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Chemotherapy Induces Oral Mucositis in Mice Without Additional Noxious Stimuli[1]

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Abstract

Oral mucositis (OM) is a serious side effect of cancer chemotherapy. The pathobiology of oral mucositis remains incompletely understood due to lack of appropriate models which recapitulate the human condition. Existing rodent models are intraperitoneal and require radiation, chemical or mechanical injury to the chemotherapy protocol to induce oral lesions. We aimed to develop an OM mouse model that is induced solely by chemotherapy and reproduces macroscopic, histopathologic and inflammatory characteristics of the human condition. Female C57BL/6 mice were given intravenous 5-Fluorouracil (5-FU) injections every 48 hours, for 2 weeks. A high daily dose of intraperitoneal administration was tested for comparison. Mice were monitored daily for weight loss. Epithelial histomorphometric analyses in tongue, esophageal and intestinal tissues were conducted coupled with assessment of apoptosis, cell proliferation, neutrophilic infiltration and the integrity of adherens junctions by immunohistochemistry. Neutropenia was assessed in peripheral blood and bone marrow. Tissues were analyzed for pro-inflammatory cytokines at the protein and mRNA levels. Daily intraperitoneal administration of 5-FU led to rapid weight loss and intestinal mucositis, but no oral inflammatory changes. Intravenous administration triggered atrophy of the oral and esophageal epithelium accompanied by reduction in cell proliferation and increased apoptosis. Coincidental with these changes were up-regulation of NF-κB, TNFα, IL-1β, GM-CSF, IL-6 and KC. Despite neutropenia, increased oral neutrophilic infiltration and reduced E-cadherin was observed in oroesophageal mucosae. We developed a novel experimental tool for future mechanistic studies on the pathogenesis of chemotherapy-induced OM.

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Introduction

Mucositis is an inflammatory condition resulting from damage to the oroesophageal, gastrointestinal and genitourinary tract mucosae, following cytotoxic cancer therapies [1]. Its severity and duration varies with the dose and the type of drug used, but in certain cases it can lead to compromised nutrition and chemotherapy dose reduction or postponement due to severe pain or diarrhea. In fact, mucositis can be of such severity that patient survival is adversely impacted [2]. Oral mucositis affects 40–80% of patients undergoing chemotherapy and even though it can be subtle at first, it gradually becomes more severe after 7 to 10 days of treatment [3].

One of the most commonly used drugs associated with mucositis is 5-fluorouracil (5-FU). 5-FU inhibits thymidylate synthase and depletes intracellular thymidine triphosphate pools [4] arresting cells in S phase [5]. 5-FU has also been proposed to interfere with the activity of ribosomal RNA binding protein, at the level of pre-ribosomal RNA processing [6]. The exact mechanisms of inflammation are not fully understood, yet some progress has been made over the last several years with the development of rat, hamster [7] and mouse [8–11] mucositis models. Current intraperitoneal (IP) mouse models are either based on a single high dose of 5-FU (100–500 mg/kg), or a smaller daily dose (30–50 mg/kg) [10–12] for up to 4 weeks. IP rodent protocols are technically simple and trigger

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intestinal mucositis reproducibly but do not significantly affect the oral mucosa, unless head and neck irradiation, [13] local mechanical or chemical injury [7,14] are included.

In murine IP models the intestinal mucosa is exquisitely more sensitive to chemotherapy than the upper alimentary tract mucosa, thus animals lose intestinal function rapidly and have to be euthanized prior to developing oral lesions [9–12]. Increased susceptibility of the intestinal mucosa may be related to proximity of the drug or the type of epithelium (simple columnar versus stratified squamous epithelium) and different epithelial turnover rate, which is about 1–4 days in small intestinal epithelium and 14–21 days in buccal mucosa and esophagus. [15] Turnover rate differences can affect both the sensitivity and rate of recovery from mucositis, but are not sufficient to explain differences in the inflammatory response to chemotherapy.

Due to lack of a clinically relevant chemotherapy-induced oral mucositis animal model the cellular and molecular events involved in its pathogenesis remain incompletely understood. The aim of this study was to compare a high dose IP 5-FU chemotherapy model to a low dose, intermittent, intravenous mode of administration of longer duration, resembling human anti-cancer regimens. We focus our investigation on the effects of 5-FU on the oral mucosa with the main goal of developing a reliable model of oral mucosal inflammation in response to chemotherapy. For the first time we also analyze the bone marrow immunosuppressive effect of 5-FU and its effect on the structural integrity of the mucosal barrier, which may have a bearing on the increased susceptibility to opportunistic infections. Finally, to begin to understand the mechanism involved in the site-specific responses to chemotherapy we perform a comparative analysis of the oral, esophageal and intestinal histopathologic and inflammatory responses.

Materials and Methods

Animals

Six to twelve-week-old female C57BL/6 mice were used (Jackson Laboratories, animal protocol 100,965–1017). Animals were housed with access to pelleted food and water ad libitum in a temperature-controlled environment with a 12-hour light/dark cycle. Mice were monitored daily for signs of morbidity and body weights were recorded every 24-48 h.

5-FU Administration

Mice received 100 mg/kg 5-FU (Sigma, St. Louis, MO, USA), daily for 4 days, via IP injection and were sacrificed on day 5. Alternatively, mice received intravenously (IV, via tail vein) 50 mg/kg 5-FU every 48 hours, from day 1 to day 13 and euthanized on day 14 by carbon dioxide exposure. This dose and frequency of administration was chosen as it is effective in substantially reducing tumor size in a xenograft mouse model [16], and is within the therapeutic intermittent dose range in humans [17,18]. Control groups received PBS, either IP or IV. Tongue, esophagus, jejunum, femurs and tibiae were retrieved at necropsy. Animal experiments were repeated thrice, unless otherwise stated.

Macroscopic and Histopathologic Examination

Tongues were stained with 1% toluidine blue in 10% acetic acid for 1 minute, followed by repeated washes with acetic acid, to reveal surface erosive or ulcerative lesions [19]. The percentage of toluidine blue positive surface area (excluding excision trauma) was calculated using the Image J software. Tissues were then fixed in 4% (v/v) paraformaldehyde solution in PBS for 2 hours at 4 °C, and processed for paraffin or OCT embedding. Epithelial thickness in tongues and esophagi, and villus length in the jejunum were measured in H&E stained tissues using Image J. Three mice per group, with 3 fields per sample and 5 measurements per field, were analyzed, in a blinded fashion. Images were obtained using a Zeiss Axio Imager M1 microscope and an EC-Plan-Neofluar 920-NA 0.5 air-objective and using the AXIOVISION-SE64 Rel. 4.9.1 program.

Determination of Cell Proliferation and Apoptosis in Mucosal Tissues

Mitotically active oroesophageal and intestinal crypt cells were detected using a Ki67-rabbit pAb (Cell Signaling technology, Danvers, MA) as described previously [20]. Cell apoptosis was evaluated using the DeadEnd™ Colorimetric TUNEL® System, according to manufacturer's instructions (Promega, Madison, WI).

RNA Extraction and Reverse-Transcription Quantitative Polymerase Chain Reaction (RT-qPCR)

Mouse tongues and jejunum were homogenized using a POLYTRON homogenizer, and the supernatants were beat by zirconia beads (Ambion, Waltham, MA) with phenol:chloroform: isoamyl alcohol (25:24:1 v/v/v, Thermo Fisher Scientific, Waltham, MA). RNA was purified using the QIAgen RNeasy® Mini Kit and concentrations/quality were determined using a NanoDrop device (Thermo Scientific, Waltham, MA). Complementary DNA was synthesized with SuperScriptIII Cells Direct® cDNA Synthesis kits (Invitrogen, Carlsbad, CA). Reverse-transcription quantitative polymerase chain reaction was performed with a Bio-Rad CFX96 cycler and the iQ® SYBR Green Supermix (Bio-Rad, Hercules, CA). Primer sets for each gene are shown in supplemental Table 1 (supplemental). Fold changes were calculated using the $\Delta\Delta^{CT}$ method.

Laser Capture Microdissection (LCM)

Tongues were snap-frozen in dry ice-cooled 2-methylbutane (Acros, Geel, Belgium), and embedded in cryomatrix compound (Thermo Fisher Scientific, Waltham, MA). A Leica CM 3050S (Leica Microsystems, GmbH, Nussloch, Germany) cryostat with installed

Table 1. Primer sets for each gene used to amplify the selected pro-Inflammatory cytokines.

	Forward	Reverse
NF-kB	5′-ATGGCAGACGATGATCCC TAC-3′	5′-TGTTGACAGTGGTATTTCTGGTG -3′
IL-1β	5′-GACACTGTTCCTGAACTCAAC T-3′	5′-ATCTTTTGGGGTCCGTCAACT-3′
TNF-α	5′-GGAACACGTCGTGGGATAATG-3′	5′-GGCAGACTTTGGATGCTTCTT-3′
GM-CSF	5′-GGCCTTGGAAGCATGTAGAGG-3′	5′-GGAGAACTCGTTAGAGACGACTT-3′
IL-6	5′-GACAAAGCCAGAGTCCTTCAGAGA G-3′	5′-CTAGGTTTGCCGAGTAGATCTC-3′
KC	5′- GCCAATGAGCTGCGCTGTCAGTGC-3′	5′-CTTGGGGACACCTTTTAGCATCTT-3′
GAPDH	5′-TCCTACCCCCAATGTGTCC-3′	5′-CTCTTGCTCAGTGTCCTTGCT-3′

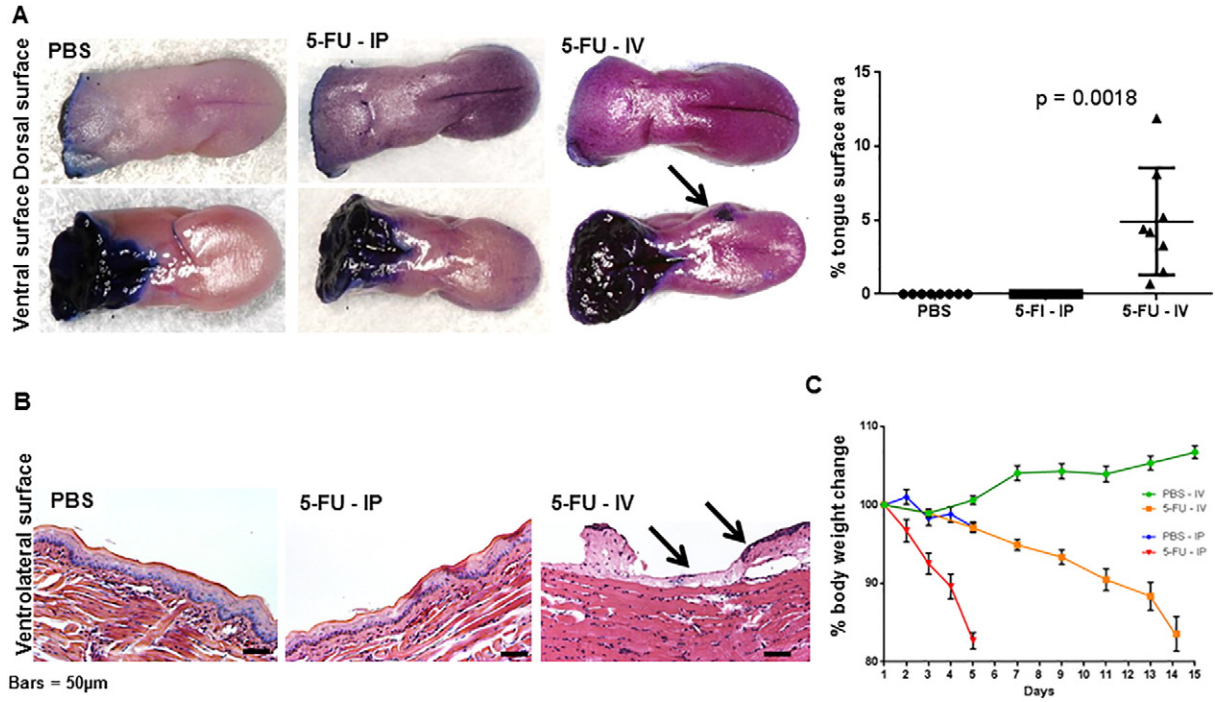

Figure 1. Effect of 5-FU on oral mucosa and body weight. Mice received either PBS IV (PBS), 5-FU IP (5-FU-IP), or 5-FU-IV. A: Tongues stained with toluidine blue. Arrow points to surface lesion. Dot plot shows percentage of toluidine blue-stained surface in each mouse. B: H&E stain of ventrolateral tongue sections in each group. Arrows show areas of erosion and ulceration. C: Body weight change in each group.

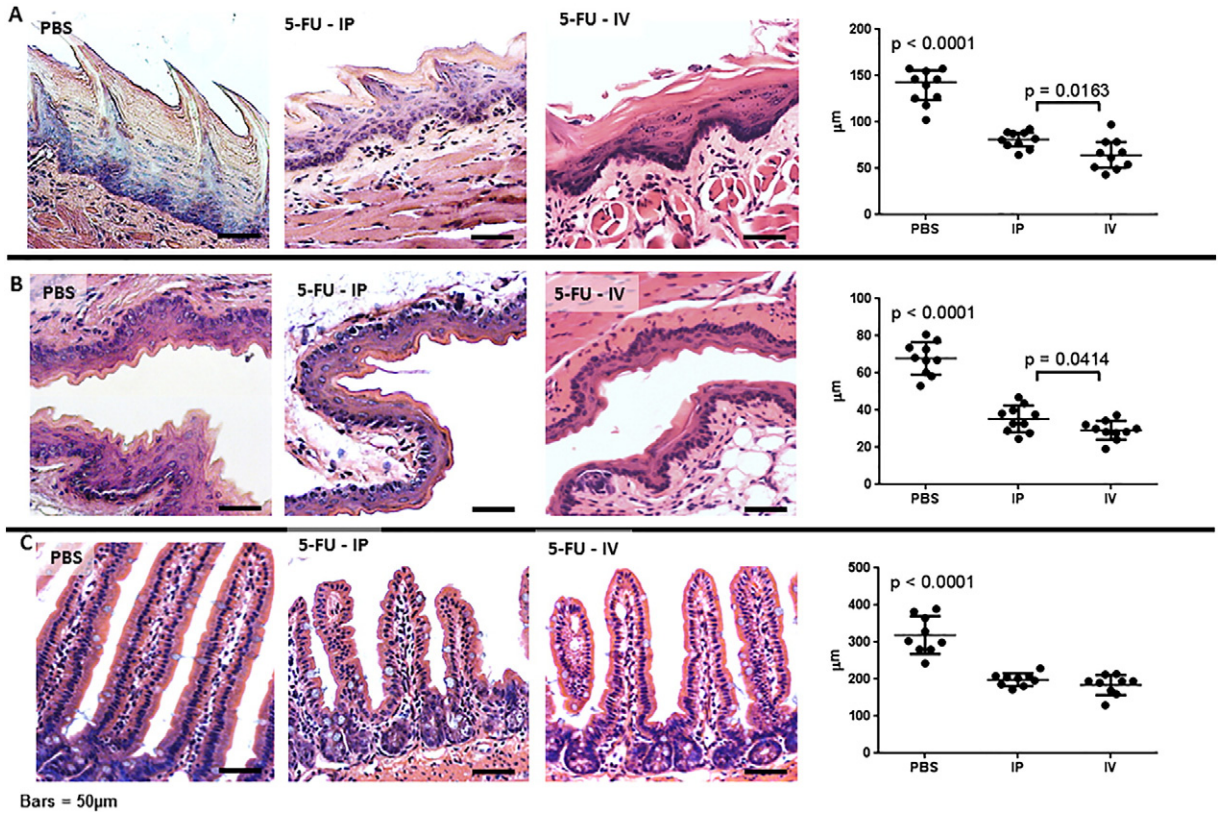

Figure 2. Effect of route of 5-FU administration on mucosal thickness and villus length. A: Dorsal tongue; B: esophagus; C: jejunum. H&E-stained tissue sections (20×). Dot plots represent epithelial thickness or villus length measurements; each dot is the average of 10 representative measurements/tissue.

CryoJane®, was used for cryosectioning. Frozen 7 μm tissue sections were mounted onto commercial CryoJane® glass slides. An Arcturus® laser capture microscope (Thermo Fisher Scientific) was used to retrieve epithelial tissue from tongue dorsal and ventral surfaces. Samples were pooled and solubilized in Cell Lysate Buffer® (Signosis, Sunnyvale, CA) for direct reverse transcription, and relative cDNA levels were quantified by RT-qPCR, as described above.

Measurement of Pro-Inflammatory Cytokine Protein Levels

Tissues were homogenized in Mammalian Protein Extraction Reagent® (Thermo Fisher Scientific, Waltham, MA) and a protease inhibitor cocktail (cOmplete® tablets, Sigma-Aldrich, St. Louis, MO), with a POLYTRON homogenizer, followed by 10 minutes disruption in a bead beater and sonication for 1 min. Samples were centrifuged at 13000 rpm for 5 min, and total protein concentration was quantified using the BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA). Samples were diluted as needed in order to standardize the amount of protein. Granulocyte-macrophage colony-stimulating factor (GM-CSF), Interleukin 1 alpha (IL-1α), IL-1β, IL-6, IL-10, keratinocyte chemoattractant (KC) and Tumor Necrosis Factor-alpha (TNFα) were simultaneously quantified in each sample using the Luminex/MAGPIX system (RCYTOMAG-80 K; Millipore, Billerica, MA).

Neutrophil Counts

To quantify bone marrow neutrophils, tibiae and femurs marrows were flushed repeatedly with 1 ml of RPMI 1640 media. A mouse neutrophil isolation kit (STEMCELL Technologies, Vancouver, Canada), based on negative magnetic cell separation, was subsequently used as described previously [21]. The Wright-Giemsa stain was used in whole blood smears from tail vein samples to calculate the neutrophil reduction fold over baseline.

Immunofluorescence Staining

Immunofluorescence staining for E-cadherin and neutrophils were described elsewhere [21,22]. Briefly, paraffin embedded (for E-cadherin) or frozen (for PMN) tissue sections were stained with an anti-E-cadherin polyclonal antibody (BD Biosciences, San Jose, CA) followed by a FITC-conjugated secondary antibody (DyLight 488, Vector Laboratories, Burlingame, CA), or for PMN with the monoclonal antibody NIMP-R14 (Hycult, Biotech, Plymouth Meeting, PA), highly specific for murine Ly-6G and Ly-6C followed by a secondary anti–rat antibody conjugated with Alexa 555 (Thermo Fisher Scientific, Waltham, MA). To visualize all cells the nuclear stain Hoechst 33,258 (Thermo Fisher Scientific, Waltham, MA) was used. Images were obtained and analyzed as above.

Statistical Analyses

Pair-wise comparison between 5-FU and control groups was performed using the non-parametric Mann–Whitney test, or Student's t-test when data points were normally distributed, using the GraphPad Prism software (version 6). Statistical significance was set at $P < .05$.

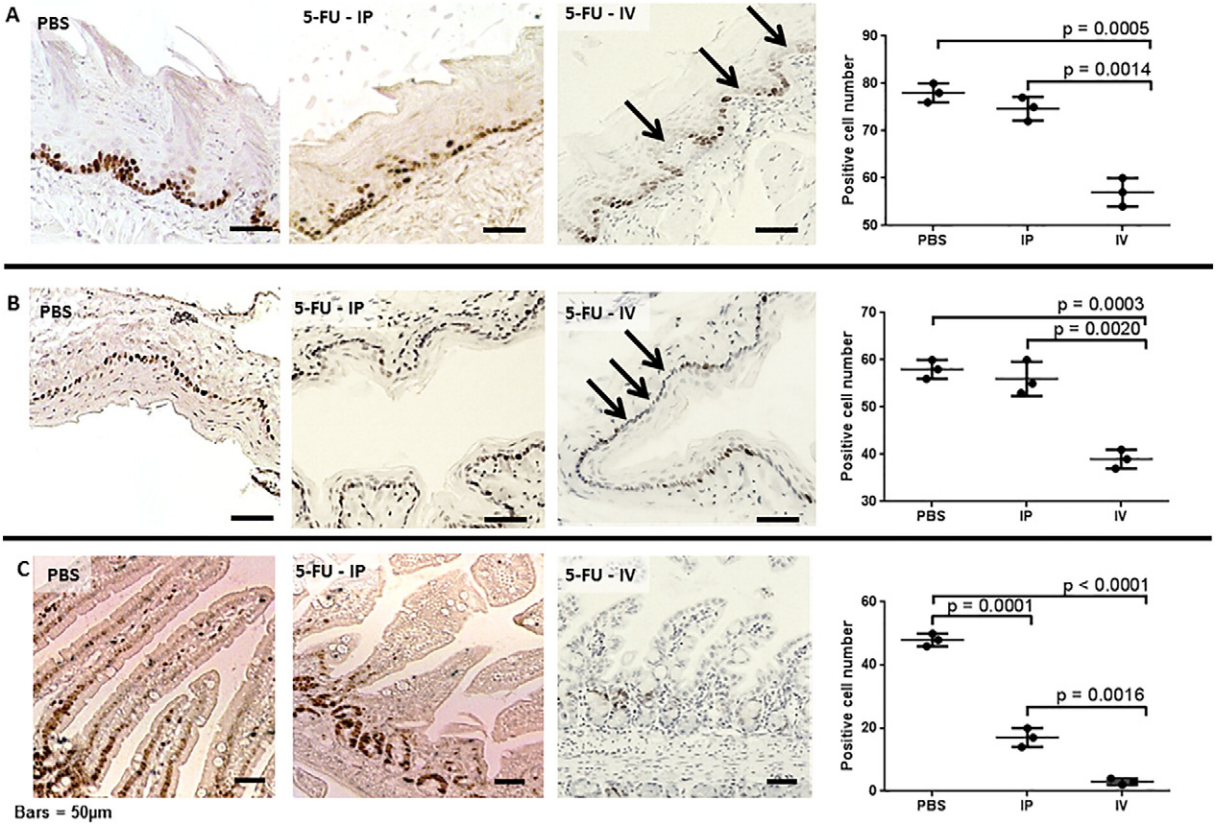

Figure 3. Ki67 staining of oral (A) esophageal (B) and intestinal mucosa (C). Arrows point to interruptions of Ki67-positive (brown) cells along the basal layer. Dot plots represent average number of positive cells/field (three representative mice/group).

Results

Intravenous 5-FU Administration Triggers Histopathologic Changes Consistent with Mucositis

Macroscopically, only IV administration triggered diffuse erythema on the dorsal surface of the tongues. Toluidine blue positive areas were found in the ventrolateral surface and represented up to 13% of this surface (Figure 1*A*). Toluidine blue positive areas corresponded to erosive or ulcerative lesions with extremely atrophic or absent epithelium (Figure 1*B*). Importantly, IV administration maintained the average body weight loss at less than 20% of baseline, similar to short-term IP administration (Figure 1*C*).

On the dorsal tongue and esophageal mucosa, both modes of 5-FU administration led to epithelial atrophy as reflected by the significant reduction in epithelial thickness, in comparison to the control group (Figure 2, *A* and *B*). However, only IV administration compromised the structural integrity of the dorsal mucosa, with filiform papillae being completely effaced and the keratinized layer having a desquamative appearance in most histologic sections examined from this group (Figure 2*A*). In the esophageal mucosa, which is keratinized in mice [23], the superficial keratin layer was reduced or absent in the IV group, compared to the IP group where a keratinized superficial layer was still intact resulting in a thicker epithelium (Figure 2*B*). In the jejunum both routes of administration triggered significant reduction in villus length and crypt destruction (Figure 2*C*).

Intravenous 5-FU Administration has a Greater Effect on Oral Mucosal Cell Proliferation and Apoptosis Markers

Consistent with the reduced thickness of the oroesophageal mucosa, a reduced number of Ki67-positive proliferating cells were detected in the basal layers of tongue and esophageal epithelium in the 5-FU-treated compared to the PBS control groups (Figure 3, *A* and *B*). This reduction was statistically significant only in the IV group, confirming the greater effectiveness of this mode of administration in affecting oroesophageal mucosal cell proliferation. In fact, in this group large basal layer segments of the tongue mucosa were completely devoid of Ki67-positive cells (Figure 3*A*, arrows). In intestinal mucosa, the Ki67-positive cells located at the crypts of Lieberkuhn and the base of villi were significantly reduced in both modes of 5-FU treatment when compared to PBS-Control. In the IV group almost complete depletion of Ki67-positive cells was observed (Figure 3*C*).

Epithelial cell apoptosis was also greatest with IV administration in both oral and esophageal mucosae (Figure 4, *A* and *B*). Interestingly, with IV administration TUNEL-positive apoptotic cells could be seen in the basal tongue epithelial layer, whereas few apoptotic cells were present in the basal layers of tongue epithelium in the IP group (Figure 4*A*, arrows). In the intestinal mucosa even though both modes of administration triggered significant increase in apoptosis, apoptotic cell numbers were significantly greater in the IP group (Figure 4*C*), possibly due to increased cytotoxicity resulting from the proximity of the drug to abdominal organs. The finding that villi in

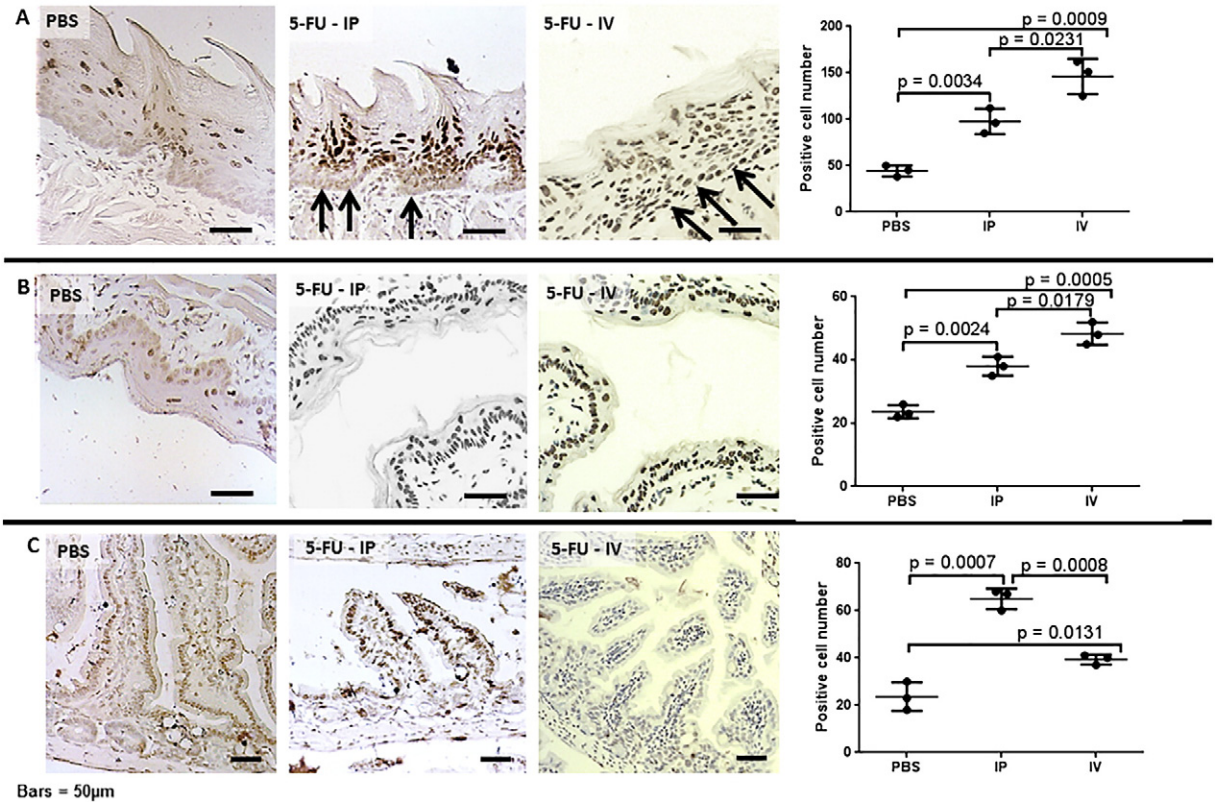

Figure 4. TUNEL staining of oral (A) esophageal (B) and intestinal mucosa (C). Arrows point to the extent of positive cell staining relative to the basal layer. Dot plots represent average number of positive cells/field (three representative mice/group).

the IP group had higher numbers of apoptotic cells but also higher numbers of proliferating cells compared to the IV group, explains the outcome that villus length and loss of intestinal function, as reflected in weight loss, was similar in the two groups (Figures 1–4).

Intravenous 5-FU Administration Triggers a High Inflammatory Response in Oral Epithelium

Consistent with the limited histopathologic changes on tongue epithelium, daily 5-FU IP administration did not increase tongue Nuclear factor-kappa B (NF-κB), IL-1β or TNFα expression (Figure 5*A*), three inflammatory markers strongly associated with oral mucositis in humans [24]. This suggested that short-term IP chemotherapy protocols, even with a high daily 5-FU dose, do not trigger a significant pro-inflammatory response in oral tissues. In contrast, 5-FU IV administration triggered an increase in NF-κB, IL-1β and TNF-α gene expression (Figure 5*B*), consistent with the mucosal erythema, erosions and ulcerations (Figure 1, *A* and *B*).

In order to dissect the role of the oral epithelial compartment in the inflammatory response to 5-FU, we extracted RNA from epithelium separated from the submucosa using LCM. As shown in Figure 5*C* NF-κB and IL-1β expression was much higher in laser dissected epithelium compared to whole tongue tissue, suggesting that epithelium is the main source of these inflammatory markers. 5-FU also triggered an exaggerated GM-CSF, IL-6 and KC response in oral epithelium, three pro-inflammatory cytokines linked to the pathogenesis of oral mucositis [1,24].

To confirm RT-qPCR findings, we tested inflammatory cytokine protein levels in tongue tissues. As seen in Figure 5*D*, IL-1β, GM-CSF and KC protein levels were significantly elevated in response to 5-FU, mirroring mRNA expression results. In addition, higher levels of IL-6 were noted, a cytokine significantly elevated in oral mucositis in humans [1,24]. High levels of IL-1α were detected in all oral samples with no differences between 5-FU and PBS groups (not shown). In comparison, in the jejunum only KC was significantly upregulated by 5-FU, suggesting a mucosal tissue-specific inflammatory response to chemotherapy (Figure 5*E*).

Intravenous 5-FU Administration Triggers Neutropenia and Compromises the Integrity of Adherens Junctions in the Oroesophageal Mucosa

We next examined neutrophil counts in the mouse IV model, since leukopenia is a well-documented side effect of 5-FU administration and oral mucositis coincides with the nadir of neutrophils in humans [25]. Peripheral neutrophil counts dropped significantly throughout the experiment (Figure 6*A*) and the same effect was noted in the bone marrow (Figure 6*B*), suggesting that 5-FU interferes with maturation of myeloid progenitors, as shown by others [26]. However we also noted neutrophils mobilized toward the oral mucosa (Figure 6*C*), suggesting that homing to this site may be induced by epithelial-derived pro-inflammatory cytokines. Neutrophil infiltration in 5-FU treated mice was accompanied by a reduction in

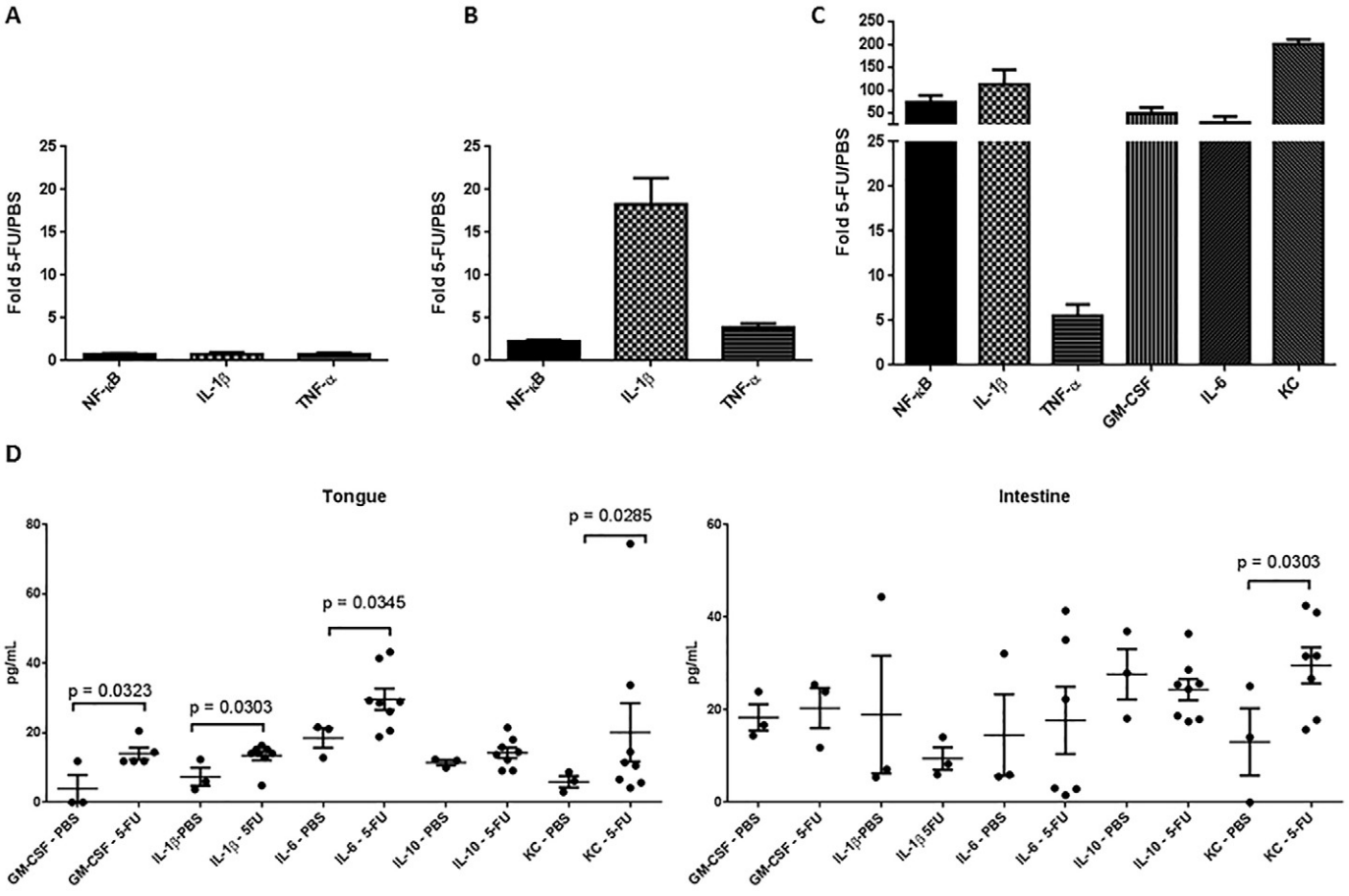

Figure 5. Inflammatory marker analysis in oral and intestinal mucosae in 5-FU IP (A) or IV (B-E) groups. RT-qPCR analysis of whole tongue (AB), or laser-captured tongue epithelium (C). Bars represent average (± SD) fold increase over PBS-treated group (5–8 mice/group). Tongues (D) and jejunum (E) were analyzed for cytokine protein levels by multiplex ELISA, after standardizing protein content. Each dot represents one mouse/group.

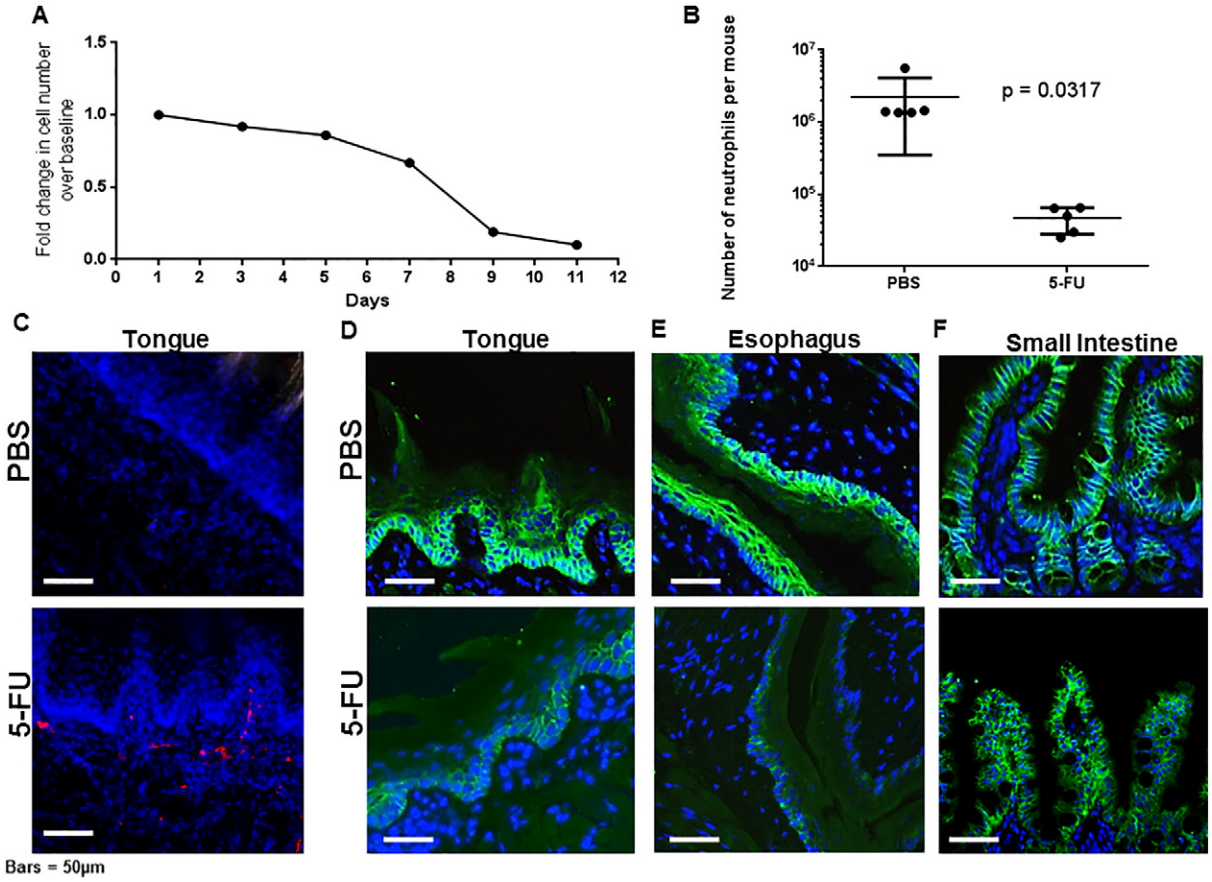

Figure 6. Neutrophils (A-C), and oral (D), esophageal (E), and intestinal (E) E-cadherin protein expression in mice treated with 5-FU or PBS, IV. A: Peripheral blood neutrophil changes over baseline in 5-FU group. B: Average number of bone marrow neutrophils isolated per mouse (5 mice/group). C: Immunofluorescence staining for neutrophils (red). D-E: Immunofluorescence staining for E-cadherin (green).

E-cadherin protein levels in oral mucosal (Figure 6*D*). Reduction in E-cadherin was also found in the esophageal (Figure 6*E*), but not the intestinal mucosa (Figure 6*F*).

Discussion

In this work we characterized the first intravenous mouse chemotherapy-induced oral mucositis model that faithfully reproduces histopathological and inflammatory responses of the human oral mucosa to 5-FU. Our model is a significant improvement over existing models because it: i) does not require additional mucosal "irritation" to induce macroscopically and histologically identifiable erosions and ulcers; ii) triggers a significant inflammatory response in oral epithelium, without severe loss of intestinal function; and iii) is the first model resembling most current human anti-cancer 5-FU regimens [27]. Because in most existing 5-FU models mechanical-, chemical- or radiation-induced injury of the mucosal surface is additionally required, the ability of chemotherapy to directly trigger ulcerations has been questioned [28]. Our work provides the first conclusive evidence that chemotherapy alone in mice can trigger oral ulcers with longer term, frequent intravenous administration. This is not surprising given the high oral bioavailability of 5-FU when given IV, coupled with its short half-life [29].

The first animal model of 5-FU-induced oral mucositis was described by Sonis and colleagues (1990) [30] and combined mucosal mechanical "irritation" with IP injections in hamsters. While the combination of mechanical irritation with chemotherapy triggers clinically severe mucosal ulcerations, the requirement for additional experimental manipulation of the oral mucosa raises the question whether this is a model of chemotherapy-induced impaired wound healing, in which the direct effects of chemotherapy on healthy epithelium cannot be dissected. Other limitations are that mechanical and chemical experimental mucosal trauma is difficult to standardize and there are no genetic knockout or transgenic hamster models that would allow mechanistic studies on the pathogenesis of this condition. Despite limitations, this rodent model is still a useful tool in preclinical testing of new mucositis chemopreventive agents as it may offer endpoints of therapy close to human clinical pathology.

Existing 5-FU chemotherapy-induced mucositis mouse [9–12] models are intraperitoneal and most focused their evaluations on lower gastro-intestinal tract mucosal changes. Our work has shown that even a high daily dose of 5-FU intraperitoneally is not sufficient to induce oral mucosal inflammatory changes in mice. In contrast to IV administration which increases bioavailability to all tissues and organs, intraperitoneal administration targets primarily organs in the peritoneal cavity, thus toxicity in these organs exceeds other sites. In humans 5-FU is administered intravenously except in cases of advanced gastric cancer, where IP administration aims to increase the concentration of chemotherapy in the peritoneal cavity. [31] Thus, it is not surprising that a recently published mouse oral mucositis model

spaced IP injections a week apart to reduce abdominal toxicity, while the study presented no histologic evidence of oral ulcers and only a weak oral mucosal inflammatory response, as tissues can recover after lengthy intermissions to 5-FU exposure [9]. Nevertheless, using a high throughput transcriptomic approach this model identified novel genes that may be up- or down-regulated in response to 5-FU in the oral mucosa [9].

Ours is the first study that used laser capture technology to separately quantify the oral epithelial from the stromal inflammatory response to chemotherapy. In our IV model, 5-FU triggered upregulation of certain pro-inflammatory cytokines in the oral but not intestinal mucosa, suggesting a mucosal site-specific response to 5-FU. In agreement to our findings others have shown that cytokines such as IL-1β and IL-6 were not induced by 5-FU in mouse small intestinal mucosa [32]. NF-κB was significantly upregulated by 5-FU in oral epithelium, consistent with the proposed central role of this signaling pathway in chemotherapy-induced oral inflammation [1,3,33]. Our findings of an exaggerated oral epithelial IL-1β, GM-CSF, IL-6 and KC response to 5-FU, suggest that these cytokines may be regulated by NF-κB [34]. It has been hypothesized that release of cytokines such as IL-1β results in tissue injury by activating matrix metalloproteinases or by promoting apoptosis [1,3,33]. We noted a significant apoptotic response in oral epithelium to 5-FU, consistent with existing pathobiology models [1,3,33]. NF-κB signaling was shown to play a protective role from mucosal cell death triggered by certain apoptotic signals [34]. Development of this mouse model allows further mechanistic studies into the regulatory networks of 5-FU-induced mucosal inflammation and apoptosis, and into the contributions of the NF-κB pathway in each biological process.

All of the pro-inflammatory cytokines we have identified as strongly induced by 5-FU in oral epithelium, are functionally associated with neutrophil activation. Thus it was not surprising to identify neutrophils infiltrating oral mucositis-affected tissues. 5-FU-triggered neutrophil infiltration has been reported in the intestinal mucosa but was attributed to upregulation of CXCL1 and CXCL2 chemokines [32,35]. These findings further highlight the different molecular regulatory circuits of the response to 5-FU in different parts of the alimentary tract. Consistent with this, we found that E-cadherin protein levels were reduced in the oroesophageal but not the intestinal mucosa. In intestinal mucositis others have found expression of occludin and claudin-1, but not ZO-1 to be significantly reduced by 5-FU [35]. Dissolution of adherens junctions by 5-FU may promote local neutrophilic transmigration but also weaken the barrier function of the oral mucosa against chemicals or opportunistic pathogens [22].

Despite the clinical significance of chemotherapy-induced oral mucositis, the specific cellular events involved in its pathogenesis are still poorly understood. To our knowledge, this is the most thorough characterization of the oral mucosal response to 5-FU published to date in a mouse model, including cell proliferation and apoptosis markers, inflammatory responses, as well as assessment of the integrity of the mucosal barrier. Importantly, this is the first study to simultaneously assess the deleterious effects of this type of chemotherapy on neutrophils, which may be associated with increased susceptibility to oral opportunistic infections in cancer chemotherapy patients [36].

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