# Properdin deficiency protects from 5-fluorouracil induced small intestinal mucositis in a complement activation independent, IL-10 dependent mechanism

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Short running head- Properdin and intestinal mucositis

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**Summary**

Intestinal mucositis is a serious complication of chemotherapy that leads to significant morbidity that may require dose or drug adjustments. Specific mitigating strategies for mucositis are unavailable, partly due to an incomplete understanding of the pathogenic mechanisms. We have previously shown an effect of properdin, a positive regulator of complement activation, in models of colitis. Here we use properdin deficient ($P^{KO}$) mice to interrogate the role of properdin and complement in small intestinal mucositis. Mucositis was induced by 5 daily injections of 5-fluorouracil (5-FU) in wild type (WT), $P^{KO}$, IL-$10^{-/-}$ and properdin/IL-$10^{-/-}$ double knockout (DKO) mice. At the time of sacrifice their jejunum was collected for histology, immunohistochemistry and cytokine and complement activation measurements. Complement became activated in mice receiving 5-FU, indicated by increased intestinal levels of C3a and C5a. Compared to WT, $P^{KO}$ mice experienced significantly less mucositis, despite C3a levels as high as inflamed WT mice and slightly less C5a. On the other hand, $P^{KO}$ mice had higher intestinal levels of IL-10. IL-10 expression was mainly by epithelial cells in both uninflamed and inflamed $P^{KO}$ mice. IL-$10^{-/-}$ mice proved to be highly susceptible to mucositis and DKO mice were equally susceptible, demonstrating that a lack of properdin does not protect mice lacking IL-10. We interpret our findings to indicate that to a significant extent, the inflammation of mucositis is properdin-dependent but complement activation independent. Additionally, the benefit achieved in the absence of properdin is associated with increased IL-10 levels, and IL-10 is important in limiting mucositis.

## Introduction

Gastrointestinal mucositis is a frequent and severe side effect of chemotherapy in cancer patients. Depending on the dose and type of chemotherapy, between 50-80% of the patients suffer from mucositis that results in vomiting, severe diarrhea, and abdominal pain[1, 2]. In particular, small intestinal mucositis leads to reductions in the drug dosage and delayed treatment that risks compromising the effectiveness of chemotherapy[3,4]. At the histological level, small intestinal mucositis is characterized by epithelial cell death, villi shortening, crypt damage and leukocyte infiltration[4]. Moreover, intestinal mucositis predisposes to infections that can lead to further significant morbidity and mortality[5,6]. Despite research into possible drugs to directly mitigate mucositis, none are yet available in the clinic, so there remains an urgent need to better understand the underlying pathogenic mechanisms to focus the effort[7].

Recent studies using animal models have implicated both the innate and adaptive immune systems mechanistically in the inflammation underlying chemotherapy-induced mucositis[8-10]. Complement is a crucial component of the innate immune system that can lead to inflammation but also modulate the development of the emerging adaptive immune response[11,12], yet a role for complement, particularly the pattern recognition receptors of the lectin and alternative pathways, has not been directly explored in mucositis. Our understanding is limited to a report where mRNA for proteins belonging to the classical and alternative pathway were found up-regulated in intestines of rats subjected to irinotecan induced mucositis[13].

Properdin, a protein of the alternative pathway, is a positive regulator of complement that can initiate and amplify ongoing complement activation[14]. Because the

loss of properdin abolishes terminal complement activation in multiple models of intestinal injury[15,16], we thought to use properdin deficient mice to explore the contribution of complement to 5-FU induced mucositis. Surprisingly, loss of properdin did not abolish terminal complement activation but was associated with reduced pathology compared to 5-FU inflamed WT controls. We further demonstrate that the protection in the absence of properdin is likely dependent on IL-10. Overall, we establish a previously unappreciated complement activation-independent, pro-inflammatory role of properdin in this model of intestinal mucositis.

## Materials and Methods

### Mice

Age and sex-matched wildtype (WT), properdin knockout ($P^{KO}$), IL-$10^{-/-}$ (Jackson Laboratories, Bar Harbour, ME) and IL-10/properdin double gene knockout (DKO, developed locally) mice, all on the C57BL/6 background and of both sexes, were used. Mice had free access to food and water and were housed on wood chip bedding under specific pathogen free conditions on a 12-hour dark/light cycle. All animals used were Helicobacter negative, assayed by PCR on stool DNA preparations. Helicobacter triggers colitis in susceptible strains of mice, including IL-10 gene knockout animals[17]. The numbers of mice used in each experiment is indicted in each figure. The experiments were undertaken with the approval of the University Committee on Laboratory Animals, Dalhousie University, who in turn adjudicate the standards of the Canadian Council on Animal Care.

### 5-FU induced small intestinal mucositis

Mucositis was induced by administering 5-FU once daily beginning on day 1 to either day 3 or 5. PBS injected mice were treated as vehicle controls. The study began with optimizing the dose of 5-FU, examining 50, 100 and 200 mg/kg, delivered intraperitoneally. The animals' weight was recorded daily and any mice that reached a loss of 20% were immediately sacrificed and were excluded from the data analyses. In some experiments, 5-FU injected WT mice were orally gavaged with the selective C5a receptor (C5aR1) antagonist PMX205[15] (200μg/mouse) or water (control) daily from day 1 to day 5. One day after the final injection of 5-FU, a stool specimen was collected and a rectal bleeding score was assigned based on stool consistency and whether blood was detectable, with the scale ranging from 0 (normal stool with no blood) to 4 (diarrhea with blood). The mice were then anaesthetized and blood was withdrawn by cardiac puncture and serum was isolated. Then the mice were killed by cervical dislocation; their jejunum was excised, flushed with cold PBS, opened longitudinally and divided into two parts. One longitudinal part was used for histology whereas the other part was homogenized.

**Histopathology**

The longitudinal strip of jejunum was fixed in buffered formalin overnight, rehydrated in 70% v/v ethanol, embedded in paraffin, and 4μm sections were stained using hematoxylin and eosin. A pathologist blinded to the treatments determined the inflammation score summed from the following parameters, villous shortening (0 – normal, 1 – mild blunting, 2 – marked blunting), apoptosis (0 – none, 1- focal, 2 - diffuse), epithelial damage (0 – normal, 1 – reactive epithelial change, 2 – superficial

erosion, 3 - ulceration), inflammation (0 – none, 1 – mild, 2 – moderate, 3 – severe) and crypt loss (0 – none, 1 – focal, 2 - diffuse).

**ELISA measurements**

The remaining longitudinal strip of the jejunum was homogenized in 50mM HEPES buffer supplemented with soy trypsin inhibitor (100μg/ml) and centrifuged at 16000xg for 30 min at 4ºC after which the supernatants were stored at -80°C until examined by ELISA. IFN-γ, IL-1β and IL-10 concentrations were measured using ELISA kits from eBiosciences (San Diego, CA). C3a and C5a levels were assessed using reagents from BD Pharmingen (Mississauga, ON). IL-4 and IL-6 were measured using ELISA kits from Peprotech (Dollard, QC). All the protocols were performed according to the manufacturer's instructions.

**Immunohistochemistry**

Four-micron thick paraffin embedded sections were deparaffinised in xylene and graded ethanol washes. To detect neutrophils, antigen retrieval was performed by heat in 10mM sodium citrate buffer (pH=6.0) and the slides incubated with rat anti-mouse Ly6G (1:4000, BD Pharmingen). For the detection of IL-10, antigen retrieval was performed by heating in Tris-EDTA buffer (pH=6.0) and sections were subsequently incubated with a blocking buffer (2% goat serum, 1% BSA, 0.1% triton X-100, 0.05% Tween 20 and 0.05% sodium azide in PBS) for 1 hour at room temperature (RT) to block non-specific binding sites. Tissue sections were then incubated with rat monoclonal anti-mouse IL-10 antibody (clone JES5-2A5, Abcam, Cambridge, MA) as the primary antibody. Goat anti-rat IgG secondary antibody was used to detect the primary antibodies. Isotype specific control monoclonal antibodies and tissues from IL-$10^{-/-}$ mice were used to confirm the

specificity of the staining. Positively stained cells were counted in the remaining intact villi only.

**Statistical Analyses**

Statistical analyses were performed using GraphPad Prism version 5 (La Jolla, CA). Parametric data are shown as mean ± standard deviation and were compared using two-tailed t-test (two groups) or one-way ANOVA with Tukey's multiple comparison-post test (more than two groups). Body weights were compared using two-way ANOVA with Bonferroni post hoc tests. Inflammation scores are shown in scatter plots with the median as a line. Differences between groups were tested using the nonparametric Mann-Whitney test. The threshold for declaring a significant difference was defined as $p \leq 0.05$.

**Results**

**Titration of 5-fluorouracil induced mucositis in mice**

In a pilot experiment to determine a dose of 5-FU for the study, WT mice were injected intraperitoneally with 50, 100 or 200 mg/kg/day or PBS for 5 days then sacrificed 24 hours later (day 6). All mice receiving 5-FU lost weight in the period of the experiment. The average maximum body weight loss in mice receiving 50 and 100mg/kg/day 5-FU was 10% and 19% respectively (Figure 1A). Mice treated with 200mg/kg lost close to 20% body weight by day 5 and were sacrificed and this dose was not considered further, nor were mice which reached this unintended endpoint used in any further data sets.

As another macroscopic measure of the treated animals' phenotype, the stool consistency was evaluated on day 6. PBS injected WT mice had firm fecal pellets and no rectal bleeding. 5-FU treatment resulted in soft stool pellets and blood in the stool with the rectal bleeding score higher in the 100mg/kg group compared to PBS controls (Figure 1B). Microscopic examination of the small intestines of mice treated with 100mg/kg 5-FU revealed extensive epithelial damage, crypt loss and villi shortening, and an inflammation score statistically higher in 100 mg/kg 5-FU treatment group compared to the 50mg/kg group and/or PBS treated controls (Figures 1C and D). Based on these clinical and histopathological responses the 100mg/kg 5-FU dose was chosen for experiments including the $P^{KO}$ mice.

**Genetic deficiency of properdin ameliorates 5-FU induced intestinal mucositis**

To evaluate whether properdin, and complement, play a role in our model of mucositis we compared the response of WT and $P^{KO}$ mice to the 100mg/kg 5-FU treatment regimen. Shown in Figure 2A, 5-FU treatment led to comparable body weight losses in the two genotypes. Despite weight loss, $P^{KO}$ mice had significantly less rectal bleeding compared to the WT mice (Figure 2B). Consistent with the bleeding score data, 5-FU inflamed $P^{KO}$ mice also experienced significantly lower inflammation scores compared to inflamed WT mice (Figures 2C and D). Noteworthy, despite the lower inflammation score in $P^{KO}$ mice, the numbers of neutrophils infiltrating the small intestine was not different between the strains (Figure 2E). In addition to neutrophil infiltration, a reduction in goblet cell numbers is another hallmark of intestinal mucositis[18,19]. We stained tissue sections with periodic acid Schiff stain to enumerate goblet cells. The two untreated mouse strains had similar numbers of small intestinal

goblet cells and while both experienced a reduction in numbers with 5-FU treatment (Figure 2F), inflamed $P^{KO}$ mice registered significantly higher numbers compared to inflamed WT controls. Thus the preservation of goblet cell numbers in $P^{KO}$ mice was associated with the evidence for reduced inflammation. Overall these results indicate that $P^{KO}$ mice have an attenuated inflammatory phenotype in 5-FU induced small intestinal mucositis.

**Reduced mucositis in properdin deficient mice is C5a independent**

Because properdin is a positive regulator of complement activation we measured complement activation products, C3a and C5a, in the intestinal homogenates of treated mice. C3a and C5a were detectable at comparable levels in uninflamed mice of both strains (Figures 3A and B). 5-FU treated WT mice possessed significantly higher levels of C3a and C5a compared to uninflamed controls, confirming that complement is activated during mucositis (Figures 3A and B). Interestingly, in inflamed $P^{KO}$ mice the increase in C3a levels reached statistical significance but the level of C5a did not, suggesting that perhaps reduced levels of C5a were mechanistically responsible for the lower inflammation in $P^{KO}$ mice (Figure 3B). Also, considering that we recently discovered that properdin impacts the inflammation in murine infectious colitis in a C5a-dependent mechanism[15], we sought to elucidate whether C5a is inflammatory in mucositis. We gavaged 5-FU inflamed WT mice with the selective C5aR1 antagonist, PMX205. Shown in Figures 3C and D, PMX205 treatments did not protect the mice from mucositis, suggesting that the apparent lower levels of C5a in $P^{KO}$ mice is not mechanistically contributing to the protection. Together these data suggest that while properdin in involved in driving mucositis, it is independent of complement activation.

**Reduced mucositis in $P^{KO}$ mice is associated with higher IL-10 levels**

To further decipher the mechanism behind the protection observed in $P^{KO}$ mice, we measured a number of relevant pro- and anti-inflammatory cytokines in intestinal homogenates. IFN-γ, IL-6, IL-17A, IL-12 and IL-13 were all below the limit of detection in all groups of mice. IL-1β, while detectable, was neither increased nor statistically different between the inflamed groups (Figure 4A). With regards to IL-10, 5-FU inflamed WT mice demonstrated significantly reduced levels compared to uninflamed mice. On the other hand, IL-10 levels were not significantly different comparing inflamed and control $P^{KO}$ mice (Figure 4B).

Since the level of IL-10 was similar between uninflamed and inflamed $P^{KO}$ mice and uninflamed WT mice, we used an immunohistological approach to identify the cellular source of IL-10, particularly to determine whether the source changes when the animals received 5-FU. Tissue sections stained with an anti-IL-10 antibody revealed that epithelial cells were the predominant source of IL-10 in healthy animals of both strains (Figure 5A). Importantly, and consistent with the ELISA data and the idea that resident cells remained the principal source of IL-10, the number of epithelial cells positive for IL-10 was significantly higher in 5-FU inflamed $P^{KO}$ mice compared to inflamed WT (Figure 5B). Taken together, lower mucositis in $P^{KO}$ mice is associated with higher numbers of epithelial cells preserved in the intestine synthesizing IL-10. This finding can be explained by a lack of inflammation in the $P^{KO}$ strain receiving 5-FU, leaving more of the epithelium intact, and importantly, implicating properdin in triggering the inflammation.

**Protection from mucositis is IL-10 dependent in properdin deficient mice**

IL-10 is a potent anti-inflammatory cytokine in multiple models of intestinal inflammation but a role for IL-10 specifically during 5-FU induced mucositis has not been reported. Consequently, we conducted experiments to explore the properdin/IL-10 relationship in our model. If properdin and IL-10 are linked mechanistically, a lack of properdin will not protect IL-$10^{-/-}$ mice from mucositis and properdin/IL-10 double knockout mice (DKO) will presumably experience similar mucositis as IL-$10^{-/-}$ mice. In preliminary experiments treating IL-$10^{-/-}$ mice with 5-FU the animals reached the weight loss endpoint early and had to be euthanized, indicating this strain is considerably more sensitive than WT to 5-FU (data not shown). Consequently, the 5-FU regimen was shortened to 3 days and the mice sacrificed 24 hours later (day 4). Despite the shorter treatment regime, significantly higher weight loss was observed in both IL-$10^{-/-}$ and DKO mice compared to WT controls (Figure 6A). When combined with IL-10 deficiency the protective effect of properdin deficiency was lost, as the inflammation in DKO mice was comparable to 5-FU treated IL-$10^{-/-}$ and higher than WT mice (Figure 6B, C). This outcome indicates that a lack of properdin fails to protect DKO animals from mucositis, and points to the importance of IL-10 in protecting the intestine from mucositis.

**Discussion**

Complement is understood to be inflammatory yet the history of investigation into complement and mucositis is scant. Bowen et. al surveyed changes in gene expression in the intestines of rats subjected to a single injection of irinotecan leading to mucositis and found that levels of mRNA for C1q and C2 of the classical pathway and factor D of the alternative pathway were considerably increased[13]. Whether the increase in mRNA of

complement proteins was a direct product of damaged epithelial cells, infiltrating leukocytes, or indirectly triggered by the inflammation (many complement proteins are acute phase reactants) or whether the complement was mechanistically involved in the pathogenesis of mucositis were not assessed. Here we report that complement indeed becomes activated during mucositis but the mechanism by which the genetic deficiency of properdin protects against mucositis is complement activation independent. We did determine that C5a is not driving mucositis since the C5aR1 antagonist failed to prevent inflammation; however, other split complement molecules such as C3a remain to be assessed.

Complement is a network of approximately 30 proteins that coordinate a cascade of enzymatic reactions ultimately generating opsonins, the anaphylatoxins and the membrane attack complex (MAC). The past understanding was that these activation products presumably positively impact the phenotype of inflammatory diseases, not limited to the intestines. However, a new understanding is emerging that these proteins, particularly the proximal activators in the cascade, have activities independent of complement activation. For example, mannose binding lectins (MBL), initiators of the lectin pathway, can modulate LPS mediated secretion of IL-8, IL-6 and MCP-1 without activating complement[22]. MBL binds the extracellular domain of TLR4 diminishing binding of LPS[23]. Properdin was recently shown to facilitate removal of apoptotic cells in a complement activation independent manner[24]. Here we demonstrate that the lack of properdin did not significantly impact complement activation apart from slightly lower C5a levels, yet protected mice from 5-FU injury. As C5a is a potent inflammatory product of complement activation, we thought to directly test the impact of C5a in the

model. We determined that C5a was not driving the inflammation by administering a C5aR1 antagonist, PMX205, which failed to protect WT mice from mucositis. Noteworthy, we showed previously that PMX205 significantly reduced DSS-induced colitis, indicating that perhaps complement/C5a has different impacts on the large versus small intestine[25]. Additionally, the finding that C5a does not influence mucositis only strengthens our conclusion that properdin is a positive regulator of mucositis in a complement activation independent manner. How properdin positively contributes to inflammation consequential to the cell injury caused by 5-FU remains to be discovered.

IL-10 has profound anti-inflammatory effects in the gastrointestinal tract, clearly demonstrated by the fact that a lack of IL-10 or the IL-10 receptor indisputably predisposes to colitis, both in experimental animal models and humans. In cancer patients, IL-10 levels were found to negatively correlate with the degree of mucositis[27] and LPS/IFN-γ stimulated monocytes from patients with high grade mucositis produced less IL-10 compared to patients without mucositis[27]. These observations may mean that individuals who make less IL-10 experience worse mucositis or alternatively, that events related to mucositis may actively suppress IL-10 production. We observed a significant reduction in the number of IL-10 producing cells and the soluble IL-10 levels in WT mice with mucositis. Using mice deficient in IL-10 we can further address whether homeostatic levels of IL-10 provide any initial barrier to mucositis[28]. We further show that IL-10 in the small intestine is mainly detectable in epithelial cells, including in $P^{KO}$ mice (proving this strain is not IL-10 deficient), suggesting local constitutive expression of IL-10 plays a role in limiting the cell injury directly due to 5-FU, and that the lack of properdin interrupts the subsequent inflammatory response. When both molecules are

absent the extent of cell injury overwhelms the properdin-dependent process or provokes an alternate mechanism leading to inflammation, and severe mucositis. We are currently investigating how IL-10 may directly prevent the apoptosis triggered by 5-FU in gut epithelium. Ultimately, the fact that DKO mice were as severely affected by 5-FU as IL-$10^{-/-}$ mice mechanistically links these two molecules in mucositis.

Knowing constitutive gut IL-10 levels are not elevated in $P^{KO}$ mice is important because there are precedents that indicate the lack of properdin may result in increased IL-10. In one example, $P^{KO}$ mice with zymosan induced peritonitis demonstrated higher levels of IL-10[29]. Properdin is composed of thrombospondin repeats type I and it possibly has activities in mucositis similar to other members of this protein family[30]. In this regard, thrombospondin I (also contains type I repeats), negatively regulates the production of IL-10 from dendritic cells through interactions with CD47 and CD36[31]. It remains to be determined in our model whether properdin uses these or other receptors to influence production of IL-10 in the small intestine and inflammation, but currently our understanding is that properdin deficiency compromised inflammation, leaving a greater number of epithelial cells intact and making IL-10.

Although our data support the conclusion that PKO mice have normal levels of IL-10, it is possible that IL-$10^{-/-}$ mice may have abnormal levels of properdin, where high properdin levels would presumably make the mice more susceptible to mucositis. While we did not measure properdin levels in the IL-$10^{-/-}$ strain, we interpret our earlier experiments exploiting this strain's susceptibility to colitis to infer there is no obvious reason to believe they have high levels of properdin[16].

Despite incomplete knowledge of the mechanisms, our findings identify properdin as a potential target for ablating mucositis, particularly considering the lack of impact properdin has on complement activation. Complement is required for controlling the escape of microbes from inflamed intestines[32] and this will presumably be intact in patients in which properdin levels are intentionally reduced. Properdin is present in very low levels in plasma (4-25μg/ml) so purposely reducing levels may be easily achievable[33].

Our study is the first experimental evidence for a mechanistic role of a complement protein during small intestinal mucositis, though not acting through the presumed role of supporting complement activation. We do demonstrate that the protective effects of properdin deficiency is linked to IL-10. Now, in addition to further investigations into the mechanisms of properdin dependent mucositis, experiments are needed to assess the impact of properdin on tumor progression and chemotherapeutic efficacy against tumors before reducing levels could be applied to mitigate mucositis in humans.

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**Conflict of Interest**

The authors have no financial conflicts of interest.

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**Figure legends**

**Figure 1: 5-FU titration and clinical illness and histopathological phenotypes in WT mice**. Mice were administered 5-FU daily, to day 5, then were sacrificed 24 hours after the final injection. (**A**) Each animal's weight was measured daily and is reported as the average percentage of the weight from the start of the treatments. Data is shown as mean ± S.E.M (n=3 mice/group). * and † represent comparison of 50 and 100mg/kg groups with no 5-FU controls on the same day, respectively. § is indicative of a difference between 50 and 100mg/kg groups. * $p<0.05$, ** $p<0.01$, †† $p<0.01$, ††† $p<0.001$ and §§ $p<0.01$. (**B**) Rectal bleeding scores. Each dot represents a single mouse and the line represents the median score. Any animal whose weight loss reached 20% was not included in further data collection or analyses. (**C**) Representative images of the jejunum prepared from PBS or 5-FU treated mice. Jejunum of mice receiving 5-FU at a dose of 100mg/kg demonstrated severe villus height shortening, epithelial damage, crypt loss and inflammation. (**D**) The total inflammation scores were calculated based on criteria outlined in the methods. Each dot represents a single mouse and the line represents the median inflammation score. * $p<0.05$ comparing the 50 versus 100 mg/kg groups.

**Figure 2: Properdin deficiency protects mice from 5-FU induced mucositis**. (**A**) Weight loss during 5-FU treatments. Data is the mean percent of each animals' weight at the start of the experiment ± S.E.M (n=6 mice/group). (**B**) Inflamed $P^{KO}$ mice registered significantly less rectal bleed and (**C, D**) intestinal inflammation, compared to WT mice. Each dot represents a single mouse and the line represents the median score. * $p<0.05$ vs inflamed WT mice. (**E**) Neutrophil infiltration into the5-FU treated mouse intestine. Similar numbers of neutrophils were detected in the two strains. (**F**) PAS positive cells/10

intact villi/section and averaged for all mice within a treatment group. The data is the mean ± S.E.M of 4-5 mice.

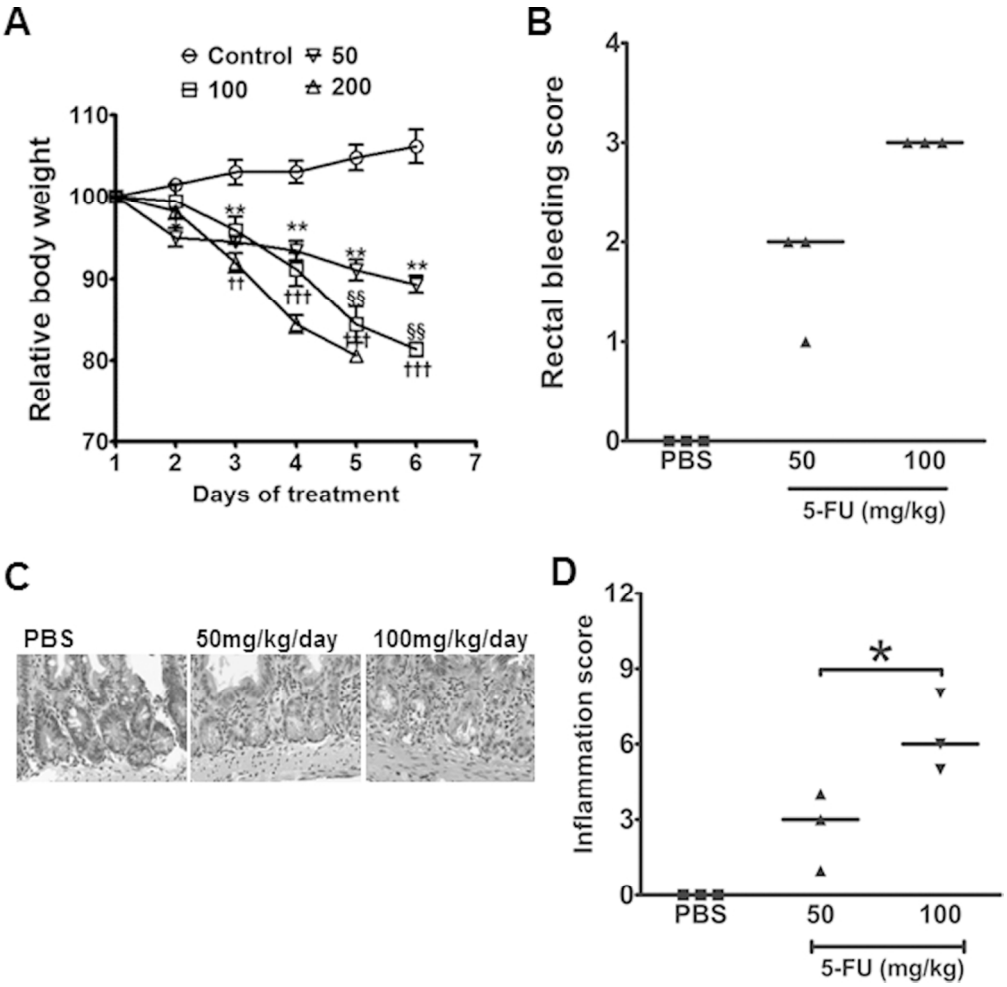
**Figure 3: Reduced inflammation in $P^{KO}$ mice is independent of complement activation.** Levels of (**A**) C3a and (**B**) C5a measured by ELISA in tissue homogenates of uninflamed and inflamed mice. Data is shown as mean ± S.E.M (n=3-10 mice/group). *p<0.05 and **p<0.01 vs control, ns = not significant. Control refers to mice that did not receive 5-FU. To investigate the role of C5a during mucositis, 5-FU inflamed WT mice were gavaged with PMX205 or water. All mice received 5-FU, control refers to mice which received water in their gavage. (**C**) Inflammation and (**D**) rectal bleeding score of control or mice receiving PMX205. Each dot represents a single mouse and the line indicates the median score.

**Figure 4: Reduced inflammation in $P^{KO}$ mice is associated with higher IL-10 levels**. Tissue homogenates from the jejunum of control (did not receive 5-FU) or 5-FU treated mice were examined for (**A**) IL-1β and (**B**) IL-10.

**Figure 5**: **IL-10 expression is detected in the epithelium**. Control in the figure refers to mice which did not receive 5-FU. (**A**) Immunohistochemical staining for IL-10 shows that IL-10 expression on epithelial cells was reduced in inflamed WT but not $P^{KO}$ mice. Isotype (control) antibody or anti-IL-10 used on sections from IL-$10^{-/-}$ mice did not demonstrate any staining (not shown). (**B**) Quantification of IL-10 positive epithelial cells/10 crypts or villi (shown separately). Cells were counted from intact villi/crypts only. Data is shown as mean ± S.E.M (n=4-5 mice/group). *p<0.05 and **p<0.01.

**Figure 6: $P^{KO}$ mice lacking IL-10 are not protected against intestinal mucositis**. To assess if IL-10 contributes to the protection observed in $P^{KO}$ mice against mucositis, $P^{KO}$

mice were crossed with IL-10$^{-/-}$ and DKO offspring were subjected to 5-FU mucositis. WT, IL-10$^{-/-}$ and DKO were administered 5-FU from day 1 to day 3 and sacrificed 24 hours later. All the mice in the figure received 5-FU. (**A**) Weight loss during 5-FU treatments. Data is shown as mean± S.E.M (n=4 mice/group) and (**B**) rectal bleed and (**C**) inflammation scores of the mice. Each dot represents a single mouse and the line represents the median score. *p<0.05, **p<0.02 versus WT control.

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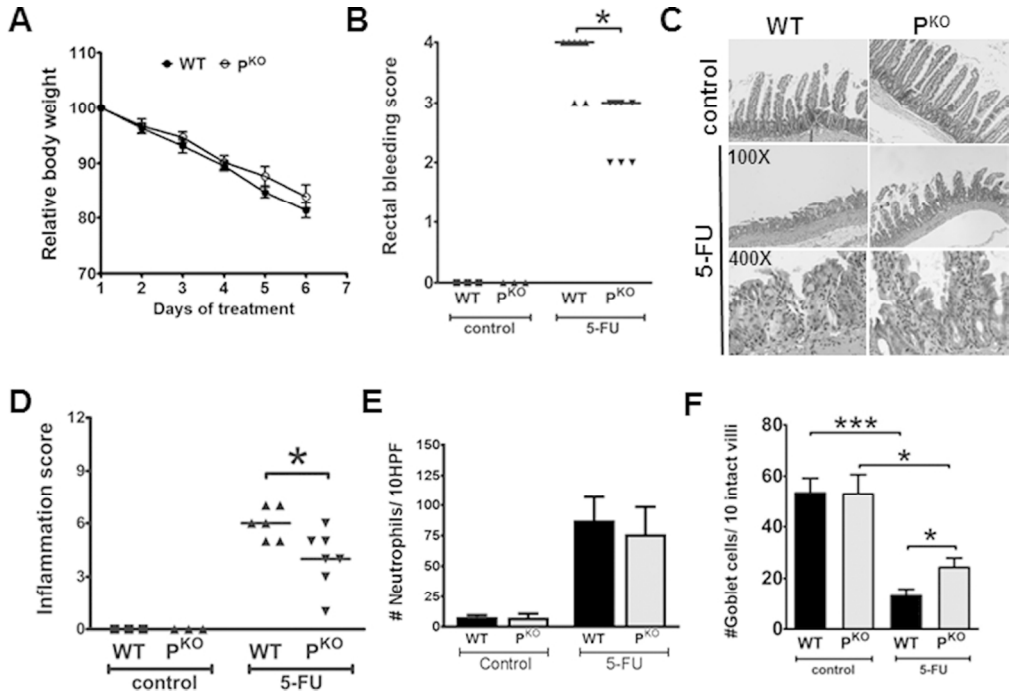


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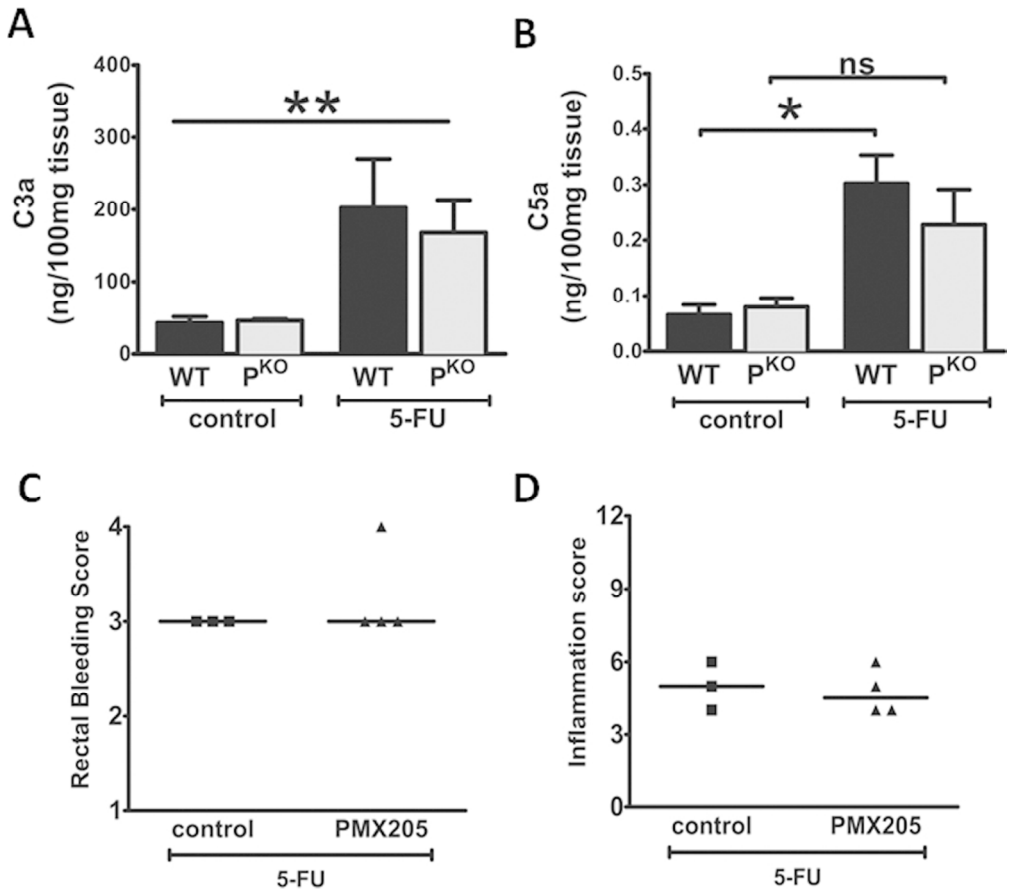


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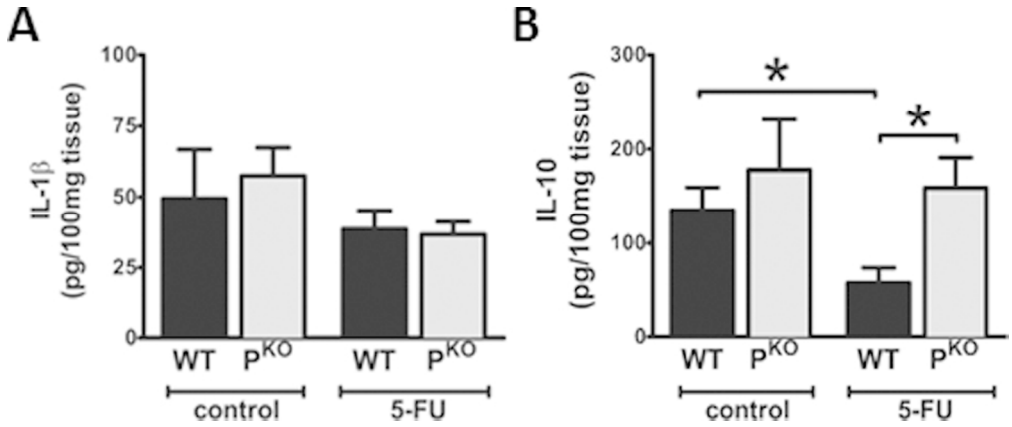


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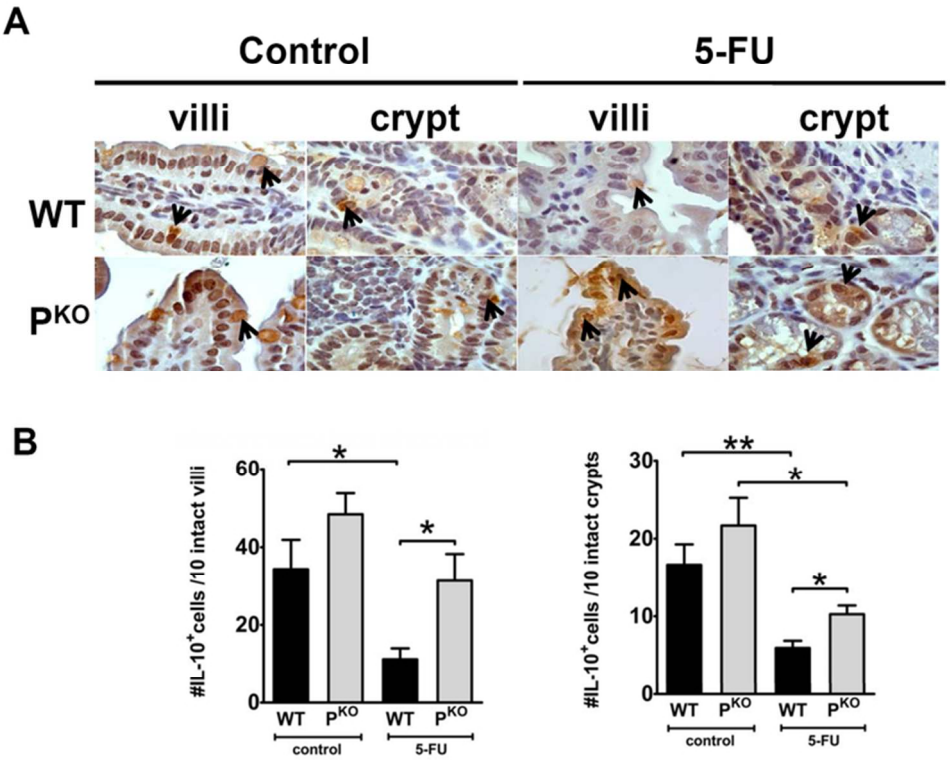


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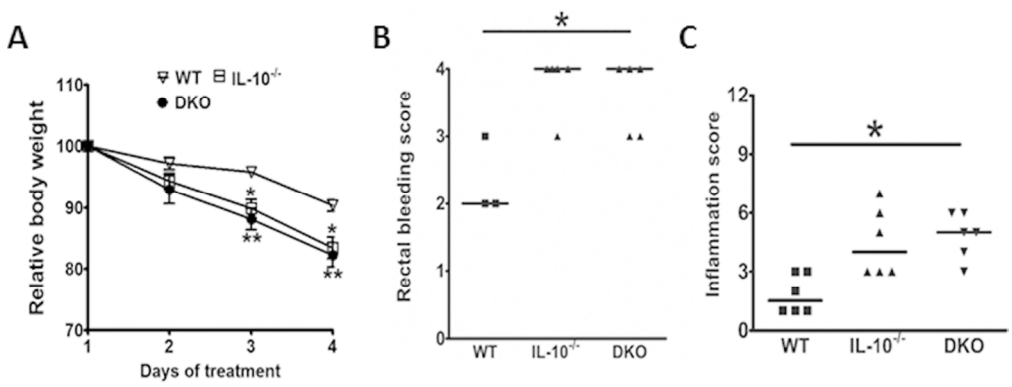


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