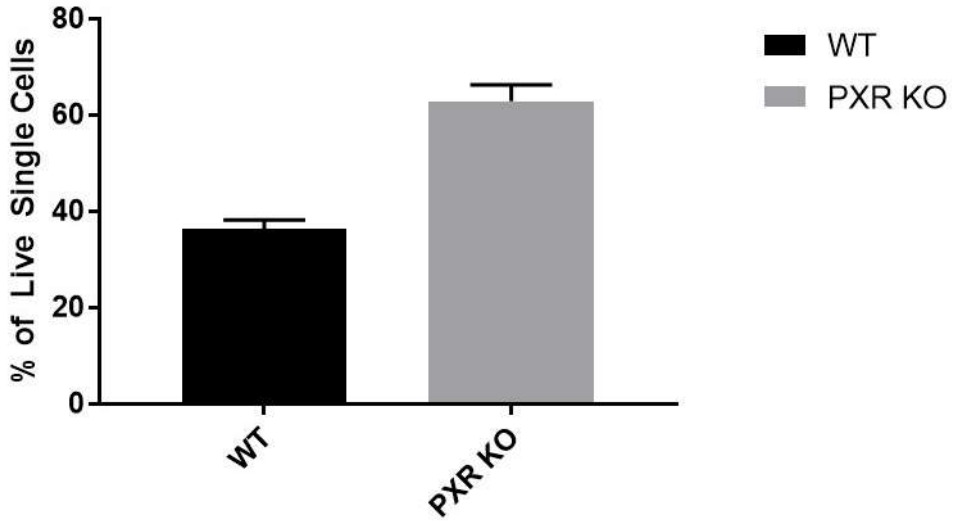


Fig.10. Frequency of LY6C+ monocytes in the colon of the indicated strains of mice.

Judging from our macrophage data, we had hypothesized that the increase in CD169+ macrophages may lead to increased recruitment of neutrophils and monocytes, and that is indeed what we observed. There was a striking increase in the number of neutrophils and monocytes (Fig.9 and 10 respectively) in the colon of PXR KO mice compared to WT mice. These data demonstrate a novel mechanism by which PXR may wield its immune-modulatory effects in the intestines. Thus, conditional ablation of PXR specifically in CD169+ macrophages by using PXR-floxed mice will be important and will likely yield interesting and mechanistic results.

We continued to work with our PXR floxed mice and began crossing them to LysM-Cre as well as CD169-Cre. While we were optimizing the transgenic mouse models we began determining the status of the macrophage subsets and other myeloid cells in the intestines of mice that were PXR deficient or normal.

To determine the function of PXR in regulating enteric infection, we began our studies with infecting PXR WT and PXR deficient mice with wildtype *Citrobacter rodentium* orally. *Citrobacter rodentium* shares approximately 67% gene homology with *Escherichia coli* (E.coli). Given that mice are resistant to E.coli infection, *Citrobacter rodentium* is a valid model to study enteric E.coli infections. Both PXR WT and PXR deficient mice were monitored for weight loss as well as fecal shedding of bacterial. To this end, we sterilely collected fecal samples from each mice every 2 days post infection. The fecal sample was weighed and resuspended in sterile 1xPBS and homogenized. Fecal slurry was then serially diluted and plated on MacConkey Agar plates to allow for selection of gram-negative and enteric bacilli bacterium.

15 days post infection, both groups were euthanized and the colon and caecum was harvested for gross anatomy analysis for colonic hyperplasia. Additionally, the distal colon was fixed with PLP and froze in OCT for histopathological and confocal microscopy analysis. 20um thick sections were cut and imaged using antibodies against CD169, CD11c, O152, and EpCAM. A Zeiss 880 confocal microscope was used to acquire the images. Imaris software was used to analyze and quantify imaging data.

As shown in Fig.11, PXR deficient mice exhibit weight reduction following *Citrobacter rodentium* infection compared to infected PXR WT control mice. Additionally, as shown in Fig.12, PXR deficient mice has a higher propensity to be colonized by and clearance of the bacteria compared to PXR WT mice. These data suggest that PXR has a functional role in regulating the clearance of enteric *Citrobacter rodentium* infection and the subsequent weight regulation. Interestingly, we did not observe any gross colonic hyperplasia difference between PXR WT and PXR deficient mice (Fig.13), which is typically indicative of an IBD phenotype. However, the lack of differences in gross colonic hyperplasia between the groups could be a result of both groups exhibiting equal levels of colonic hyperplasia. Therefore, to better determine the overall level of disease pathogenesis, we conducted confocal microscope of 20 um thick sections to determine crypt hyperplasia, which is another measure of IBD phenotype characterized by over active intestinal stem cell proliferation. In contrast to our gross anatomy observation, we found that PXR deficient mice have increase crypt length compared to PXR WT mice as shown in Fig.14.

To further confirm colonization of both PXR WT and PXR deficient mice, we conducted immunofluorescent staining of colon cross-sections against O152 antigen, which is present on E.coli and *Citrobacter rodentium*. As shown in Fig.15, both PXR WT and PXR deficient mice were both colonized by *Citrobacter rodentium* and consistent with our fecal shedding and crypt hyperplasia data, PXR deficient mice exhibit greater immune-reactive staining for O152 antigen compared to PXR WT. Additionally, it is observed that in the PXR KO mice, *Citrobacter rodentium* is able to translocate further down the crypt and lamina propria compared to PXR WT, where majority of the bacteria resides in the luminal space.

Next we observed the in situ phenotype of myeloid cells (dendritic cells and CD169+ macrophages) between PXR KO and PXR WT following infection. We found that PXR KO mice exhibit cluster of CD11c+ dendritic cells closer to the luminal space (Fig.16) compared to PXR WT where the CD11c+ dendritic cells appear to be equally distributed along the laminal propria. Additionally, with respect to CD169+ colonic macrophages, we found that greater clustering around the basement membrane closer to the muscularis (Fig.17). These data suggest a differential role of CD11c+ dendritic cells and CD169+ colonic macrophages following enteric infection in the absence of PXR.

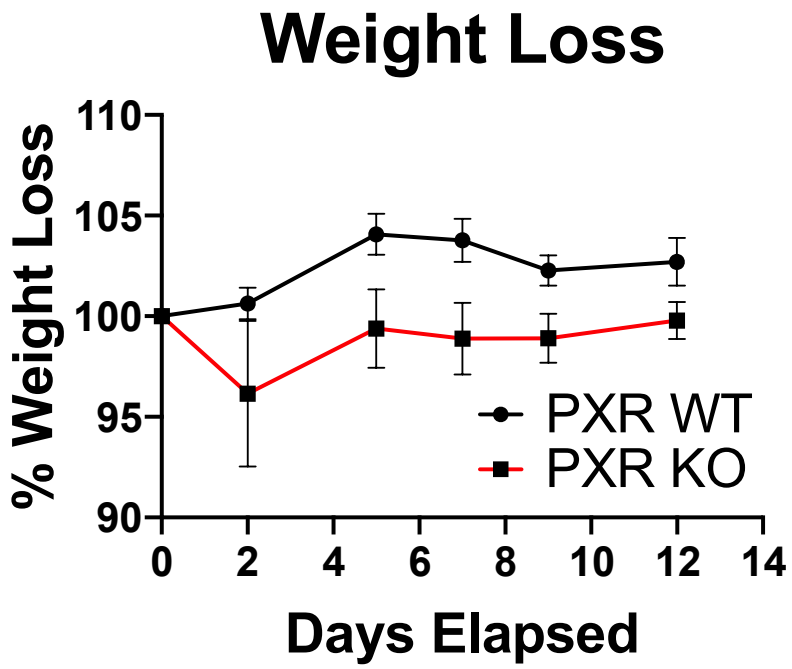


Fig.11. Weight loss between PXR WT and PXR KO mice following 2 x 10^9 CFU *Citrobacter rodentium DBS100* oral infection. N = 5 mice/group

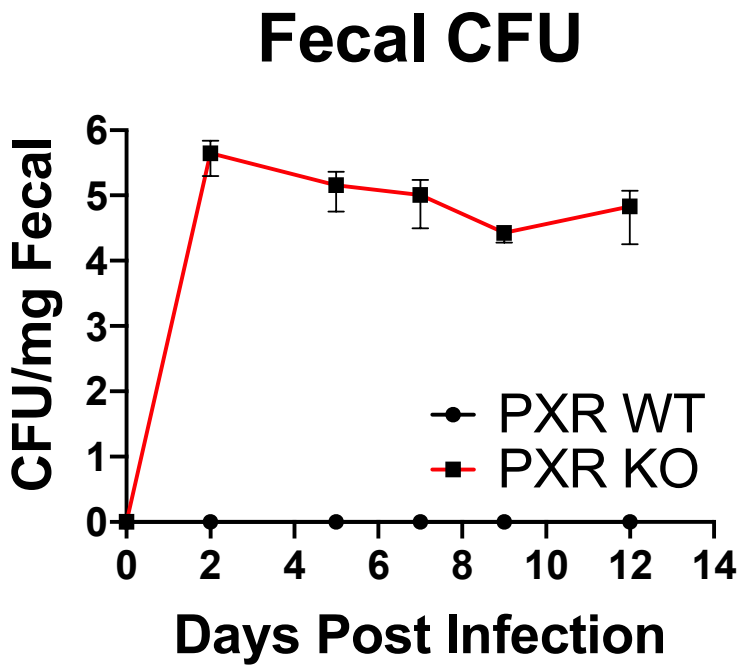


Fig.12. Fecal shedding of *Citrobacter rodentium DBS100* between PXR WT and PXR KO mice. N = 5 mice/group

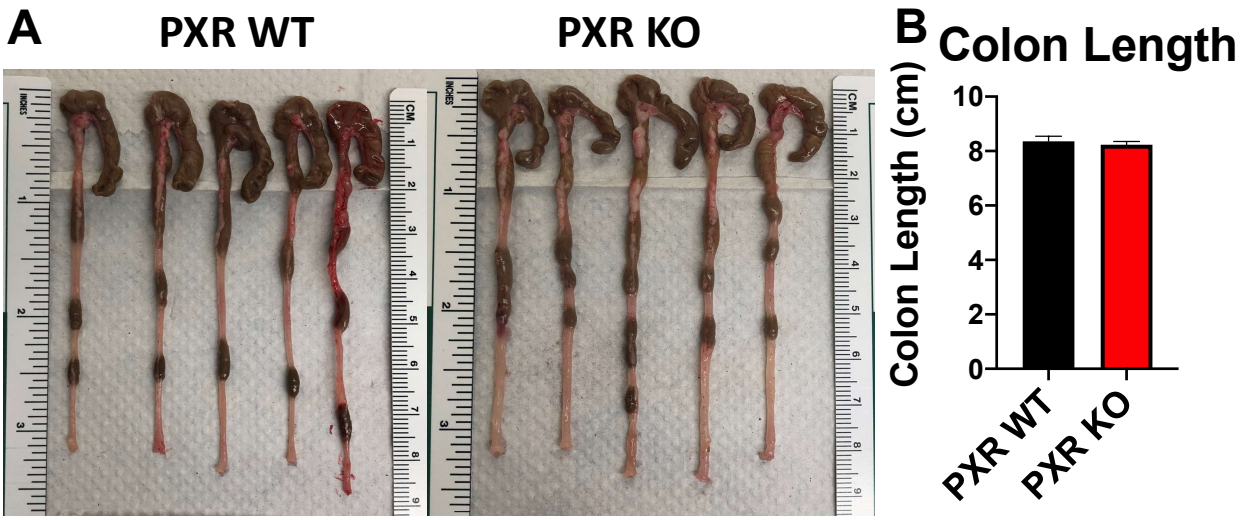


Fig.13. A) Gross anatomy of PXR WT (left) and PXR KO (right) colon 15 days post infection with *Citrobacter rodentium DBS100* infection. B) analysis of total colon length. N = 5 mice/group

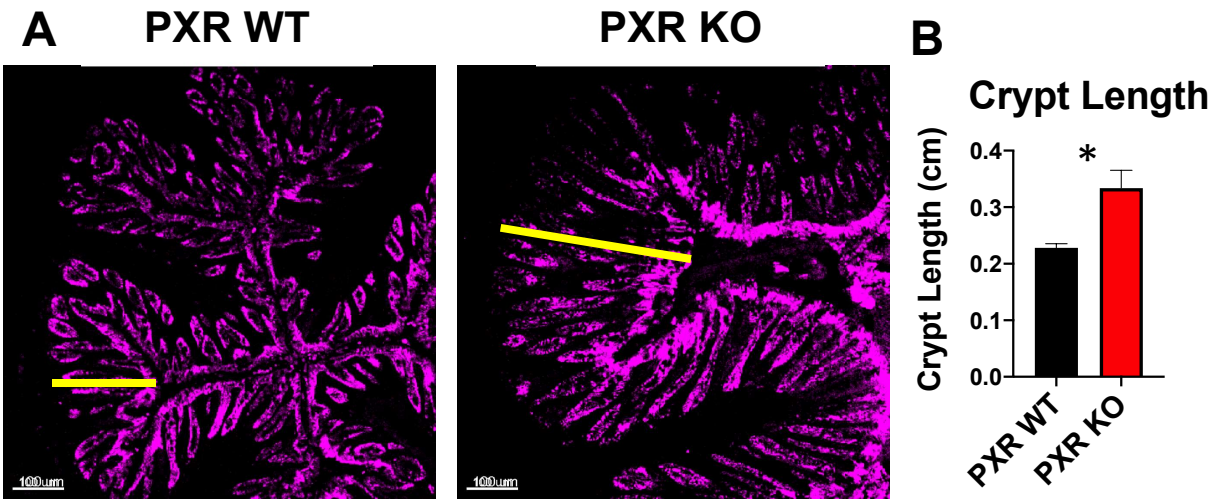


Fig.14. A) Confocal images of PXR WT (Left) and PXR KO (Right) colon cross-sections immunostained with anti-Epcam (purple) for epithelial cells 15 days post infection with *Citrobacter rodentium DBS100* infection. B) Analysis of crypt hyperplasia. Student T-test, * $p<0.05$. N = 5 mice/group

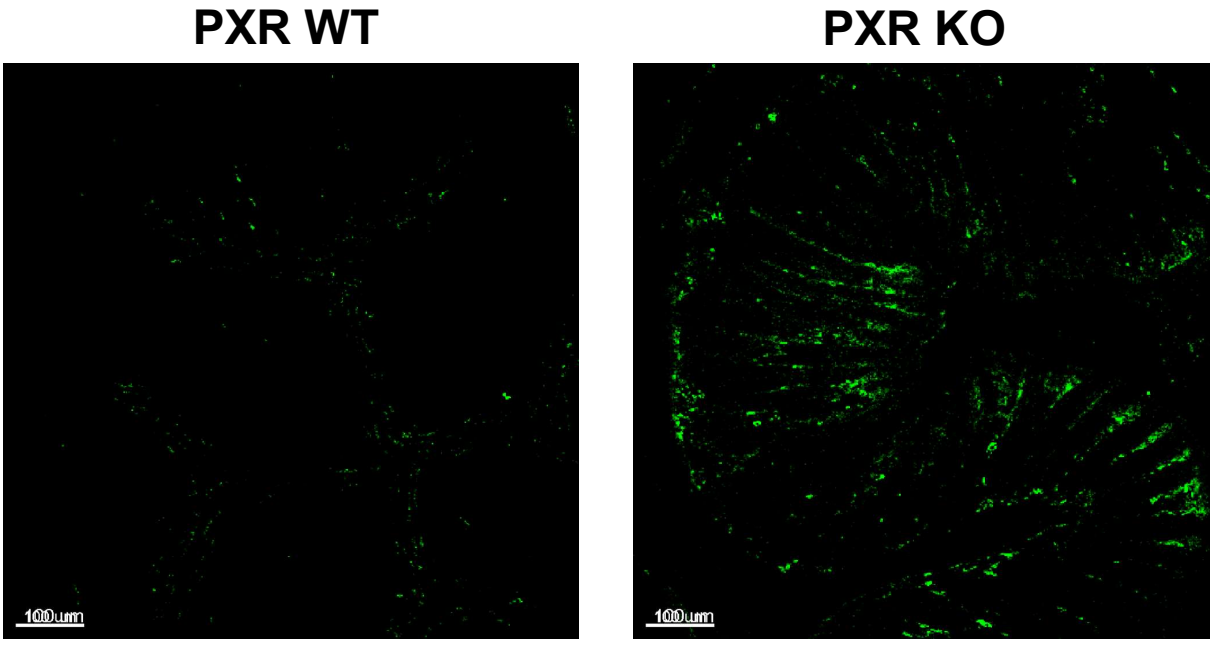


Fig.15. Confocal images of PXR WT (Left) and PXR KO (Right) colon cross-sections immunostained with anti-O152 antigen (green) for *citrobacter rodentium* 15 days post infection with *Citrobacter rodentium DBS100* infection. N = 5 mice/group

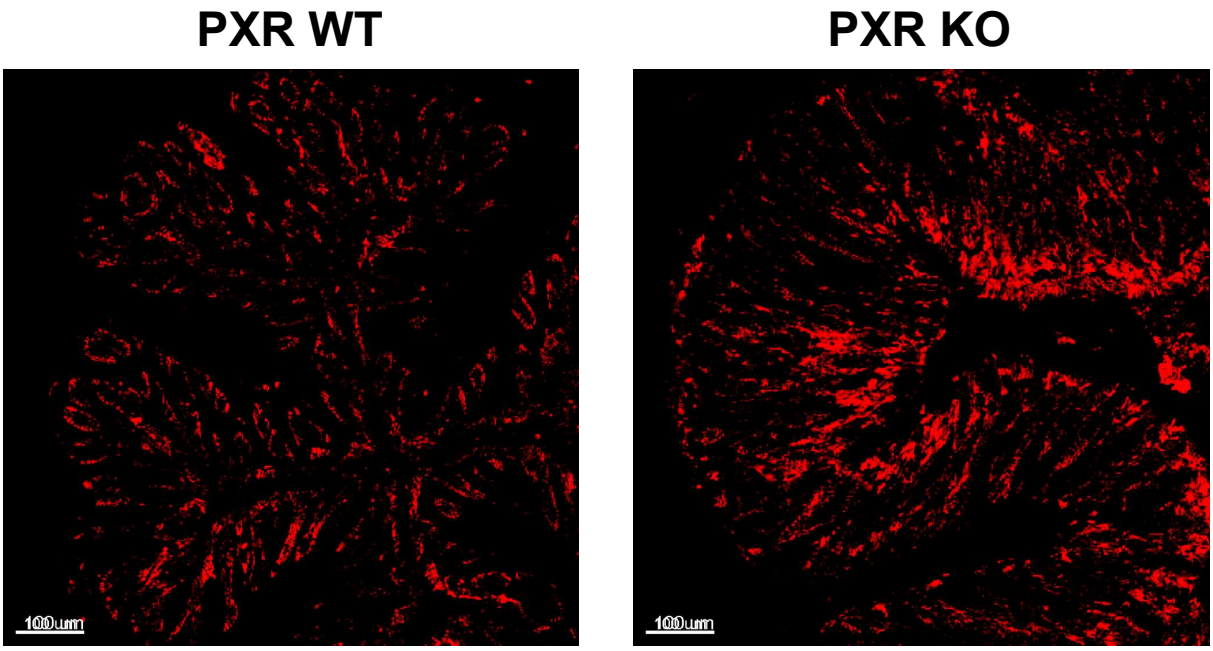


Fig.16. Confocal images of PXR WT (Left) and PXR KO (Right) colon cross-sections immunostained with anti-CD11c (red) for colonic dendritic cells 15 days post infection with *Citrobacter rodentium DBS100* infection. N = 5 mice/group

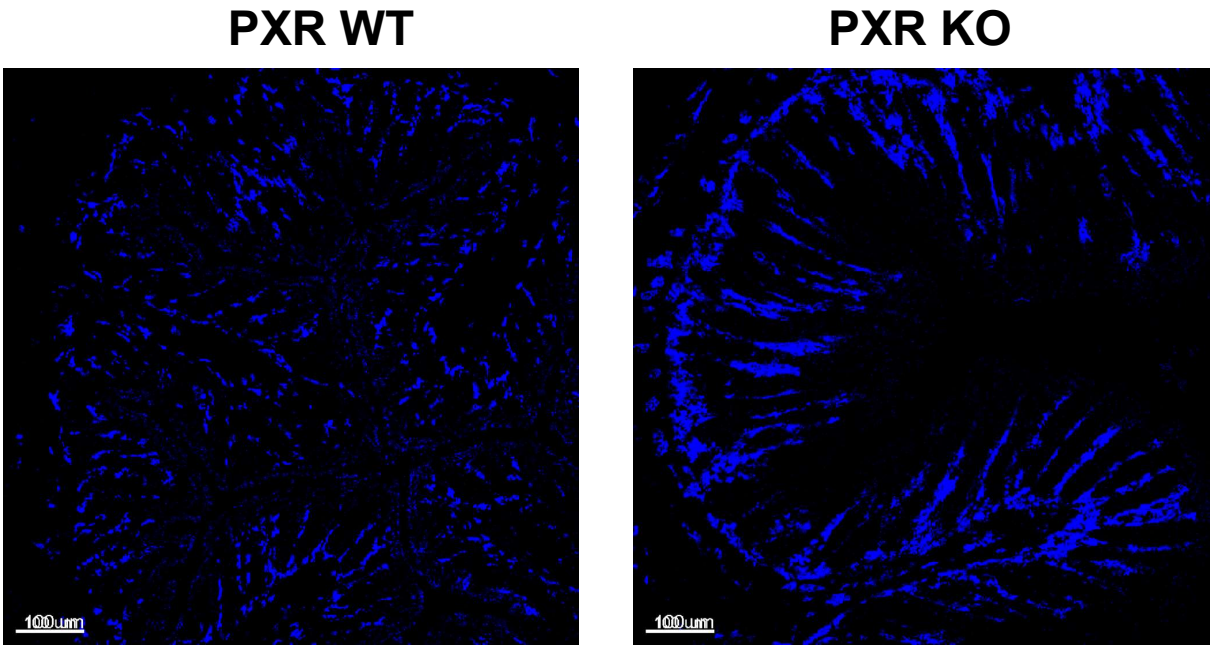
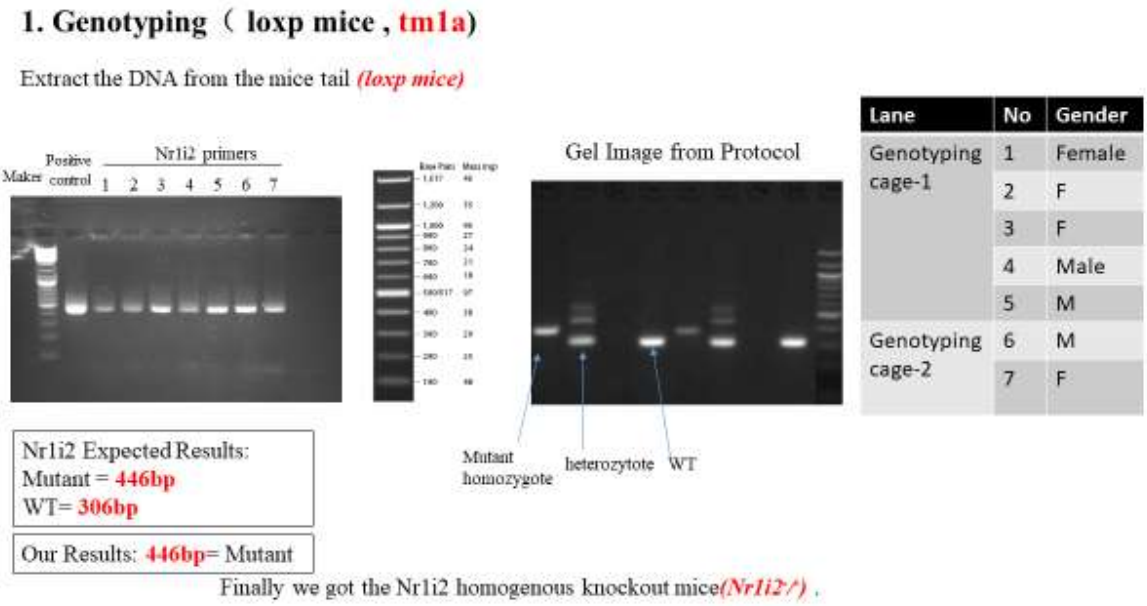


Fig.17. Confocal images of PXR WT (Left) and PXR KO (Right) colon cross-sections immunostained with anti-CD169 (blue) for colonic CD169+ macrophages 15 days post infection with *Citrobacter rodentium DBS100* infection. N = 5 mice/group

Thus, these studies clearly showed that CD169+ colonic macrophages play a critical role in regulating intestinal inflammation following enteric infection. Additionally, PXR played a partial role in regulating macrophage function and bacterial localization in the colon. It was clear to us that CD169+ macrophages may regulate infection induced inflammation thus, gut disease. We had now crossed all the PXR-floxed mice with the respective Cre models, however, our repeated attempts to use these mice resulted in disappointing results. Our expression studies repeatedly yielded results that clearly showed that the PXR-floxed mice were leaky and lacked PXR expression fidelity when crossed to many different Cre lines such as LysM-Cre, or CD169-Cre. When we repeatedly tested the progeny PXR ablation was not complete. Attempting to generate the conditional PXR KO mice turned into a herculean task, which required extensive amount of effort and funds, however, by the end of year 2 we realized that this mouse model will not be usable. This was a turning point for our studies and at this very juncture the COVID-19 pandemic hit NY area, and it considerably slowed our progress down. In spite of the pandemic restrictions we continued to make progress.

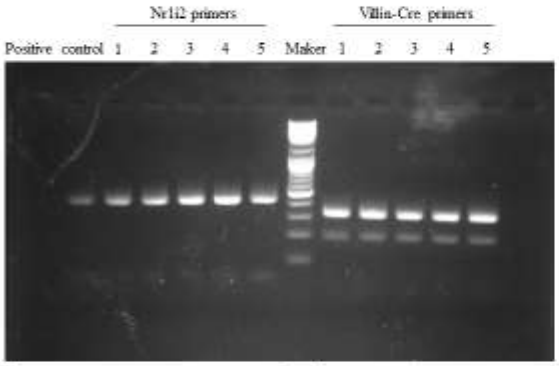


Since our PXR floxed mice crossed to macrophage specific Cre lines yielded negative results we thoroughly investigated the PXR floxed, and PXR floxed–Villin-Cre mice. Our genotyping results above clearly show that the mice harbor the floxed allele , thus, the issue was unrelated to spontaneous recombination and excision of the floxed allele.

P

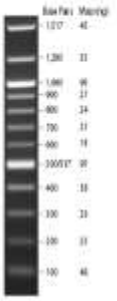
2. Genotyping（cre-loxp,tm1b）

Extract the DNA from the mice tail *(cre-loxp mice)*



Gel Image from Protocol

The genotyping protocol of Villin-cre mice can't distinguish the hemizygous from homozygous transgenic animals.



Lane	No	Gender
cag-1	1	Female
	2	F
cag-2	3	Male
	4	F
	5	M

Nr1i2 Expected Results
Mutant = **446bp**
WT= **306bp**

Villin-cre Expected Results:
Transgene = **195 bp**
Internal positive control = **324 bp**

Our Results: Nr1i2 **446bp**= Mutant , Villin-cre **195 bp**=Transgene

Now we got the Nr1i2 homogenous knockout and villin–cre heterozygous or homozygous mice *($Cre^{+/-}$/$loxp^{+/-}$)*.

When PXR floxed mice are crossed to the Villin-Cre mice to ablate PXR gene in intestinal epithelial cells the genotyping results again show the correct bands (see above).

q-PCR to detect the target gene of PXR in the indicated mice

Wild type

Loxp mice

Treat with or without PCN **(PXR agonist)** :
1. intraperitoneal injection (i.p.), 100 mg/kg
2. oral gavage, 200 mg/kg
3. i.p 100 mg/kg plus oral gavage 100 mg/kg

Small intestine

Large intestine

liver

Target genes of PXR

The relative mRNA expression

Gene	Tissue typo	1			2			3		
		WT	loxp	fold(WT/loxp)	WT	loxp	fold(WT/loxp)	WT	loxp	fold(WT/loxp)
Mdr-1b	si	93.71	11.81	8	1.11	1.06	1.05	1.97	0.89	2.21
	li	11.71	2.04	6	1.54	0.98	1.57	1.2	0.99	1.21
	liver	1.05	6.05	-	1.26	1.18	1.07	0.74	0.81	0.91
cyp3a11	si	1.2	0.06	20	2.77	2.24	1.01	8.48	2.41	3.52
	li	40.57	6.76	6	35.07	3.52	9.96	3.35	0.79	4.24
	liver	1.86	2.16	—	4.74	2.03	2.34	0.6	2.26	0.27
Gstm3	si	1.52	0.04	38	2.47	1.67	1.48	12.45	0.82	15.18
	li	1.29	1.26	—	1.31	1.18	1.11	1.34	1.31	1.02
	liver	31.15	2.9	10	15.43	2	7.72	0.25	1.39	0.18
Gstm2	si	2.09	0.7	3	2.05	0.93	2.20	1.84	0.61	3.02
	li	0.91	0.89	—	0.71	1.37	0.52	1.09	1.07	1.02
	liver	3.75	1.73	2	1.34	1.16	1.56	0.45	0.75	0.60
Gsta4	si	0.81	0.44	2						
	li	1.05	0.95	—						
	liver	2.06	1.41	1.5						
Ugt1a1	si	2.03	0.31	6.5						
	li	0.81	0.93	—						
	liver	1.33	1.23	—						

However, when we performed quantitative PCR (qPCR) for PXR target genes in different components of intestines and liver as shown above the PXR floxed/cre mice only exhibit partial reduction in indicated target gene expression following treatment of mice with a potent PXR agonist PCN.

At this time we began addressing some of the other basic questions we could address in the absence of an effective PXR floxed animal model, which was weather CD169+ macrophages in the colon regulate intestinal infection induced inflammation. To this end we utilized our CD169-DTR mouse model where we are able deplete this particular macrophage subset following the treatment of diphtheria toxin (DT). We infected DT treated WT or CD169-DTR mice with Citrobacter. Interestingly CD169+ macrophage depleted mice lost significantly more weight than WT mice indicating the essential role of CD169+ colonic macrophages in providing protection against infection induced colitis (Fig.18).

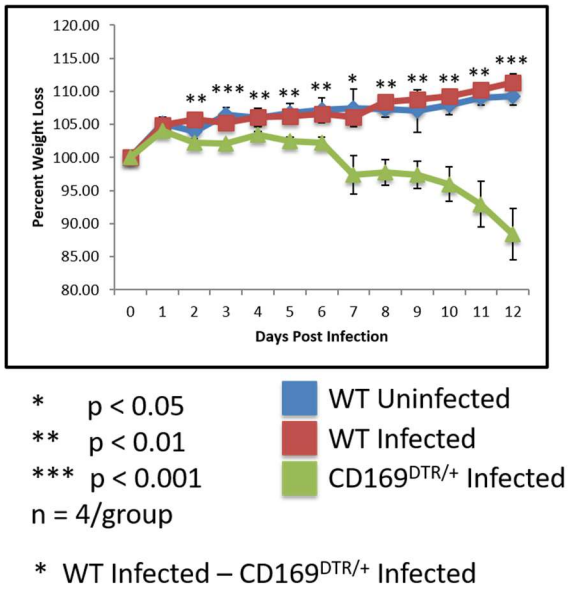


Fig.18. WT or CD169-DTR mice were treated with DT and 48 hrs later orally infection with *Citrobacter rodentium DBS100* infection. N = 5 mice/group

Interestingly, we did not observe any differences in fecal CFU of Citrobacter (Fig. 19) at any day after infection, which further indicated that the CD169+ macrophage were primarily regulating infection induced inflammation and not pathogen clearance.

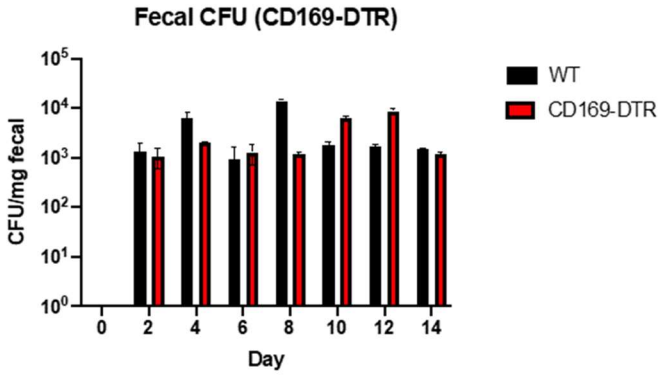


Fig.19. WT or CD169-DTR mice were treated with DT and 48 hrs later orally infection with *Citrobacter rodentium DBS100* infection. N = 5. Fecal stools were assessed for Citrobacter content by standard plating.

Finally, in the absence of a proper PXR floxed mouse model we investigated the possible mechanism by which PXR may be mediating the suppressive functions of colonic CD169+ macrophages. As we have shown previously IL10 pathway in epithelial cells is regulated by PXR. Thus, in the next set of experiments we crossed the CD169-Cre mice with IL10r floxed mice. The progeny CD169-IL10Ra CKO mice were aged to determine if they would exhibit signs of colitis. Interestingly, the CKO mice indeed showed shorter colon length (Fig.20) and the CD169+ colonic macrophage network was discontinuous and disrupted in the CKO mice compared to WT mice (Fig.21).

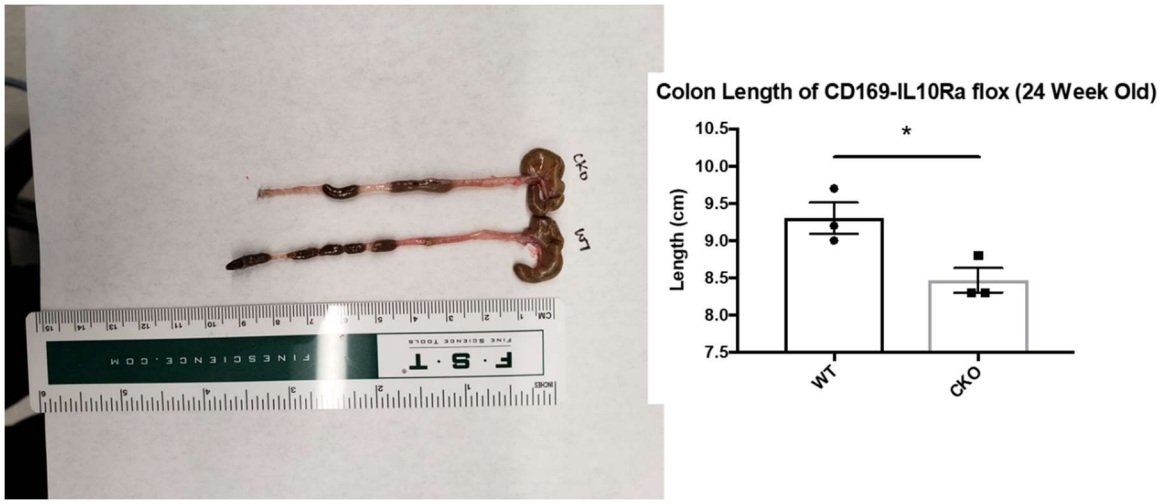


Fig.20. WT or CD169-IL10Ra CKO colon length at 24 weeks of age.

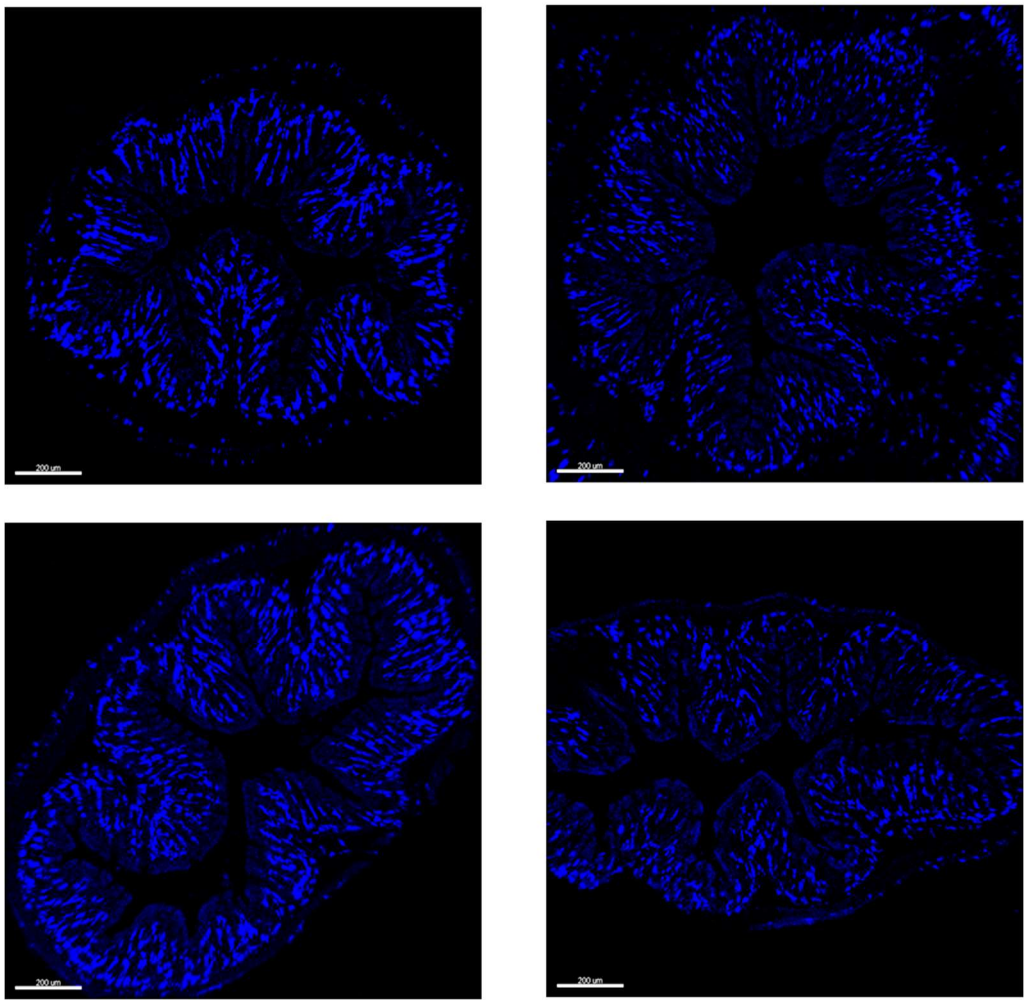


Fig.21. WT (left row) or CD169-IL10Ra CKO (right row) colon was imaged by confocal microscopy at 24 years of age. The blue stain shows CD169 staining.

What opportunities for training and professional development has the project provided?

- Jennifer Rutowski received further training in confocal imaging and multicolor flow cytometric analysis. She continues to attended several training sessions for lab and animal handling safety. She has also been trained on novel multicolor imaging platform called CODEX that uses the newly acquired Keyence microscope.
- Graduate Student Stephen Yeung was partially funded by this grant before he received a fellowship and was put on a T32 training grant. However, he has continued to work on this project.

How were the results disseminated to communities of interest?

The studies are ongoing and it is too early for publications and thus, nothing to report yet. I have presented part of this work in local and national seminars.

IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Until now the data generated above confirms our hypothesis that PXR has an important role in regulating inflammation in the gut and this regulation likely involves innate immune cells. The current model that we can propose is that CD169+ macrophages regulate the recruitment of neutrophils and monocytes that cause immunopathology in the gut. We found this to be true even in steady state conditions. Inflammatory cues such as intestinal infection will only exasperate this effect. Thus, targeting this macrophage subset may be a novel therapeutic strategy against IBD. In addition, our latest findings show that infection induced inflammation, as well as the localization and clearance of intestinal pathogens may regulate the outcome of disease (IBD) onset and chronicity of inflammation. PXR in macrophages (and perhaps intestinal epithelial cells) appears to regulated this process, which is a novel finding.

What was the impact on other disciplines?

We feel that these results once published will have a significant impact on the field of immunology. Our results clearly show that PXR has many parallel function in addition to xenobiotic sensing and clearance. PXR functions as an important regulator of colonic macrophages, especially the CD169 subset of cells.

What was the impact on technology transfer?

- Nothing to Report

What was the impact on society beyond science and technology?

- Nothing to Report

CHANGES/PROBLEMS:

Changes in approach and reasons for change

As discussed extensively above in our results section, we had crossed all the PXR-floxed mice with the respective Cre models, however, our repeated attempts to use these mice resulted in disappointing results. Our expression studies repeatedly yielded results that clearly showed that the PXR-floxed mice were leaky and lacked PXR expression fidelity when crossed to many different Cre lines such as LysM-Cre, or CD169-Cre. When we

repeatedly tested the progeny PXR ablation was not complete. Attempting to generate the conditional PXR KO mice turned into a herculean task, which required extensive amount of effort and funds, however, by the end of year 2 we realized that this mouse model will not be usable. This was a turning point for our studies and at this very juncture the COVID-19 pandemic hit NY area, and it considerably slowed our progress down. In spite of the pandemic restrictions we continued to make progress.

Actual or anticipated problems or delays and actions or plans to resolve them

In spite of these frustrating issues with the PXR floxed mouse model we changed our strategy and investigated other mechanisms that PXR regulates in colonic immune cells. We are now regenerating the PXR floxed mice.

Changes that had a significant impact on expenditures

- No, we do did use all of the funds available by the end of the three year funding period. This was due to the fact that our project was all performed in vivo using many mice and mouse models.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

- Nothing to Report

Significant changes in use or care of human subjects

- Nothing to Report

Significant changes in use or care of vertebrate animals.

- Nothing to Report

Significant changes in use of biohazards and/or select agents

- Nothing to Report

PRODUCTS:

Publications, conference papers, and presentations

- Nothing to Report

Journal publications.

- Nothing to Report

Other publications, conference papers, and presentations.

- Nothing to Report

Website(s) or other Internet site(s)
- Nothing to Report

Technologies or techniques
-Nothing to Report

Inventions, patent applications, and/or licenses
-Nothing to Report

Other Products
-Nothing to Report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Kamal Khanna, Ph.D.
Project Role: Principal Investigator
Nearest Person month worked: 4
Funding Support: 30% of Dr. Kamal Khanna's salary support is provided by the SOM

Name: Jennifer Rutowski
Project Role: Research Assistant I
Nearest personal months: 12
Contribution to Project: Ms. Rutowski is the technician in the lab and she has assisted technically in virtually every experiment especially the ones dealing with flow cytometry and processing of tissues for imaging and animal husbandry.

Name: Stephen Yeung
Project Role: Graduate Student
Nearest person month worked: 4
Contribution to Project: Has performed all the experiments above with the help of Ms. Rutowski
Funding Source: Mr. Yeung was funded on this grant until he received a fellowship and was placed on a NIH T32 training grant, under which he is still funded.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

None

What other organizations were involved as partners?

Nothing to report

SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: Nothing to report

QUAD CHARTS: Nothing to report

APPENDICES

None