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# Tumor-Induced Systemic Immune Modulation Protects Against Experimental Colitis

**Lama Awwad**

Lamaaw@technion.ac.il

Technion – Israel Institute of Technology

**Ido Livneh**

Technion – Israel Institute of Technology

**Ami Aronheim**

Technion – Israel Institute of Technology

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# Abstract

Inflammatory bowel disease (IBD) is characterized by chronic, relapsing intestinal inflammation driven by dysregulated immune responses and impaired epithelial homeostasis. Although chronic inflammation is a recognized driver of tumorigenesis, whether established tumors can reciprocally modulate intestinal inflammation remains largely unexplored. Here, we investigated the systemic effects of peripheral tumor growth and tumor microenvironment on experimental colitis. Using dextran sodium sulfate (DSS)-induced colitis in tumor mice bearing, we found that tumor-bearing mice developed significantly milder disease, with reduced Disease Activity Index scores, decreased colonic expression of pro-inflammatory cytokines, and preserved intestinal architecture. Administration of serum from tumor-bearing mice reproduced this protective phenotype, indicating that circulating tumor-induced factors are sufficient to attenuate intestinal inflammation. We next examined TNF-α and IFN-γ, previously identified as mediators of tumor-induced cardioprotection effects and cardiac remodeling. Remarkably, a single administration of recombinant TNF-α and IFN-γ before DSS exposure significantly reduced clinical disease severity and histopathological damage. Protection persisted following a second DSS challenge, demonstrating sustained effects beyond the initial inflammatory episode. Cytokine treatment was also associated with increased colonic expression of anti-inflammatory and reparative macrophage-associated markers, including IL-13 and CD206, together with reduced expression of iNOS. Collectively, our findings reveal an unexpected systemic interaction between cancer and intestinal inflammation, whereby the tumor microenvironment generates circulating signals that suppress experimental colitis. TNF-α and IFN-γ recapitulate this protective response, potentially by immunomodulation of macrophages toward anti-inflammatory resolution and tissue repair.


# Introduction

Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is a chronic, relapsing inflammatory disorder of the gastrointestinal (GI) tract that affects millions of people worldwide (1, 2). The global incidence and prevalence of IBD have increased substantially over recent decades, particularly in newly industrialized countries, making it a growing public health challenge (3). Although CD and UC differ in their anatomical distribution and histopathological features, both are characterized by recurrent episodes of intestinal inflammation, disruption of the epithelial barrier, and progressive tissue injury that compromise intestinal homeostasis and markedly reduce patients' quality of life (4). Despite significant advances in therapeutic strategies, including biologics and small-molecule inhibitors, many patients fail to achieve sustained remission or experience disease relapses, underscoring the need for a deeper understanding of the mechanisms governing intestinal inflammation and its resolution (2). The pathogenesis of IBD is multifactorial and arises from a complex interplay between genetic susceptibility, environmental factors, the intestinal microbiota, and the host immune system. Under physiological conditions, intestinal immune homeostasis is maintained through a tightly regulated balance between pro-inflammatory and immunoregulatory mechanisms, allowing effective defense against pathogens while preserving tolerance to commensal microorganisms. In IBD, disruption of this balance results in persistent activation of innate and adaptive immune responses, excessive

production of inflammatory mediators, epithelial barrier dysfunction, and progressive tissue damage(4). Although these processes are initiated and perpetuated within the intestinal mucosa, accumulating evidence indicates that intestinal inflammation is also influenced by systemic immune signals that modulate immune cell activation, trafficking, and function beyond the local tissue environment (5). This broader view suggests that inflammatory responses in the gut are dynamically integrated with systemic immune regulation rather than being exclusively governed by local intestinal events.

The link between IBD and cancer has been extensively investigated, although this relationship has been studied primarily from the perspective of inflammation-driven tumorigenesis. Chronic intestinal inflammation is a well-established risk factor for colitis-associated colorectal cancer, and experimental models have been widely used to dissect the mechanisms by which colitis promotes tumor initiation and progression (6). In contrast, whether established tumors can reciprocally influence the course of intestinal inflammation, particularly through systemic soluble mediators, remains poorly understood.

Tumors are increasingly recognized as systemic regulators of host physiology rather than isolated local lesions. Through the release of cytokines, chemokines, metabolites, and extracellular vesicles, tumors can remodel immune responses, alter hematopoiesis, and influence the function of distant organs(7). Such tumor-induced systemic effects have been implicated in cancer-associated cachexia, immune suppression, and organ dysfunction, highlighting the capacity of malignant disease to reshape whole-body homeostasis.

Recent studies have demonstrated that peripheral tumors exert profound systemic immunomodulatory effects capable of altering disease progression in distant organs. We previously showed that tumor growth attenuates cardiac inflammation and improves cardiac function and pathology in experimental models through systemic immune reprogramming, suggesting that tumor-associated factors can influence inflammatory responses beyond the tumor microenvironment(8, 9). Based on these observations, we sought to determine whether the systemic immunomodulatory effects induced by a peripheral tumor could alter the course of experimental colitis. We further aimed to investigate whether tumor-associated circulating factors contribute to this effect and identify the molecular mediators responsible for the observed protection.

# Results

Growing evidence indicates that tumors exert systemic effects that extend far beyond the primary tumor site by reshaping immune responses and influencing the function of distant organs. We have previously demonstrated that peripheral tumor growth alleviates inflammatory cardiac disease through systemic immune modulation(8, 9), we sought to determine whether this phenomenon could also influence the course of inflammatory bowel disease (IBD). To address this question, PyMT breast cancer cells were orthotopically implanted into the mammary fat pad of female mice. Five days later, experimental colitis was induced by administering 2% dextran sodium sulfate (DSS) in the drinking water for 9 days. Four experimental groups were included: naïve controls, tumor-bearing mice, DSS-treated mice, and tumor-

bearing mice receiving DSS. Mice were euthanized when tumors reached the predefined humane endpoint (average tumor volume of approximately 1,500 $mm^3$), after which clinical, histological, and molecular parameters of colitis were evaluated (Fig. 1A). It should be noted that tumors were non-measurable at the time of DSS treatment initiation.

Tumor burden was first evaluated to determine whether DSS-induced colitis affected tumor growth. Tumor weights at the experimental endpoint were comparable between tumor-bearing mice with or without DSS treatment, indicating that colitis did not influence tumor progression (Fig. 1B). To assess the severity of colitis, Disease Activity Index (DAI) scores were monitored throughout the experiment. Tumor-bearing mice exposed to DSS developed significantly milder disease than mice receiving DSS alone, as reflected by significantly lower DAI scores (Fig. 1C).

To determine whether the improved clinical phenotype was accompanied by reduced intestinal inflammation, the colonic expression of the pro-inflammatory cytokines IL-1β, TNF-α, and IFN-γ was quantified by RT-qPCR. Expression of all three inflammatory markers was significantly reduced in tumor-bearing DSS-treated mice compared with mice receiving DSS alone, approaching the levels observed in naïve and tumor-bearing control mice (Fig. 1D-F). Consistently, colon sections stained with hematoxylin-eosin (H&E) and histopathologically evaluated showed that the colon of tumor-bearing mice exhibited reduced inflammatory cell staining, and the colon epithelial cells displayed high regenerative activity compared to their DSS-treated counterparts (Fig. 1G-H).

Collectively, these findings demonstrate that the presence of PyMT cancer cells markedly attenuates experimental colitis, leading to reduced clinical disease severity, suppression of intestinal inflammatory responses, and preservation of colonic tissue architecture.

Having suggested that pre-injection of cancer cells has a beneficial effect on experimental colitis, we next sought to examine whether the observed protective effect is mediated by circulating soluble factors. To address this question, serum was collected from either naïve or tumor-bearing donor mice and administered to recipient mice 5 days before inducing colitis using DSS. (Fig. 2A) Recipients treated with serum derived from tumor-bearing mice developed significantly milder disease than those receiving serum from naïve donors, as evidenced by a significant reduction in DAI scores following DSS administration (Fig. 2B). Histological examination of colonic tissue further supported these clinical findings. While mice receiving naïve serum exhibited the characteristic pathological features of DSS-induced colitis, including extensive inflammatory cell infiltration, epithelial destruction, crypt damage, and mucosal ulceration, administration of serum from tumor-bearing donors markedly preserved colonic architecture and reduced inflammatory infiltrates (Fig. 2C). Consistently, quantitative histopathological scoring confirmed a significant reduction in tissue injury in mice treated with tumor-derived serum compared with control serum-treated mice (Fig. 2D).

Collectively, these findings demonstrate that the protective effect induced by peripheral tumors is transferable through the circulation, strongly supporting the presence of tumor-derived soluble mediators that systemically suppress intestinal inflammation.

Next, we sought to identify candidate mediators responsible for this beneficial effect. Previous studies from our lab, investigating the systemic communication between tumors and the remodeled heart identified TNF-α and IFN-γ as prominent tumor-induced circulating cytokines that mediate, at least in part, the cardioprotective effects of peripheral tumors in murine models of Duchenne Muscular Dystrophy (DMD), transgenic mice model, and pressure overload-induced heart failure(10). Although these cytokines are classically recognized for their pro-inflammatory activities, our findings demonstrated that, in the context of tumor-induced systemic immune remodeling, they can exert unexpected systemic protective effects in distant tissues.

Based on these observations, we hypothesized that TNF-α and IFN-γ may similarly contribute to the attenuation of intestinal inflammation. To test this hypothesis, recombinant TNF-α and IFN-γ were administered intravenously (IV) five days before DSS exposure, followed by induction of experimental colitis as described previously (Fig. 3A). Administration of TNF-α and IFN-γ markedly attenuated disease severity throughout DSS-induced colitis, as reflected by a significantly lower DAI score compared with control mice (Fig. 3B (1 Cycle)). Histological examination further revealed substantial preservation of colonic architecture, characterized by reduced inflammatory cell infiltration, diminished epithelial damage, and improved crypt integrity in cytokine-treated mice compared with control mice (Fig. 3C (1 Cycle)). Consistently, quantitative histopathological analysis demonstrated a significant reduction in overall tissue injury following cytokine administration (Fig. 3D (1 Cycle)).

Together, these observations suggest that administration of TNF-α and IFN-γ is sufficient to reproduce the protective phenotype observed in both tumor-bearing mice and recipients of tumor-derived serum, supporting the hypothesis that these cytokines contribute, at least in part, to the tumor-induced systemic response that attenuates intestinal inflammation.

To determine whether the protective effects of TNF-α and IFN-γ are transient or persist beyond the initial inflammatory episode, we employed a recurrent DSS colitis model that more closely mimics the relapsing-remitting nature of human IBD. Mice received recombinant TNF-α and IFN-γ five days prior to the first DSS cycle. However, rather than being euthanized after the initial DSS exposure, mice were allowed to recover for 5 days on regular drinking water before undergoing a second cycle of DSS administration (Fig. 3A). Remarkably, mice pre-treated with TNF-α and IFN-γ remained significantly protected throughout both inflammatory episodes. Disease severity was consistently reduced compared with control mice, as shown by DAI scores (Fig. 3B (2 Cycles)). Consistently, histological analysis of colon tissues exhibited a reduced histopathological score in cytokine-treated mice as compared to control mice (Fig. 3C-D (2 Cycles)).

These results demonstrate that the protective effects of TNF-α and IFN-γ extend beyond the initial inflammatory insult, remaining evident during subsequent episodes of DSS-induced colitis.

Having established that administration of TNF-α and IFN-γ recapitulates the protective effect on tumor growth in DSS-induced colitis, we next sought to determine whether this response was associated with changes in the intestinal immune environment. Given the central role of macrophages in regulating

intestinal inflammation, tissue repair, and resolution(11), we examined the expression of macrophage-associated markers in colonic tissue following cytokine treatment. qRT-PCR analysis revealed increased expression of markers associated with anti-inflammatory and reparative macrophage phenotypes (IL-13, CD206) in the colons of TNF-α and IFN-γ-treated mice compared with control mice (Fig. 4). Consistently, lower levels of the pro-inflammatory macrophage marker (iNOS) expression were measured in cytokine-treated mice compared with control mice. Notably, expression of the macrophage marker F4/80 remained unchanged between the groups, suggesting that the observed changes reflect an alteration in the macrophage-associated phenotype rather than a change in overall macrophage abundance (Fig. 4A). This shift in macrophage-associated gene expression was consistent with the reduced disease severity observed following cytokine treatment, suggesting that exposure to these tumor-induced cytokines alters the local intestinal immune environment toward a phenotype associated with inflammatory resolution and tissue repair. TNF signaling is mediated through two receptors with distinct and context-dependent functions. While TNFR1 is predominantly associated with pro-inflammatory signaling, TNFR2 has been implicated in tissue repair, immune regulation, and restoration of intestinal epithelial homeostasis following injury (12, 13).

We therefore examined whether cytokine pre-treatment was associated with persistent alterations in the local inflammatory environment of the colon. Despite the initial systemic exposure to TNF-α and IFN-γ, colonic expression of both TNF-α and IFN-γ was significantly reduced in cytokine-pretreated mice compared with controls. Interestingly, this reduction in local inflammatory cytokine expression was accompanied by differential regulation of TNF receptors, with decreased TNFR1expression and increased TNFR2 expression (Fig. 4B). These findings indicate that exposure to TNF-α and IFN-γ is associated with a sustained remodeling of the local intestinal inflammatory response, characterized by reduced pro-inflammatory cytokine expression and a shift in the relative expression of TNF receptor pathways.

Together, these findings support a model in which transient systemic exposure to TNF-α and IFN-γ before the onset of colitis preconditions the immune system and promotes a reparative macrophage-associated response in the colon, characterized by increased expression of CD206 and IL-13. This altered immune state is associated with reduced intestinal inflammation, preservation of colonic architecture, and sustained protection during recurrent inflammatory challenge (Fig. 5).

# Discussion

In the present study, we identify an unexpected protective interaction between peripheral tumor growth and intestinal inflammation. Tumor-bearing mice developed substantially milder DSS-induced colitis, characterized by reduced clinical disease activity, decreased expression of inflammatory mediators, and attenuated histopathological damage. This protective phenotype was transferable through serum obtained from tumor-bearing mice, supporting the involvement of circulating mediators in tumor-induced protection. Building on our previous identification of TNF-α and IFN-γ as components of a tumor-induced systemic immune response(10), we further demonstrate that administration of these cytokines before

the induction of colitis was sufficient to recapitulate the protective phenotype. Importantly, the protection persisted during a second inflammatory challenge despite cytokine administration being restricted to the period preceding the first DSS exposure. Cytokine treatment was also associated with increased colonic expression of markers characterizing anti-inflammatory and reparative macrophage states. Together, these observations support a model in which early tumor-induced inflammatory signals modify the systemic and intestinal immune environment, establishing a protective state that alters the response to subsequent inflammatory injury.

Current therapeutic strategies for IBD are primarily directed toward controlling established inflammation, inducing remission, and preventing subsequent relapse. The therapeutic landscape has expanded considerably and now includes conventional anti-inflammatory and immunosuppressive agents as well as biologics and small molecules targeting TNF, integrins, IL-12/23, JAK signaling, and S1P receptors(14). Several of these agents are highly effective as maintenance therapies and reduce relapse once remission has been achieved. Nevertheless, these strategies generally depend on sustained or repeated therapeutic intervention and are fundamentally based on suppressing or interrupting pathways that contribute to ongoing disease. The approach revealed by our findings is conceptually different. TNF-α and IFN-γ were administered before intestinal injury, yet their beneficial effect remained detectable well beyond the treatment period and during a subsequent inflammatory challenge. Thus, rather than continuously suppressing an established inflammatory response, our results raise the possibility that immune conditioning can alter how the host responds to future inflammatory insults.

At first glance, the protective effects of TNF-α and IFN-γ appear paradoxical. Both cytokines are conventionally associated with inflammatory pathology(15, 16), and TNF represents one of the most successful therapeutic targets in IBD(17). However, cytokines are highly pleiotropic mediators whose biological consequences depend not only on their molecular identity but also on dose, timing, duration of exposure, receptor distribution, cellular target, and tissue context(18). Sustained or excessive cytokine signaling may promote chronic inflammation and tissue injury, whereas appropriately controlled exposure to the same signaling pathways may produce fundamentally different biological outcomes. Our observations therefore argue against a simple classification of cytokine as intrinsically “pro-inflammatory” or “anti-inflammatory” and instead emphasize the context in which cytokine signaling occurs. This principle is increasingly relevant to the development of cytokine-based therapies. Cytokines have a long history as therapeutic agents, but their clinical application has often been limited by pleiotropic activity, systemic toxicity, unfavorable pharmacokinetics, and activation of unwanted cellular populations. More recent approaches seek to exploit, rather than simply inhibit, cytokine biology by controlling dose, receptor selectivity, tissue distribution, and signaling duration(18). IL-2 provides a particularly instructive example. Whereas high-dose IL-2 promotes potent effector immune responses and was developed as an anticancer immunotherapy(19), low-dose IL-2 preferentially supports regulatory T-cell expansion and function, and has demonstrated immunoregulatory activity in autoimmune and inflammatory disorders(20). Moreover, researchers are developing engineered cytokines and receptor-biased variants to preferentially engage selected immune populations while limiting undesirable systemic activity. These developments illustrate a broader shift in cytokine

therapeutics: the biological effect of a cytokine can potentially be redirected by controlling how, where, and for how long its signal is delivered.

Our findings extend this concept to TNF-α and IFN-γ. Importantly, we do not propose that systemic administration of either cytokine should constitute a treatment for IBD. Instead, our results suggest that brief, defined exposure to these cytokines can initiate downstream processes that differ from those associated with chronic cytokine activity. The therapeutically relevant question may therefore not be whether to administer TNF-α and IFN-γ, but which cellular and molecular programs the exposure initiates, and whether those programs can be reproduced selectively without the adverse consequences of systemic inflammatory cytokine signaling.

The persistence of protection following cytokine administration is particularly intriguing in this context. Mice received TNF-α and IFN-γ only before the initial DSS challenge yet remained protected during a second cycle of intestinal inflammation following a recovery period. This raises the possibility that the initial inflammatory stimulus establishes a more durable alteration in immune responsiveness. Conceptually, this phenomenon resembles biological preconditioning, in which limited exposure to an initial stimulus alters the response to a subsequent challenge. Related principles are observed in phenomena such as hormesis(21), endotoxin tolerance(22), ischemic preconditioning(23), and trained innate immunity(24).

The differential regulation of TNF receptors observed in the colon may provide additional insight into the paradoxical protective effects of TNF-α pre-exposure. TNF signaling is highly dependent on receptor context, with TNFR1 primarily associated with pro-inflammatory responses, whereas TNFR2 has been implicated in immune regulation, tissue repair, and restoration of intestinal homeostasis (12, 13). Notably, cytokine-pretreated mice exhibited reduced colonic expression of TNFR1 and increased TNFR2 expression, accompanied by decreased expression of TNF-α and IFN-γ. These findings suggest that transient cytokine exposure may reshape the intestinal inflammatory environment, potentially favoring regulatory and tissue-repair processes during subsequent inflammatory challenges. However, whether altered TNF receptor signaling directly contributes to the observed protection remains to be determined.

The macrophage-associated changes observed following cytokine administration provide a potential cellular basis for this sustained response. Intestinal macrophages are highly plastic and play central roles not only in inflammatory amplification but also in resolution, epithelial support, clearance of cellular debris, and tissue repair(25). TNF-α and IFN-γ treatment increased the colonic expression of markers associated with anti-inflammatory and reparative macrophage states, coinciding with reduced clinical and histopathological disease. These findings are notable considering our previous observations in cardiac disease, where tumor-induced cytokine signaling promoted a reparative macrophage phenotype that contributed to improved cardiac function(10). The emergence of a similar macrophage-associated signature in the intestine raises the possibility that macrophage remodeling represents a conserved downstream component of tumor-induced systemic immune modulation across distinct organs.

The timing of the tumor-induced response provides another important mechanistic clue. DSS exposure began only five days after implantation of PyMT cells, before a solid tumor mass had developed. Thus, protection does not appear to require the physiological consequences of an advanced tumor burden. Instead, the timing is consistent with an early host immune response to malignant cells. In our previous work, early recognition of implanted tumor cells initiated an NK-cell-dependent systemic response involving TNF-α and IFN-γ, ultimately promoting macrophage remodeling and protection in models of cardiac disease(10). Altogether, these findings raise the possibility that an NK cell-cytokine-macrophage axis represents a broader systemic program through which early host recognition of malignancy can influence inflammatory responses in distant organs.

Interestingly, the interaction between IBD and cancer in this study appeared to be asymmetric. Whereas tumor growth markedly altered the course of DSS-induced colitis, DSS treatment did not significantly affect tumor burden at the experiment timepoint (Fig. 1B). This finding. Is noteworthy because the relationship between intestinal inflammation and cancer has traditionally been investigated predominantly in the opposite direction, namely in the context of chronic inflammation promoting colitis-associated colorectal carcinogenesis(6). Here, acute intestinal inflammation did not alter the growth of a peripheral mammary tumor, while the tumor substantially modified inflammatory disease in a distant organ. This distinction emphasized that tumor-host communication can operate across organ systems without necessarily producing reciprocal effects over the same temporal window. Nevertheless, it will be important to determine whether prolonged or recurrent intestinal inflammation influences tumor progression over longer periods in different tumor models.

The translational implications of these findings extend beyond TNF-α and IFN-γ themselves. Our results suggest an additional dimension worth considering: tissue pre-conditioning. A short cytokine exposure may potentially initiate a beneficial immune program whose duration considerably exceeds the period of cytokine signaling itself. If researchers can identify the downstream pathways responsible for this durable response, they may be able to reproduce the protective state without administering the upstream inflammatory cytokines. Such a strategy would shift from continuous suppression of inflammation to controlled induction of endogenous mechanisms that enhance resilience to future inflammatory injury.

Several limitations should be acknowledged. DSS-induced colitis reproduces important aspects of epithelial injury and intestinal inflammation but does not encompass the full immunological complexity of human IBD. Although TNF-α and IFN-γ reproduce the protective phenotype, loss-of-function studies are required to establish whether they are necessary for tumor-mediated intestinal protection. Similarly, the macrophage-associated signature observed after cytokine treatment does not establish a causal role for macrophages. Our previous cardiac studies and the parallels observed here support the proposed involvement of an NK cell-cytokine-macrophage axis, but this remains to be directly demonstrated in the intestinal setting. Finally, the molecular basis and duration of the protective state are currently unknown. Determining whether it involves transcriptional or epigenetic reprogramming, altered hematopoiesis, changes in tissue-resident immune populations, or other mechanisms will be critical for understanding its biological and therapeutic significance.

In conclusion, our findings reveal an unexpected form of systemic communication between cancer and intestinal inflammation in which an early tumor-induced immune response protects against subsequent inflammatory injury. Transient exposure to TNF-α and IFN-γ reproduces this effect and provides protection that persists through recurrent inflammatory challenge, accompanied by a shift toward an anti-inflammatory and reparative macrophage-associated signature in the colon. These observations challenge a binary view of inflammatory cytokines as exclusively pathogenic and instead highlight dose, timing, duration, and cellular context as critical determinants of their biological effects. More broadly, they suggest that controlled inflammatory signaling can, under defined conditions, establish a durable protective immune state. Deciphering the mechanisms underlying this response may ultimately reveal strategies for harnessing endogenous immune adaptation to prevent recurrent tissue injury in chronic inflammatory disease.

## Materials and Methods

# Mice

All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the Technion-Israel Institute of Technology, Faculty of Medicine (Approval No. IL1020824), and were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. The number and gender of animals used in each experiment is indicated in the corresponding figure legends.

Age-matched female and male C57B/6 (8–12 weeks old, 20–25 g) were bred and maintained at the Pre-Clinical Research Authority of the Ruth and Bruce Rappaport Faculty of Medicine. All surgical procedures were performed under isoflurane anesthesia. Mice were anesthetized with isoflurane and euthanized by cervical dislocation before tissue harvesting.

## Cancer cell injection

The polyoma middle T (PyMT) murine breast carcinoma cell line was derived from the primary tumor-bearing transgenic mice expressing PyMT under the control of the murine mammary tumor virus promoter. These cells were kindly provided by Prof. Tsonwin Hai (Ohio State University, Columbus, OH, USA). Cells were tested and found to be free of mycoplasma contamination. Cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), 1% streptomycin and penicillin at 37°C, in a humidified atmosphere containing 5% $CO_2$.

PyMT cancer cells ($10^6$) were implanted into the mammary fat pad of female mice or subcutaneously into the flanks and orthotopically injected into male mice, respectively. Tumor volume was monitored over time with a caliper and calculated using the formula: $\text{width}^2 \times 0.5 \times \text{length}$. According to the

Institutional Animal Care and Use Committee, the humane endpoint is defined when the tumor size reaches 1500 $mm^3$.

## Blood serum extraction

Blood was obtained from the facial vein with a 4-μm sterile Goldenrod Animal Lancet (MEDIpoint, Inc., Mineola, NY, USA). Blood was collected and allowed to clot at room temperature for 2 h, followed by 15 min of centrifugation at 2000× *g*. Serum was immediately aliquoted and stored at

−20°C for future use.

# DSS-induced Colitis

Experimental colitis was induced using dextran sodium sulfate (DSS). A well-established murine model of IBD that recapitulates key clinical and histopathological features of UC (26). Briefly, Mice received 2% DSS in their drinking water for 9 consecutive days, followed by regular drinking water as indicated in the experimental design. The 2% DSS concentration was selected to induce moderate colitis, allowing the evaluation of disease-modifying effects while minimizing excessive morbidity.

Disease progression was monitored throughout the experiment by assessing body weight, stool consistency, and the presence of fecal blood. At the experimental endpoint, mice were euthanized, and colons were collected for histopathological and molecular analysis.

## Recombinant cytokine administration

Recombinant murine TNF-α (Thermo Fisher Scientific, catalog no. 315-01A), and IFN-γ (Thermo Fisher Scientific, catalog no. 315-05) were diluted in Saline. A total volume of 100 μL was administered to each mouse by intravenous tail-vein injection. For the two-factor treatment, mice received recombinant TNFα and IFNγ at final concentrations of 50 ng/mL each, corresponding to 5 ng of each cytokine per mouse. Control mice received an equivalent volume of vehicle. Cardiac function was evaluated before treatment and at the indicated time points after injection.

## Hematoxylin and Eosin (H&E) stain

Colons were fixed in 4% formaldehyde and left overnight before being embedded in paraffin, serially sliced at 4-μm intervals, and mounted on slides. H&E staining was performed according to the standard protocol. Images were acquired using a 3DHistech Panoramic 250 Flash III (3DHISTECH Ltd., Budapest, Hungary). Each colon section was fully scanned.

Quantification of tissue damage was performed using a histopathological scoring system that included inflammatory cell infiltrates and structural changes in the colon, such as ulcerations and crypt damage

as described before(27).

# RNA extraction

Total RNA was extracted from colons using an Aurum total RNA fatty or fibrous tissue kit (No. 732–6830, Bio-Rad, Hercules, CA), according to the manufacturer's instructions. Next, cDNA was synthesized from 1000 ng of purified mRNA using the iScript cDNA synthesis kit (No. 170–8891, Bio-Rad).

# qPCR

Real-time polymerase chain reaction was performed with QuantStudio3 (Thermofisher Scientific, 5823 Newton Drive, Carlsbad, CA, 92008, the United States). Serial dilutions were included for each gene to generate a standard curve. Values were normalized to housekeeping gene GAPDH expression levels. All Oligonucleotides sequences are listed in supplemental table S1.

# Statistics

Data are presented as mean ± SEM. All mice were included in each statistical analysis. Animals were randomly selected for each experimental group. Statistical significance was determined by comparing the two means using a Student's t-test. Statistical analysis was performed with GraphPad Prism 8 software (La Jolla, CA). Values of $P < 0.05$ were accepted as significant.

## Declarations

# Supplemental table S1

Oligonucleotides sequence

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# Author Contribution

L.A. conceptualized the study, acquired data, analyzed and interpreted data, and wrote and edited manuscripts. I.L. Data analysis and interpretation. A.A. Study conceptulization, data analysis and interpretation; writing, editorial revisions, and acquired funding.

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# Data Availability

The corresponding author will make the data, analytic methods, and study materials available upon request.

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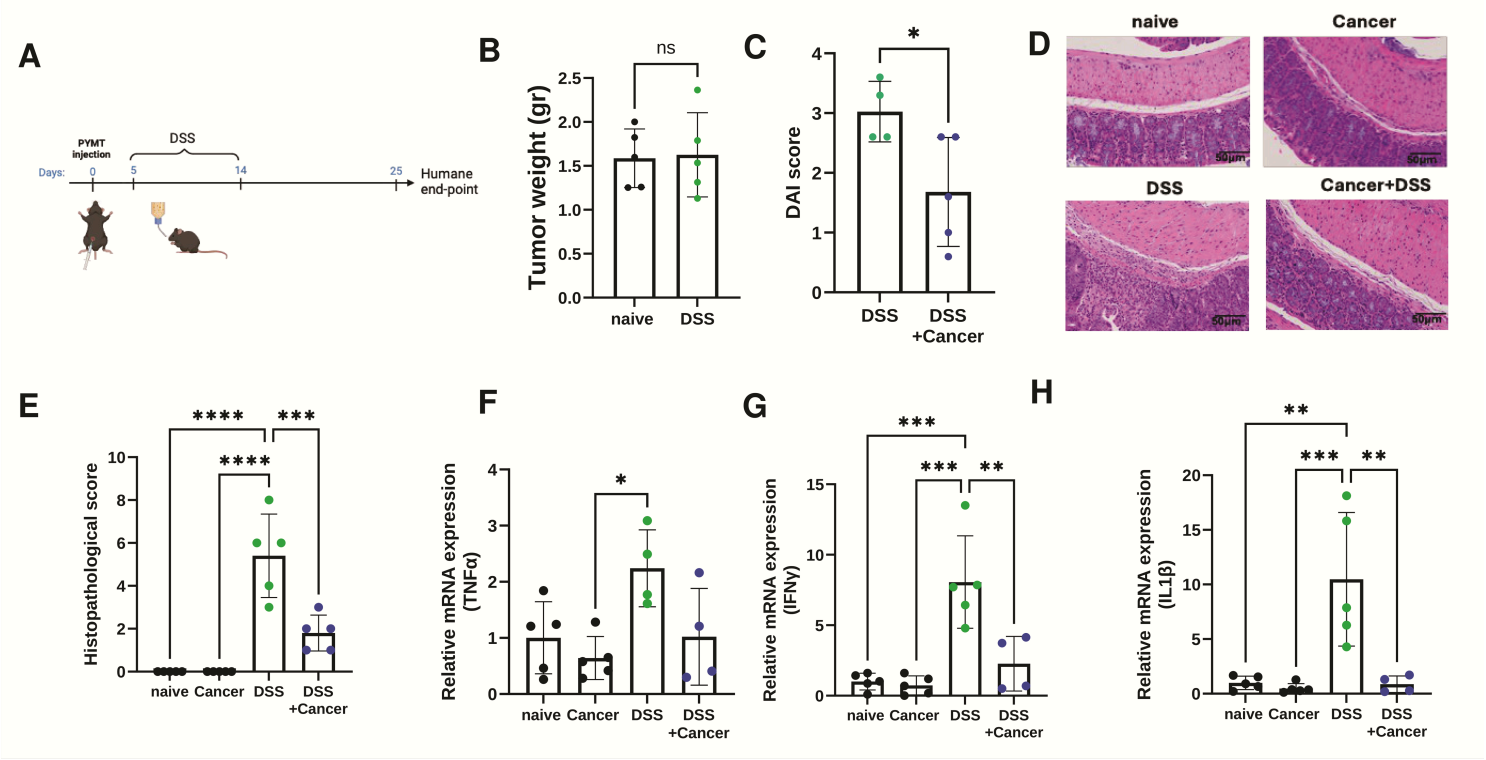
# Figures




## Figure 1

**Tumor Growth decreases colon inflammation severity following DSS treatment. A.** Schematic timeline. Mice were divided into four groups: naïve, Cancer, DSS, and DSS+Cancer. On day 0, two groups (Cancer and DSS+Cancer) received PYMT cancer cells. 5 days after, the DSS and DSS+Cancer groups received DSS in their drinking water for 9 days, then replaced with regular water. All mice were sacrificed on day 29 for further analysis. **B.** Tumor weight of naïve and cancer groups at endpoint. **C.** DAI score of DSS-receiving groups. **D.**Representative colon sections of four groups stained with H&E stain. Scale Bar = 50mm. **E.** Quantification of H&E staining. Colon mRNA levels of inflammation hallmark gene markers TNFα (F), IFNγ (E), and IL1b (H) were determined using qRTPCR, and normalized using GAPDH housekeeping gene. Data are presented as mean ± SD. Student's t-test (A-C) or One-way ANOVA followed by Tukey posttest (E-H). ns=non-significant. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

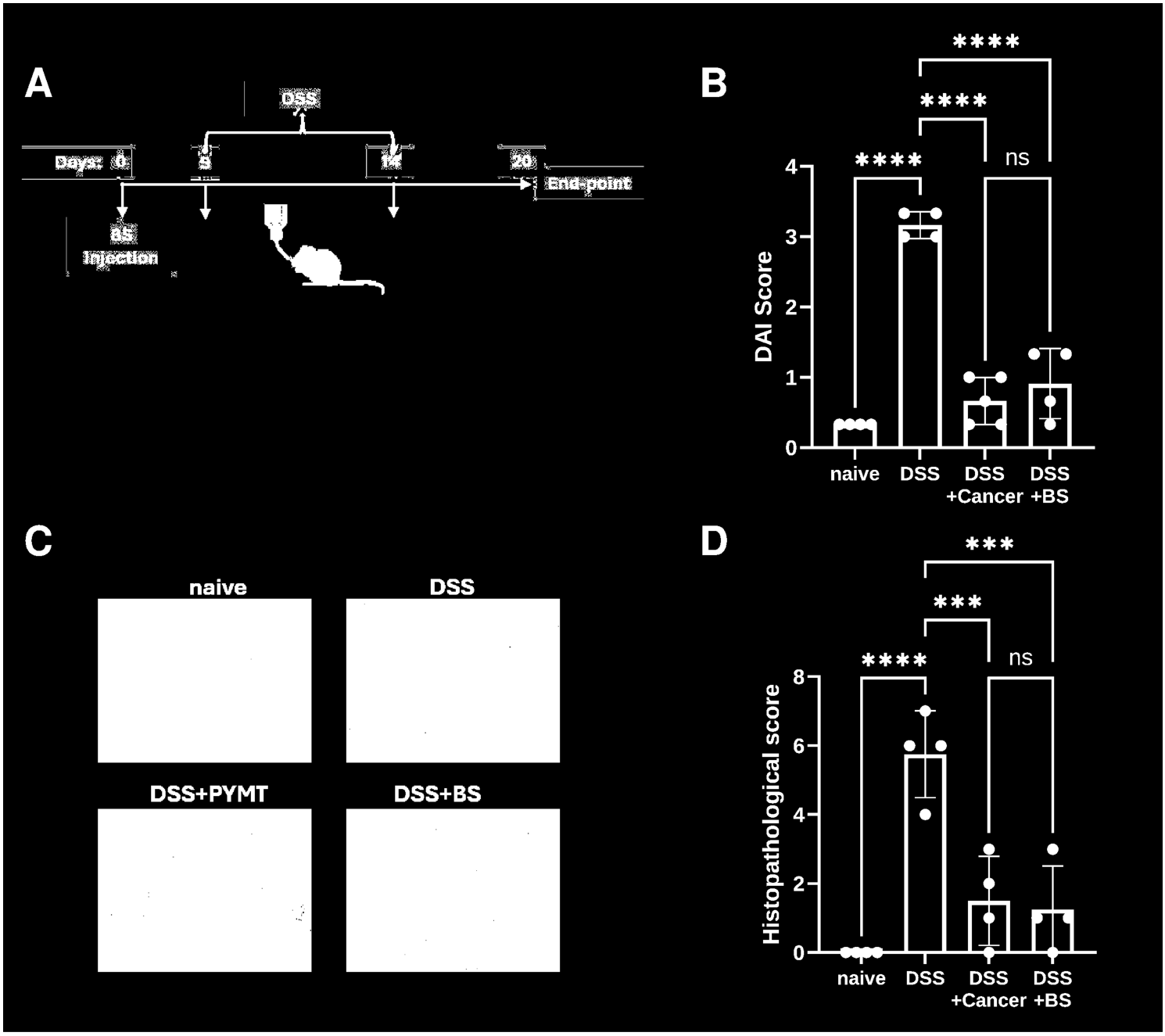



**Figure 2**

**Blood serum (BS) derived from tumor-bearing mice dampens colon inflammation following DSS treatment. A.** Schematic timeline. Mice were divided into four groups: naïve, DSS, DSS+cancer, and DSS+BS. On day 0, the DSS+cancer group received cancer cells; DSS+BS were IV injected with BS derived from tumor-bearing mice. 5 days later, the DSS and DSS+Cancer groups received DSS in their drinking water for 9 days, then switched to regular water. All mice were sacrificed on day 20 for further analysis. **B.** DAI score of all experimental groups. **C.** Representative colon sections of four groups stained with H&E stain. Scale Bar = 50mm. **E.**Quantification of H&E staining. Data are presented as mean ± SD. Data are presented as mean ± SD. One-way ANOVA followed by Tukey posttest. ns=non-significant, ***P<0.001, ****P<0.0001.

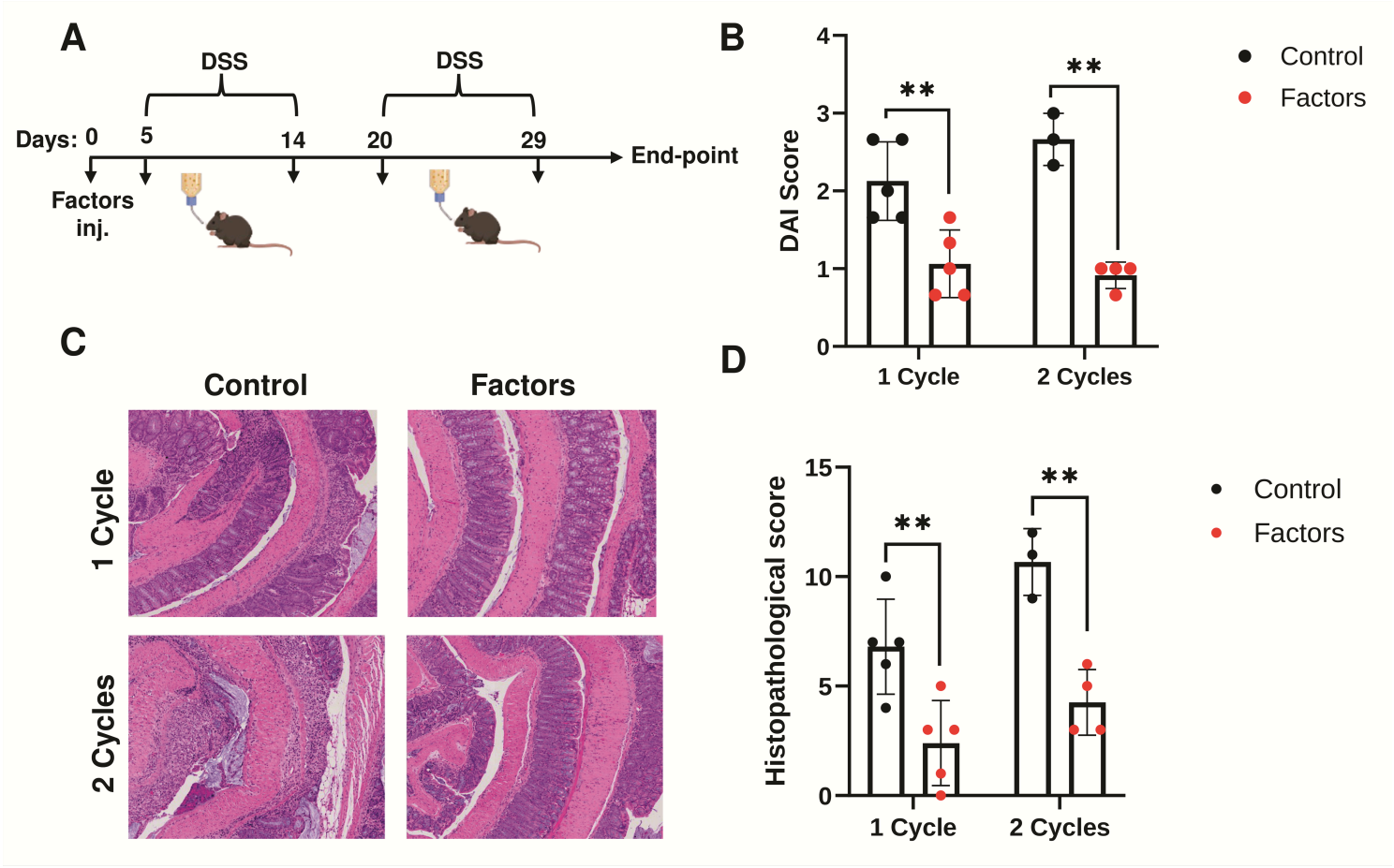



Figure 3

**IV injection of purified recombinant TNF-a and IFN-g decreases colon inflammation following DSS treatment.**

**A.** Schematic timeline. Mice received DSS in either 1 cycle or 2 cycles. In each cycle 2 groups of mice participated in the experiment: Control or factor-injected mice. **B.** DAI score of all experimental groups. **C.** Representative colon sections of four groups stained with H&E stain. Scale Bar = 50mm. **E.**Quantification of H&E staining. Data are presented as mean ± SD. Data are presented as mean ± SD. Data are presented as mean ± SD. Student's t-test. **P<0.01.

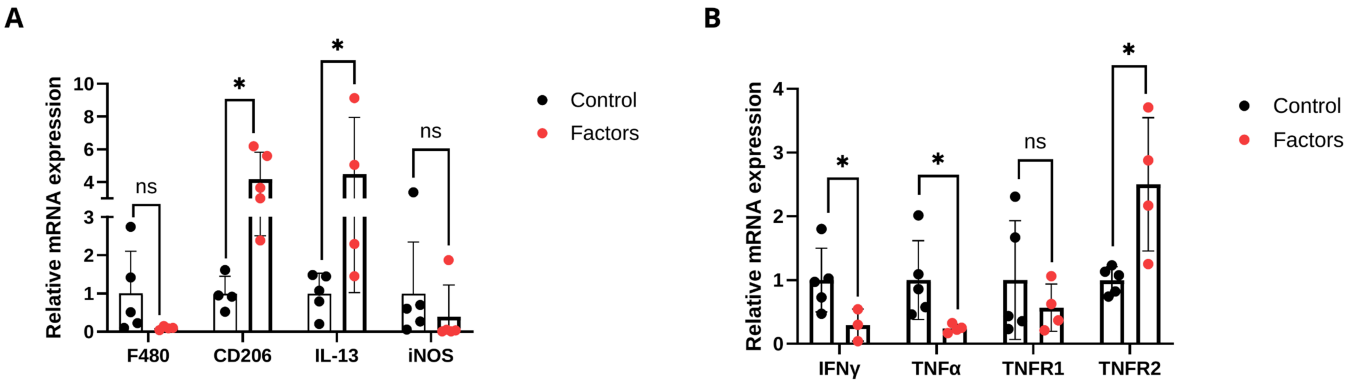



**Figure 4**

**Figure 4**

**Factors' injection induces a macrophage switch toward an anti-inflammatory state. A.** Colon mRNA levels of macrophage hallmark gene markers CD206, IL-13, and iNOS were determined by qRTPCR, and normalized using GAPDH housekeeping gene. Data are presented as mean ± SD. Student's t-test. ns=non-significant. *P<0.05, **P<0.01.

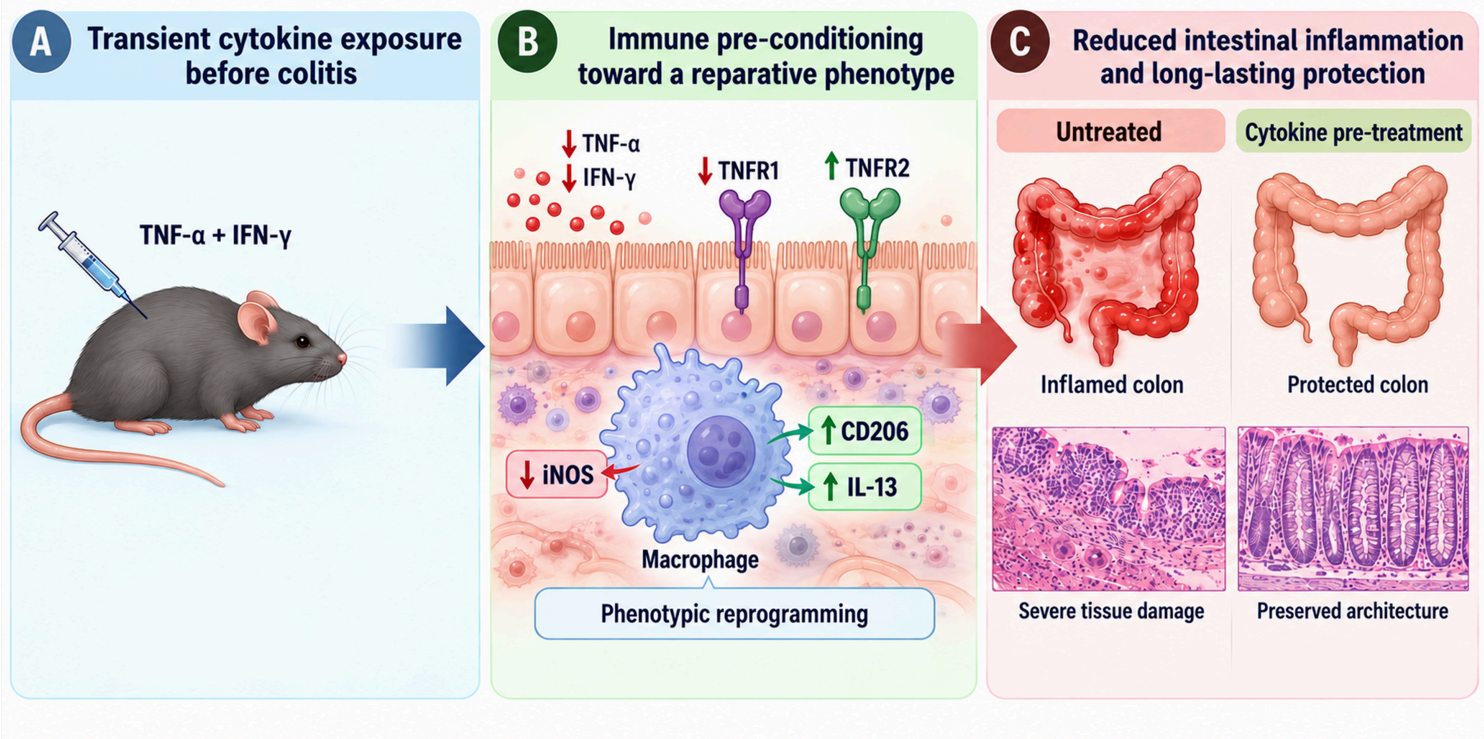



**Figure 5**

Proposed model of cytokine-mediated protection against intestinal inflammation. (A) Transient systemic exposure to TNF-α and IFN-γ before the induction of DSS colitis preconditions the immune response. (B) Cytokine pretreatment promotes a reparative macrophage-associated phenotype in the colon, characterized by increased expression of CD206 and IL-13. (C) This immune preconditioning attenuates intestinal inflammation, preserves colonic architecture, and provides sustained protection during recurrent inflammatory challenges.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalTable1.docx