Published in final edited form as:

Cytokine. 2012 January ; 57(1): 113–119. doi:10.1016/j.cyto.2011.09.027.

Intestinal Inflammatory Cytokine Response in Relation to Tumorigenesis in the *Apc^{Min/+}* Mouse

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Abstract

The etiology of colon cancer is a complex phenomenon that involves both genetic and environmental factors. However, only about 20% have a familial basis with the largest fraction being attributed to environmental causes that can lead to chronic inflammation. While the link between inflammation and colon cancer is well established, the temporal sequence of the inflammatory response in relation to tumorigenesis has not been characterized. We examined the timing and magnitude of the intestinal inflammatory cytokine response in relation to tumorigenesis in the *Apc^{Min/+}* mouse. *Apc^{Min/+}* mice and wildtype mice were sacrificed at one of 4 time-points: 8, 12, 16, and 20 wks of age. Intestinal tissue was analyzed for polyp burden (sections 1, 4 and 5) and mRNA expression and protein concentration of MCP-1, IL-1 β , IL-6 and TNF- α (sections 2 and 3). The results show that polyp burden was increased at 12, 16 and 20 wks compared to 8 wks ($P < 0.05$). Gene expression (mRNA) of MCP-1, IL-1 β , IL-6 and TNF- α was increased in sections 2 and 3 starting at wk 12 ($P < 0.05$), with further increases in MCP-1, IL-1 β and IL-6 at 16 wks ($P < 0.05$). Protein concentration for these cytokines followed a similar pattern in section 3. Similarly, circulating MCP-1 was increased at 12 wks ($P < 0.05$) and then again at 20 wks ($P < 0.05$). In general, overall polyp number and abundance of large polyps were significantly correlated with the inflammatory cytokine response providing further support for a relationship between polyp progression and these markers. These data confirm the association between intestinal cytokines and tumorigenesis in the *Apc^{Min/+}* mouse and provide new information on the timing and magnitude of this response in relation to polyp development. These findings may lead to the development of inflammatory mediators as important biomarkers for colon cancer progression. Further, these data may be relevant in the design of future investigations of therapeutic interventions to effectively target inflammatory processes in rodent models.

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Keywords

colon cancer; inflammation; mouse models; monocyte chemoattractant protein 1; pro-inflammatory cytokines

1. Introduction

Colon cancer is a significant global health concern; despite advances in detection, surgery and chemopreventive treatment it remains the third most common malignancy and the fourth most common cause of cancer mortality worldwide [1–3]. The etiology of colon cancer is a complex phenomenon that involves contribution from both genetic and environmental factors. However, only about 20% of colon cancer cases can be attributed to genetic factors [4] with the vast majority of cases being ascribed to environmental causes that can lead to chronic inflammation. For example, inflammatory bowel disease (IBD), ulcerative colitis (UC) and Crohn's colitis, all of which display chronic inflammation of the gastrointestinal mucosa, are associated with increased risk for the development of colon cancer [5, 6].

The link between inflammation and colon cancer is well established; inflammation has been linked to every step involved in the development and progression of colon cancer, including cellular transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis [7–11]. Furthermore, chronic inflammation has been associated with “sickness behaviors” including circadian disruptions, anorexia, cachexia, fatigue, and decreased physical activity all of which can lead to a decreased quality of life, as well as poorer prognosis and survival in cancer patients [12–14]. The inflammatory cytokines interleukin 1 β (IL-1 β), interleukin 6 (IL-6), and tumor necrosis factor- α (TNF- α), largely produced by macrophages, have all been associated with poor outcome in colon cancer [7–10]. In fact, we have previously shown that IL-6 overexpression using electron gene transfer techniques can increase polyp burden in the *Apc^{Min/+}* mouse model of intestinal tumorigenesis [15]. Similarly monocyte chemoattractant protein 1 (MCP-1), a major chemokine for macrophage recruitment, has been associated with increased grade of the tumor in certain cancers [16, 17]. While the link between inflammatory cytokines and colon cancer is well recognized, the timing and magnitude of this response in relation to tumorigenesis has not been described.

The *Apc^{Min/+}* mouse model has been the most widely used genetically engineered mouse model for cancer studies that involve the gastro-intestinal tract [18, 19]. It was the first mouse model to be generated by mutation of the adenomatous polyposis coli (Apc) gene through random chemical carcinogenesis [20]. This gene is similarly mutated in patients with familial adenomatous polyposis [21]. It has been shown to be responsive to treatment with anti-inflammatory agents, including both anti-inflammatory dietary supplements as well as non-steroidal anti-inflammatory drugs (NSAIDs) [18, 19]. Despite this, intestinal inflammation has not been characterized in this model; to our knowledge there are no studies that have examined the relationship between inflammatory cytokines and tumor burden in the *Apc^{Min/+}* mouse.

The purpose of this study was to examine the temporal sequence and magnitude of the inflammatory cytokine response in relation to tumorigenesis in the *Apc^{Min/+}* mouse. This may lead to the development of inflammatory mediators as important biomarkers to assess progression of this disease, and further, allow for the determination of appropriate timing of effective treatments to target inflammatory processes in mouse models.

2. Materials and Methods

2.1 Animals

Apc^{Min/+} male mice on a C57BL/6 background (Jackson Laboratories) were purchased and bred with female C57BL/6 mice in the University of South Carolina's Center for Colon Cancer Research (CCCR). Offspring were genotyped as heterozygotes by RT-PCR for the *Apc* gene by taking tail snips at weaning. The primer sequences were sense: 5'-TGAGAAAGACAGAAGTTA-3'; and antisense: 5'-TTCCACTTTGGCATAAGGC-3'. Female *Apc*^{Min/+} offspring were randomly assigned to one of four different timepoints: 8, 12, 16 or 20 wks of age (n=8–12/group). Wildtype C57BL/6 mice were used as age matched controls (n=6–11/group). Mice were maintained on a 12:12 h light-dark cycle in a low-stress environment (22°C, 50% humidity and low noise) and provided food and water ad libitum. All animal experimentation was approved by the University of South Carolina's Institutional Animal Care and Use Committee.

2.2 Tissue collection

Mice were sacrificed at their respective group age (8, 12, 16 or 20 wks) for tissue collection using isoflurane overdose. All mice were sacrificed in the mornings between 9:00 and 11:00am. The small intestine was carefully dissected distally to the stomach and proximal to the cecum. The large intestine (section 5) was removed from the distal end of the cecum to the anus. Mesentery tissue was removed with tweezers, and the small intestine was cut into four equal sections (sections 1–4). All intestinal sections were flushed with PBS, opened longitudinally, and flattened with a cotton swab. Sections 1 and 4 of the small intestine and the large intestine (section 5) were fixed in 10% buffered formalin (Fisher Scientific, Pittsburg, PA) for 24 h. Sections 2 and 3 were divided into 2 equal parts and mucosal scrapings were performed in iscoves medium (Invitrogen, Carlsbad, CA) (containing 5% fetal bovine serum and a cocktail enzyme inhibitor (10 mM EDTA, 5 mM benzamidine HCl, and 0.2 mM phenylmethyl sulfonyl fluoride)) and TRIzol reagent (Invitrogen, Carlsbad, CA) for protein and gene expression analysis, respectively. Samples were stored at –80°C until analysis of inflammatory mediators. Blood was collected from the inferior vena cava using a heparinized syringe and spun in a microcentrifuge at 4,000rpm for 15 min. Plasma was then stored at –80°C until assayed for MCP-1.

2.3 Polyp counts

Formalin-fixed intestinal sections from all animals were rinsed in deionized water, briefly stained in 0.1% methylene blue, and counted by the same investigator who was blinded to the treatments. Polyps were counted under a dissecting microscope, using tweezers to pick through the intestinal villi and identify polyps. Polyps were categorized by size (>2 mm, 1–2 mm, and <1 mm).

2.4 Expression of inflammatory markers

Procedures for RNA isolation from mucosal scrapings were performed as previously described [22, 23]. Briefly, mucosal tissue was homogenized under liquid nitrogen with a polytron, and total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA). The extracted RNA (2.5 µL of sample) was dissolved in DEPC-treated water and quantified spectrophotometrically at 260-nm wavelength. RNA quality was assessed on an Agilent 2100 BioAnalyzer. RNA was reverse transcribed into cDNA in a 50 µL reaction volume containing 19.25 µL RNA (1.5 µg) in RNase-free water, 5 µL 10X RT Buffer, 11 µL 25 mM MgCl₂, 10 µL deoxyNTPs mixture, 2.5 µL random hexamers, 1 µL RNase inhibitor, and 1.25 µL multiscribe reverse transcriptase (50U/µL). Reverse transcription was performed at 25°C for 10 min, 37°C for 60 min, and 95°C for 5 min, followed by quick

chilling on ice and storage at -20°C until subsequent amplification. Quantitative Real-Time Polymerase Chain Reaction (RT-PCR) analysis was done per manufacturer's instructions (Applied Biosystems, Foster City, CA) using TaqMan® Gene Expression Assays (IL-1 β , IL-6, TNF- α and MCP-1). DNA amplification was carried out in 12.5 μL Taqman Universal PCR Master Mix (AmpliTaQ Gold DNA Polymerase, Passive Reference 1, Buffer, dNTPs, AmpErase UNG), 1 μL cDNA, 9 μL RNase-free water, and 1.25 μL 18S primer (VIC) and 1.25 μL primer (FAM) (for endogenous reference and target gene) in a final volume of 25 μL /well. Samples were loaded in a MicroAmp 96-well reaction plate. Plates were run using Applied Biosystems Sequence Detection System. After 2 min at 50°C and 10 min at 95°C , samples were coamplified by 50 repeated cycles of which one cycle consisted of 15 s denaturing step at 95°C and 1 min annealing/extending step at 60°C . Data were analyzed using Applied Biosystems software using the CT, cycle threshold, which is the value calculated and based on the time (measured by PCR cycle number) at which the reporter fluorescent emission increases beyond a threshold level (based on the background fluorescence of the system), and it reflects the cycle number at which the cDNA amplification is first detected. All samples were run in duplicate. Quantification of cytokine gene expression for IL-1 β , IL-6, TNF- α and MCP-1 were calculated using the delta CT method as described by Livak and Schmittgen (2001) [24]. Briefly, delta CT (CT(FAM) - CT(VIC)) is calculated for each sample and control. Delta delta CT (delta CT(control) - delta CT(sample)) is then calculated for each sample and relative quantification is calculated as $2^{\text{delta delta CT}}$. Initial exclusion criteria consist of FAM CT ≤ 40 and VIC CT ≤ 23 . Multiple plates were used for each marker, however an equal number of samples from each time-point were run on the same plate and all plates were run under the same conditions. The intra assay variability for all gene expression assays was $< 1\%$.

2.5 Concentration of inflammatory markers

Mucosal tissue was homogenized using a polytron and samples were centrifuged at 10,000 rpm at 4°C for 10min, and the supernatants removed and stored at 4°C prior to the assay of IL-1 β , IL-6, TNF- α and MCP-1 via ELISA (R&D Systems, Minneapolis, MN). The assay was performed according to the manufacturer's instructions. Total soluble protein was also determined using supernatant of homogenized samples via bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL.). Cytokine levels are expressed as a pg per 100 μg total protein. Plasma levels of MCP-1 were also measured using ELISA techniques (R&D Systems, Minneapolis MN). As described above, multiple plates were used for each inflammatory marker with an equal number of samples from each time-point on the same plate. Intra assay and inter assay variability were $< 3\%$ and $< 5\%$, respectively for all proteins via ELISA.

2.6 Statistical Analysis

Polyp data was analyzed using a one-way ANOVA (Time) with Student Newman-Keuls post-hoc analysis. All inflammatory mediator data were analyzed using a two-way ANOVA (Time $\times$ Strain) with Student Newman-Keuls post-hoc analysis where appropriate. The correlation coefficients between polyp number and inflammatory mediator levels were determined by Spearman correlation analyses. Analysis was done using commercial software (SigmaStat, SPSS, Chicago, IL). Statistical significance was set with an alpha value of $p < 0.05$. Data are presented as mean ($\pm$ SEM).

3. Results

3.1 Polyp incidence

Polyps were counted on formalin-fixed, methylene blue-stained intestinal sections. Overall polyp number was increased at 12 (60.3 ± 6.3), 16 (63.4 ± 7.6) and 20 wks (47 ± 4.4) versus 8 wks (26.4 ± 3.2) ($P < 0.05$) (Figure 1A) and colon polyps at 20 wks (1.4 ± 0.4) were greater

than 8 wks (0.28 ± 0.18), but this did not reach statistical significance ($P < 0.1$) (Figure 1B). There were also differences in the polyp size distribution over time (Figure 1C) that were consistent with disease development; 8 and 12 wk old mice showed the greatest number of small polyps ($P < 0.05$), 16 wk old mice had the greatest distribution of medium polyps ($P < 0.05$), and large polyps were most abundant at 16 and 20 wks ($P < 0.05$).

3.2 Tissue expression of inflammatory markers

Gene expression of inflammatory markers (MCP-1, IL-1 β , IL-6 & TNF- α) were examined in regions 2 and 3 of the intestines of *Apc^{Min/+}* over time (Figure 2). Regions 2 and 3 were chosen so as to represent a section of the intestine with low and high polyp incidence, respectively. All data was normalized to fold-increase from age-matched wildtype mice.

MCP-1—MCP-1 was elevated at 16 and 20 wks in both sections 2 and 3 (~ 20–35 fold) ($P < 0.05$) and there was further elevation at 20 wks in section 2 only ($P < 0.05$) (Figure 2A).

IL-1 β —In both sections 2 and 3, there was an increase in IL-1 β at 12, 16 and 20 wks (~ 4–10 fold) ($P < 0.05$) and an additional increase was observed at 16 and 20 wks ($P < 0.05$) (Figure 2B).

IL-6—IL-6 was significantly elevated at 16 and 20 wks in section 2 (~ 15–30 fold) ($P < 0.05$), and at 12, 16 and 20 wks in section 3 (~ 10–30 fold) ($P < 0.05$). The greatest magnitude of IL-6 expression was observed at 16 wks in both sections ($P < 0.05$) (Figure 2C).

TNF- α —In sections 2 and 3, TNF- α expression was increased at 12, 16 and 20 wks (~ 4–6 fold) ($P < 0.05$) (Figure 2D).

3.3 Tissue concentration of inflammatory markers

The concentration of pro-inflammatory cytokines (IL-1 β , IL-6 and TNF- α) and MCP-1 were examined in regions 2 and 3 of the intestines of *Apc^{Min/+}* at 8, 12, 16 and 20 wks of age (Figure 3). The data was normalized to fold-increase from age-matched wildtype mice.

MCP-1—In section 2, MCP-1 was increased at 16 and 20 wks (~ 3–4 fold) ($P < 0.05$) and in section 3 at 12, 16 and 20 wks (~ 5–7 fold) ($P < 0.05$) and there was further elevation at 20 wks ($P < 0.05$) in both sections (Figure 3A).

IL-1 β —In section 2, IL-1 β was increased at 20 wks (~ 1.5 fold) ($P < 0.05$), and in section 3 at 12, 16 and 20 wks (~ 2 fold) ($P < 0.05$) (Figure 3B).

IL-6—There were no differences in protein concentration of IL-6 across age in section 2. However, in section 3, IL-6 concentration was increased at 12, 16 and 20 wks (~ 2–3 fold) ($P < 0.05$) (Figure 3C).

TNF- α —Similar to IL-6 there were no differences in protein concentration of TNF- α across age in section 2. In section 3, there was an increase in TNF- α only at 12 wks (~ 4 fold) ($P < 0.05$) (Figure 3D).

3.4 Plasma MCP-1

Plasma concentration of MCP-1, a measure of systemic inflammation, was also measured in *Apc^{Min/+}* mice and wildtype mice over time (Figure 4). MCP-1 was significantly increased in *Apc^{Min/+}* at 12, 16 and 20 wks ($P < 0.05$) and there was an additional increase at 20 wks ($P < 0.05$).

3.5 Correlations

In order to further address the relationship between polyp progression (overall polyp number & abundance of large polyps) and inflammatory mediators we performed correlation analyses. Mice from all time-points were included in these analyses. Figure 5 shows the relationship between large polyp number and mRNA expression of MCP-1, IL-1 β , IL-6 and TNF- α in section 3 of the intestines. All four inflammatory mediators were positively associated with the number of large polyps ($P < 0.01$); MCP-1 showed the strongest correlation ($R^2 = 0.721$; Figure 5A) followed by IL-1 β ($R^2 = 0.659$; Figure 5B), IL-6 ($R^2 = 0.566$; Figure 5C) and TNF- α ($R^2 = 0.398$; Figure 5D). This was generally consistent with the relationship observed between these mediators and the abundance of large polyps in section 2 ($P < 0.001$) and overall polyp number in sections 2 and 3 ($P < 0.001$) (data not shown). Similarly, there were positive correlations for protein concentration of pro-inflammatory mediators and polyp progression ($P < 0.05$) (data not shown) although this relationship was not as strong as that observed for gene expression.

4. Discussion

Epidemiological evidence links colon cancer to chronic intestinal inflammation [25]. Further support for a role of inflammation in colon cancer comes from studies using mouse models that have been shown to be responsive to treatment with anti-inflammatory agents, including both anti-inflammatory dietary supplements as well as non-steroidal anti-inflammatory drugs (NSAIDs) [19]. Despite this, the timing and magnitude of the inflammatory cytokine response in relation to tumorigenesis has not been characterized. This study used an established mouse model of intestinal tumorigenesis to examine this response in the *Apc^{Min/+}* mouse. Our findings indicate that an elevation in the intestinal inflammatory cytokine (MCP-1, IL-1 β , IL-6 and TNF- α) response occurs at 12 wks of age in association with the rapid increase in polyp number. Further elevations in these mediators with age were associated with increased polyp size. In fact, all inflammatory mediators were positively correlated with the abundance of large polyps as well as overall polyp number (data not shown). These data also generally show a greater increase in the cytokine response in intestinal section 3, a region of high polyp incidence, compared to section 2 that is considered a region of relatively low polyp incidence [26]. These findings contribute to the growing evidence on the association between inflammatory cytokines and colon cancer and provide important new data on the magnitude and timing of this response in relation to tumorigenesis in the *Apc^{Min/+}* mouse.

The *Apc^{Min/+}* mouse model has been the most widely used genetically engineered model for cancer studies that involve the gastro-intestinal tract. In fact over 600 articles have been published using this model. It has been shown to be responsive to treatment with anti-inflammatory agents, including both anti-inflammatory dietary supplements as well as non-steroidal anti-inflammatory drugs (NSAIDs) [19]. For example, Greenspan et al. reported that the NSAID Sulindac can decrease intestinal polyp number in the *Apc^{Min/+}* mouse that is associated with a decrease in proliferative and inflammatory prostaglandins in the small intestine and colon [27]. Similarly, another study reported that Celecoxib reduced the formation of intestinal polyps that was associated with lower levels of COX-2 activity [28]. And we have shown that the anti-inflammatory dietary component curcumin can offset intestinal inflammatory cytokines and tumorigenesis in this mouse model [23]; a decrease in mRNA expression of IL-1 β , IL-6, TNF- α and MCP-1 as well as protein concentration of IL-1 β and MCP-1 was observed in the intestines following 14 wks of curcumin treatment. Another study reported that the dietary component Silibinin can decrease intestinal tumorigenesis in the *Apc^{Min/+}* mouse in association with a decreased expression of TNF- α , IL-1 β but not MCP-1. However, the timing and magnitude of the intestinal pro-inflammatory response in relation to tumor progression in this model has not been

characterized. This is surprising given the preponderance of studies that have used this model to test anti-inflammatory agents [19]. Most studies have focused on the effects of various anti-inflammatory treatments over the life-span of these mice without consideration for the temporal sequence of inflammation in relation to cancer. To our knowledge there is only one study that has examined any inflammatory mediators in the *Apc^{Min/+}* mouse over time. Kettunen et al., (2003) examined the intestinal immune response in *Apc^{Min/+}* mice at 5, 8 and 15 wks of age and reported an increase in prostaglandin E₂ (PGE₂) at 15 wks but not TNF- α or IL-12 [29]. However, MCP-1, IL-6 and IL-1 β were not measured in this study and there were no attempts made to measure gene expression of any inflammatory mediators.

We show here for the first time the timing and magnitude of the inflammatory cytokine response in intestinal tissue across time in the *Apc^{Min/+}* mouse. In addition to the inflammatory cytokines that have well documented effects on tumor progression [7–11], MCP-1 was examined as it has been identified as a major chemokine for macrophage recruitment in several human tumors including the colon [30, 31] and has been associated with increased inflammation and tumor grade in various cancers [16, 17]. Our data show an increased mRNA expression of MCP-1, IL-1 β , IL-6 and TNF- α that is evident at 12 wks of age and is consistent with the increase in polyp number that occurs at this time; total polyp number (sections 1, 4 and 5) increases from 26 ± 3 at 8 wks to 60 ± 6 at 12 wks. Any further increases in the inflammatory response appear to be associated with a change in polyp size; 12 wk old *Apc^{Min/+}* mice had the largest number of small polyps (<1mm) whereas 20 wk old mice had the greatest number of large polyps (>2mm). In fact, a positive correlation was observed between the abundance of large polyps and all inflammatory mediators. Similarly, protein concentration of these cytokines generally follows the same trend. It is worth noting that section 3, a section of high polyp incidence shows a greater magnitude of inflammation than section 2, a section of low polyp incidence [26] that provides further evidence for the association between inflammation and tumor burden in this model. Whether the changes in polyp number and size that occurs in this model is a result of the elevated inflammatory response or vice versa cannot be determined from this investigation. Future studies are necessary to identify whether inflammation is a cause and/or effect of the changes in polyp number and size across time in this mouse model.

These findings may have important implications for colon cancer. Firstly, these results may contribute to the future development of biomarkers to assess colon cancer progression. The current literature supports a positive relationship between systemic IL-6 and increasing tumor stages and tumor size, metastasis and decreased survival [32] in colon cancer. Similarly, MCP-1 has been correlated with increased grade of the tumor [17] advanced tumor stage, lymph node involvement [33] and poor prognosis in breast cancer [16, 31], but no studies of this nature have been carried out with colon cancer. However, our data shows a strong relationship between polyp progression and MCP-1 in a mouse model of colon cancer. Future studies are necessary to fully evaluate the potential of these inflammatory cytokines as prognostic indicators for colon cancer. Secondly, these data provide important new information that can be used for the determination of appropriate timing of effective treatments that can be used in the design of future investigations that target inflammatory processes in mouse models of colon cancer. Based on these data it is likely that anti-inflammatory treatments would be more effective as a preventive approach (i.e administered before 12 wks) to reduce polyp number and an intervention approach (i.e administered after 12 wks) to reduce polyp size in this model.

In summary, the *Apc^{Min/+}* mouse is the most widely used model in studies that examine the effect of anti-inflammatory treatments including bioactive food components and NSAIDs on intestinal tumorigenesis. Despite this there is very little information on the temporal

sequence of inflammation in relation to tumorigenesis in this model. This is the first systematic investigation of the timing and magnitude of the inflammatory cytokine (IL-1 β , IL-6 and TNF- α) and MCP-1 response in the *Apc^{Min/+}* mouse. These data contribute to the growing evidence on the association between inflammation and colon cancer and provide important new data that could be used in the development of biomarkers as well as in the design of future investigations of anti-inflammatory treatments.

Acknowledgments

This work was supported by a grant from the National Cancer Institute (R21 CA135377) to E.A.M.

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Figure 1. Polyp number in the *Apc^{Min/+}* mouse at 8, 12, 16 and 20wks of age
 Differences in (A) total polyp number (B) colon polyp number and (C) polyp size distribution were observed over time (n=6–12/group). P<0.05 * greater than 8 wks, # greater than 12 wks, ! greater than 16wks and @ greater than 20 wks.

Figure 2. mRNA gene expression of inflammatory mediators in the *Apc^{Min/+}* mouse at 8, 12, 16 and 20wks of age

Differences in mRNA gene expression of (A) MCP-1, (B) IL-1 β , (C) IL-6, and (D) TNF- α were observed in sections 2 and 3 of the intestines over time (n=6–12/group). P<0.05 * greater than 8 wks, # greater than 12 wks, ! greater than 16wks and @ greater than 20 wks.

Figure 3. Protein concentration of inflammatory mediators in the *Apc*^{Min/+} mouse at 8, 12, 16 and 20wks of age

Differences in protein concentration of (A) MCP-1, (B) IL-1 β , (C) IL-6, and (D) TNF- α were observed in sections 2 and 3 of the intestines over time (n=6–12/group). P<0.05 * greater than 8 wks, # greater than 12 wks, ! greater than 16wks and @ greater than 20 wks.

Figure 4. Plasma MCP-1 is increased in the *Apc*^{Min/+} mouse model of intestinal tumorigenesis Wildtype and *Apc*^{Min/+} mice were sacrificed at various time-points (8, 12, 16 or 20 wks) and plasma was analyzed for MCP-1 concentration using ELISA. * MCP-1 was increased at 12, 16 and 20 wk versus WT (P<0.05). P<0.05 * greater than 8 wks and # greater than 12 wks.

Figure 5. Inflammatory mediators are positively correlated with polyp progression in the *Apc^{Min/+}* mouse

Correlations were performed between abundance of large polyps and mRNA expression of MCP-1 (A), IL-1 β (B), IL-6 (C) and TNF- α (D) in section 3 of the intestines. All inflammatory mediators showed a positive relationship with abundance of large polyps ($P < 0.01$).