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Role of tumor necrosis factor- α in the extra-intestinal thrombosis associated with colonic inflammation

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Abstract

Background—Inflammatory bowel diseases (IBD) are associated with a hypercoagulable state and an increased risk of thromboembolism, with accelerated thrombus formation occurring both within the inflamed bowel and in distant tissues. While the IBD-associated prothrombogenic state has been linked to the inflammatory response, the mediators that link inflammation and thrombosis remain poorly defined. The objective of this study was to assess the role of TNF- α in the enhanced extra-intestinal microvascular thrombosis that accompanies colonic inflammation.

Methods—TNF- α concentration was measured in plasma, colon and skeletal muscle of control mice and in mice with dextran sodium sulfate (DSS)-induced colitis. A light/dye injury method was used to induce microvascular thrombosis in cremaster microvessels. The effects of exogenous TNF- α on thrombus formation were determined in control mice. DSS-enhanced thrombus formation was evaluated in wild type (WT) mice treated with an anti-TNF- α antibody ($\pm$ an anti-IL-1 β antibody) and in TNF- α receptor deficient (TNFr^{-/-}) mice.

Results—DSS colitis enhanced thrombus formation in cremaster arterioles. A similar response was produced by TNF- α administration in control mice. TNF- α concentration was elevated in plasma, colon and skeletal muscle. Immunoblockade of TNF- α or genetic deficiency of the TNF- α receptor blunted the thrombotic response of arterioles to DSS colitis. Additional protection was noted in mice receiving antibodies to both TNF- α and IL-1 β .

Conclusions—Our findings implicate TNF- α in the enhanced microvascular thrombosis that occurs in extra-intestinal tissue during colonic inflammation, and suggests that the combined actions of TNF- α and IL-1 β accounts for most of the colitis-enhanced thrombotic response.

Keywords

thrombus formation; interleukin-1 beta; microcirculation; inflammation

Introduction

There is growing evidence that inflammation and coagulation are interdependent processes that are linked in a manner that enables each process to activate and propagate the other (1–

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3). For example, inflammation can lead to an imbalance between pro- and anti-coagulant mechanisms that favor coagulation. The induction of this procoagulant state likely involves endothelial cells, leukocytes and platelets, which are activated in response to the inflammatory stimulus. The anticoagulant role of endothelial cells is diminished during inflammation and results from an increased expression of tissue factor (the initiator of coagulation), down-regulation of the anticoagulant protein C pathway, and inactivation of nitric oxide by superoxide. Activated leukocytes also exhibit an increased tissue factor expression and can release proteases that degrade antithrombin as well as cleave and inactivate thrombomodulin on endothelial cells. Similarly, the activation and binding of platelets to endothelial cells, leukocytes and to other platelets in the microvasculature of inflamed tissue also promotes a procoagulant state, via an enhanced expression of tissue factor, the generation/activation of coagulation factors (eg, factor Xa) and enhanced thrombin production. (1–4)

Inflammation-induced coagulation and thrombosis has been implicated in a variety of diseases, including atherosclerosis, sepsis and inflammatory bowel diseases (IBD). For example, patients with IBD exhibit a hypercoagulable state and are at increased risk for thromboembolism (TE).(2,3,5,6) The IBD-induced thrombosis occurs in both the arterial and venous circulations, and is usually manifested as deep vein thrombosis (skeletal muscle) or pulmonary embolism, although thromboses have been detected in brain, retina, and liver. (2) Animal models of IBD also exhibit a procoagulant, prothrombotic phenotype that includes defects in the protein C and antithrombin III anticoagulant systems.(7,8) Enhanced thrombus formation in vascular beds (e.g., skeletal muscle) distant from the inflamed bowel is also evident in animal models of IBD.(8–10) While several parallels have been described between the procoagulant/prothrombotic phenotype that accompanies gut inflammation in IBD patients and animal models, the identity of the chemical and/or cellular mediator(s) produced/released from inflamed tissue that induces this phenotype remains largely unknown.

Pro-inflammatory cytokines are considered to be an important link between the inflammation and hypercoagulable, prothrombotic state observed in some pathological conditions, such as sepsis.(4) A role for cytokines in mediating inflammation-induced coagulation/thrombosis is supported by reports describing the ability of cytokines such as IL-1 β , TNF- α , and IL-6 to enhance the expression of tissue factor, downregulate thrombomodulin, reduce the density of endothelial protein C receptors, and inhibit fibrinolysis on endothelial cells.(4,7,11,12) Recent work from our laboratory has implicated IL-1 β in the enhanced extra-intestinal thrombosis that accompanies experimental colitis.(13) We noted that exogenous IL-1 β dose-dependently enhanced thrombosis in arterioles of control mice and that the enhanced thrombus formation in cremaster arterioles of WT mice with DSS-induced colitis was significantly attenuated in WT colitic mice treated with an IL-1 β blocking Ab and in colitic IL-1 receptor deficient mice. The partial (~50%) attenuation of thrombus formation observed with IL-1 β directed interventions suggests that, while the cytokine significantly contributes to the colitis-enhanced thrombotic response, other chemical and/or cellular mediators are also involved.

The major goal of this study was to determine whether TNF- α contributes to the enhanced extra-intestinal thrombosis in experimental colitis. To achieve this objective, the following experiments were performed: 1) measure TNF- α concentration in colon, skeletal muscle and plasma of control and DSS-colitic mice, 2) determine whether different concentrations of exogenous TNF- α alter the thrombotic responses of cremaster muscle arterioles in control WT mice to light/dye-induced endothelial injury, 3) examine whether mice lacking the gene for the TNF- α receptor (TNF α R $^{-/-}$) exhibit an altered thrombosis response to DSS colitis, and 4) evaluate the effects of TNF- α immunoblockade on DSS-enhanced thrombus formation. In

additional experiments, the combined effects of TNF- α and IL-1 β blocking antibodies on thrombus formation in DSS colitic mice were studied. Our findings support a role for TNF- α in mediating the enhanced extra-intestinal thrombosis that is associated with experimental IBD and suggest that this response is largely the result of the combined prothrombotic actions of TNF- α and IL-1 β .

Materials & Methods

Animals

A total of 72 male C57BL/6J mice and a total of 9 male TNF receptor deficient (TNF α ^{-/-}) mice were purchased from Jackson Laboratory for this study. All mice were housed under specific pathogen-free (SPF) conditions in standard cages and fed standard laboratory chow and water until the desired age (6–8 week). All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Louisiana State University Health Sciences Center and were performed according to the criteria outlined by the National Institutes of Health.

Experimental colitis model

Colitis was induced by an intake of 3 % (wt/vol) dextran sodium sulfate (DSS) (40,000 MW) (MP Biomedicals, Solon, OH) dissolved in filtered drinking water (14). The first day of DSS feeding was defined as day-0 and the mice were maintained on the DSS until day-6. Control mice received filtered water alone.

Assessment of colitis progression

Body weights, fecal status, presence of occult blood in the stools and peri-anal bleeding were observed and recorded every day while the mice received DSS. Occult blood was detected using guaiac paper (ColoScreen®; Helena Laboratories) (14). Disease activity index (DAI), a measure of disease severity ranging between 0 and 4, was calculated from data collected on stool consistency, presence or absence of fecal blood, and weight loss, as previously described (14). The DAI was measured daily to confirm that DSS treatment resulted in clinical responses that are consistent with colitic disease activity.

Cytokine (TNF- α) concentration

TNF- α levels in plasma, colon and skeletal muscle (quadriceps) were measured using a cytometric bead array (CBA). To obtain the plasma samples, the right carotid artery was cannulated with a PE 10 tube (BD, Franklin Lakes, NJ). A blood sample was withdrawn and collected in an Eppendorf tube, which was then centrifuged at 5,000 rpm $\times$ 10 min to separate the plasma. Tissue samples were promptly mixed with phosphate-buffered saline (PBS) containing a protease inhibitor (Sigma Chemicals, St. Louis, MO) and thoroughly homogenized. The homogenate was centrifuged at 10,000 rpm $\times$ 5 min to separate the supernatant. TNF- α concentrations in the supernatants and plasma samples were measured with the CBA as per the manufacturer's instruction (BD Biosciences, San Jose, CA). The detection limit of the CBA for mouse TNF- α is 10 pg/ml.

Relative mRNA expression of TNF- α

The relative mRNA expression of TNF- α in skeletal muscle (quadriceps) was performed, as previously described (15), using predeveloped assays for real-time PCR according to the manufacturer's instructions (Applied Biosystems, Foster City, CA).

Surgical preparation for intravital microscopy

On day-6 of DSS (colitis) or water (control) treatment, mice were anesthetized using 50 mg/kg body wt (i.p.) pentobarbital, with supplemental doses of 12.5 mg/kg, given as needed. The right internal jugular vein was cannulated for intravenous administration of FITC-dextran and the right carotid artery was cannulated for measurement of systemic blood pressure. Body temperature was maintained at 36.5 – 37.5 °C during the entire experiment with a homeothermic blanket and monitored with a rectal temperature probe. An incision was made in the scrotal skin to expose the left cremaster muscle. A lengthwise incision was made on the surface of the cremaster muscle. The testicle and epididymis were separated from the muscle. The muscle was spread out on the pedestal and the edges of the muscle were moderately extended with sutured threads (16). The surface of the exposed cremaster muscle was suffused continuously with bicarbonate-buffered saline (BBS), with a pH 7.35 – 7.45.

Microscopic observation

The cremaster microcirculation was observed using an upright microscope (BX51WI, Olympus, Japan) with a 40X water immersion objective lens (LUMPlanFI/IR 40x/0.80 w, Japan). The light and fluorescent microscopic images were projected onto a monitor (Sony TRINITRON PVM-2030, Japan) through a color video camera (Hitachi VK-C150, Japan) or a charge-coupled device (CCD) video camera (Hamamatsu XC-77, Japan), respectively. The images were recorded using a DVD recorder (JVC SR-MV50, NJ). A video timer (Panasonic Time-Date Generator WJ-810, Japan) was connected to the monitor to record time and date. The diameters of the cremaster vessels were measured by video analysis software (ImageJ 1.37v, NIH, Public Domain software) on a personal computer (G4 Macintosh, Apple, CA). Red blood cell velocity (V_{RBC}) in the microvessels was measured using an optical Doppler velocimeter (Microcirculation Research Institute, Texas A & M University, College Station, TX). Blood flow was calculated from the product of mean red blood cell velocity [$V_{mean} = V_{RBC} / 1.6$] and cross-sectional area, assuming cylindrical geometry (17). Wall shear rate (WSR) was calculated based on the Newtonian definition: $WSR = 8 (V_{mean} / D_v)$.

Light/dye-induced thrombosis

Second or third-order venules and arterioles (1–3 per mouse), meeting the characteristics of a diameter of 35–50 μ m, at least 100 μ m in length, and a wall shear rate ≥ 500 /s, were randomly selected in each cremaster muscle to study thrombus formation. Then, 10 ml/kg of 5 % FITC-dextran (150,000 MW) (Sigma chemicals, St. Louis, MO) was slowly injected into the venous cannula and allowed to circulate for 10 min. Photoactivation of FITC-dextran (excitation: 495 nm, emission: 519 nm) within the microvessels was achieved by epi-illumination using a 175-W xenon lamp (Lambda LS, Sutter, CA) and a fluorescein filter cube (HQ-FITC, Chroma Technology Company, VT). The excitation power density was measured daily (ILT 1700 Radiometer, SED033 detector, International Light, MA) and maintained within 1 % of 0.74 W/cm², as previously described(18,19). Epi-illumination was continuously applied to the vessels, and thrombus formation was quantified by determining: 1) the time of onset of platelet deposition/aggregation within the microvessel (onset time), and 2) the time required for complete flow cessation for ≥ 60 sec (cessation time). Epi-illumination was discontinued once blood flow ceased in the vessel under study. The results of each vessel type (venules, arterioles) were averaged from 2–4 thrombi produced in each mouse.

Experimental protocols

In total, 47 control mice (23.9 ± 0.3 g body weight) and 9 TNFr^{-/-} mice (24.9 ± 0.4 g body weight) were used to study light/dye-induced thrombus formation. The mice were evaluated in the following experimental groups: 1) WT mice receiving an intrascrotal injection of recombinant mouse TNF- α (Calbiochem, La Jolla, CA) at a concentration of either 0.1, 0.5 or 2.5 μ g/mouse dissolved in 0.2 ml of normal saline, 2) WT mice receiving a combination of mouse TNF- α (0.5 μ g/mouse) and recombinant mouse IL-1 β (1.0 μ g/kg; Calbiochem, La Jolla, CA), administered 4 prior to photoactivation 3) water-treated WT mice, 4) DSS-treated WT mice, 5) TNFr^{-/-} mice treated with DSS, 6) DSS-treated WT mice receiving (i.p.) 100 μ g/mouse of an anti-mouse TNF- α monoclonal antibody (TN3-19.12; Santa Cruz Biotechnology, Santa Cruz, CA), and 7) DSS-treated WT mice receiving (24 hr prior to photoactivation) a combination of blocking antibodies (100 μ g/mouse each, dissolved 0.2 ml of normal saline) directed against TNF- α (TN3-19.12) and IL-1 β (R&D System, Minneapolis, MN). (13). In a separate group of control (n=5) and colitic (n=5) mice, we quantified the TNF- α concentration of colon, skeletal muscle, and plasma. Moreover, the expression of TNF- α mRNA in skeletal muscle was compared between water (n=5) and DSS (n=5) fed mice.

Statistics

Data were analyzed using standard statistical analysis, i.e., one-way ANOVA and Fisher's post hoc test. Statistical significance was set at $p < 0.05$. All values are reported as means $\pm$ SE from 5 – 9 mice.

Results

All mice receiving 3% DSS in drinking water exhibited a significant increase in disease activity index (DAI) score, without mortality. No statistical differences in the DAI score measured on day-6 of DSS-treatment were noted between the different experimental groups. Exposure of the cremaster muscle to epi-illumination for 30 min, in the absence of FITC-dextran injection, did not elicit any signs of platelet aggregation or thrombus formation in either venules or arterioles; nor did it significantly alter the values for red blood cell velocity or shear rate in the microvessels.

Figure 1 shows the changes in time of onset of thrombosis and the time to flow cessation induced by light/dye injury in cremaster muscle arterioles and venules of WT control and DSS colitic mice. As previously reported (8,9,19), the time required for the onset of thrombosis (5.48 ± 0.51 vs 0.36 ± 0.07 min) and the time to complete flow cessation (30.32 ± 1.79 vs 3.98 ± 0.49 min) is much longer in arterioles than in venules. While no differences were noted in either the time of onset or the time to flow cessation between venules of control and colitic mice, both variables were significantly reduced (consistent with accelerated thrombosis) in cremaster arterioles of colitic mice compared to their control counterparts. Since the enhanced extra-intestinal thrombosis induced by DSS was not observed in venules, all subsequent experiments focused on the thrombosis responses to light/dye injury in arterioles.

Figure 2 summarizes the TNF- α concentrations detected in colon, skeletal muscle and plasma of control and DSS colitic mice. The inflamed colon exhibited a large increase in TNF- α concentration, compared to normal colon (5.9 ± 2.6 vs 369.1 ± 99.3 pg/ml). While plasma TNF- α in control WT was under the detectable level for the assay, plasma TNF- α in DSS colitic WT tended to be significantly elevated (10.2 ± 0.9 pg/ml). TNF- α concentration measured in skeletal muscle of control mice was 9.3 ± 1.6 pg/ml, but a significantly elevated concentration was detected in skeletal muscle tissue of DSS colitic mice (85.8 ± 17.5 pg/g).

This increase in muscle TNF was noted despite a lack of change in TNF- α mRNA expression in muscle tissue of DSS colitic mice, compared to their control (water) counterparts.

Figure 3 compares the changes in light/dye-induced thrombus formation in cremaster muscle arterioles of control WT mice receiving an intrascrotal injection of either 0.1, 0.5 or 2.5 $\mu\text{g}/\text{mouse}$ of TNF- α dissolved in 0.2 ml of normal saline, or the combination of 0.1 $\mu\text{g}/\text{kg}$ IL-1 β and 0.5 $\mu\text{g}/\text{mouse}$ of TNF- α . Intrascrotal injection of 0.2 ml of normal saline alone did not affect the thrombosis responses, compared to the responses in control (non-colitic) WT mice not receiving an injection (data not shown). While the lowest dose (0.1 $\mu\text{g}/\text{mouse}$) of TNF- α did not significantly alter thrombus formation, both higher doses (0.5 and 2.5 $\mu\text{g}/\text{mouse}$) of TNF- α accelerated both the time of onset of thrombosis and the time to flow cessation. The responses to the two higher doses did not differ from each other. The combined administration of IL-1 β and TNF- α further enhanced both the onset of thrombosis ($p=0.005$) and the time to complete flow cessation ($p=0.01$), compared to mice receiving TNF- α alone (0.5 $\mu\text{g}/\text{mouse}$).

Figure 4 compares the thrombosis responses of muscle arterioles to DSS colitis between WT and TNF- α receptor deficient (TNF α ^{-/-}) mice. These experiments reveal that the enhanced extra-intestinal thrombosis response to DSS colitis is significantly blunted in TNF α ^{-/-} mice, when compared to WT mice. However, the thrombosis responses were not restored to WT control (non-colitic) levels.

Figure 5 illustrates the effects of TNF- α immunoblockade on the enhanced extra-intestinal thrombosis responses associated with DSS colitis. Treatment with the TNF- α blocking antibody resulted in a significantly blunted thrombosis response similar to that noted in TNF α ^{-/-} mice. In view of the partial protection afforded by TNF- α immunoblockade and the results of our recent study showing partial protection in this model following IL-1 β immunoneutralization(13), we examined the thrombosis responses in mice treated with a combination of antibodies that block TNF- α as well as IL-1 β . These experiments revealed that immunoblockade of both cytokines completely prevented the accelerated thrombus formation in muscle arterioles that is elicited by DSS colitis.

Discussion

Colonic inflammation in humans and experimental animals is associated with enhanced extra-intestinal thrombus formation. Animal studies have also corroborated the existence of a hypercoagulable state during chronic gut inflammation and support the view that this condition results from an imbalance between pro- and anti-coagulant systems, such as impaired protein C and antithrombin III anticoagulant systems(7,8). Although different components of the coagulation cascade, including tissue factor, activated protein C, and thrombin, have been directly implicated in the enhanced extra-intestinal thrombosis associated with experimental colitis(8–10), less attention has been devoted to defining the circulating factors that link gut inflammation to accelerated thrombus formation in distant tissues. In a recent study from our laboratory, we obtained evidence implicating the cytokine IL-1 β as a potential mediator of the enhanced extra-intestinal thrombosis response in experimental IBD.(13) While the study revealed IL-1 β as a quantitatively important contributor to the colitis-associated thrombosis response, the cytokine accounted for about 50% of the colitis-enhanced response, suggesting that other mediators also play a role. Inasmuch as TNF- α is another major cytokine that has been implicated in both human and experimental IBD(20), the present study was directed towards defining the contribution of TNF- α to the enhanced extra-intestinal thrombosis that accompanies experimental IBD.

Our study provides several lines of evidence that support a role for TNF- α in the enhanced thrombosis observed in cremaster muscle arterioles of mice with DSS-induced colonic inflammation: 1) the concentration of TNF- α was significantly elevated in the inflamed colon, plasma and distant skeletal muscle tissue in mice with DSS colitis, compared with their control counterparts, 2) exogenously administered TNF- α accelerated both the time of onset and time to flow cessation (representing the initiation and propagation/stabilization phases of thrombus formation, respectively)(2) in cremaster arterioles after light/dye injury in control (non-colitic) mice, 3) mice that are genetically deficient in the receptor for TNF- α exhibit a blunted thrombosis response in colitic mice, and 4) immunoblockade of TNF- α in WT colitic mice attenuates the thrombosis response. Our observation that TNF- α levels are greatly elevated muscle tissue, with a small but detectable increase in plasma TNF- α of colitic mice is consistent with changes in IL-1 β concentrations noted in DSS colitic mice (13). The increased production of cytokines in skeletal muscle, coupled to the finding of increased leukocyte rolling and adhesion in cremaster muscle venules of DSS colitic mice(13), suggests that the muscle microvasculature assumes an inflammatory phenotype that involves the local generation of TNF- α and other cytokines. It remains unclear how the inflamed colon elicits this prothrombotic, pro-inflammatory state in a distant vascular bed. Perhaps blood cells (leukocytes, platelets), microparticles formed by these cells, or soluble mediators that exit the inflamed colonic vasculature are carried via the blood stream to the distant tissue (muscle) to induce the phenotypic changes.

TNF- α exerts a variety of actions on the coagulation and anticoagulation pathways that may account for its ability to mediate the thrombosis associated with colonic inflammation. This cytokine, along with IL-1 β , has been shown to reduce the expression of thrombomodulin on the surface of endothelial cells, consequently impairing the activation of protein C(11). Incubation of endothelial cell monolayers with purified recombinant TNF- α elicits a time- and dose-dependent increase in tissue factor procoagulant activity(11). The cytokine also acts directly on endothelial cells to release both tissue-type (tPA) and urokinase-type (uPA) plasminogen activators, while also increasing plasminogen activator inhibitor (PAI-1), with inhibition of fibrinolysis and inadequate removal of fibrin as the net result(4,22). Indirectly, TNF- α can lead to impaired anticoagulation by causing leukocytes to release elastase, which could cleave and inactivate thrombomodulin on vascular endothelial cells and antithrombin III(23). The actions of TNF- α on tissue factor, thrombin generation, and the protein C pathway are consistent with reports implicating each of these factors in the accelerated extra-intestinal thrombosis observed in DSS colitis(8–10).

The finding that TNF- α mediates nearly half of the extra-intestinal thrombosis response to DSS, coupled to our recent observation that IL-1 β directed interventions (genetic deletion or immunoblockade) also affords partial protection against thrombosis in the same experimental model of colitis,(13) suggests that the actions of the two cytokines (TNF- α & IL-1 β) may be linked as either series- or parallel-coupled responses. While each cytokine could independently elicit coagulation/thrombosis by engaging with their respective cell surface receptors, it is also possible that one of the cytokines could promote thrombosis by causing an increased production/release of the other cytokine. To address this issue, we examined whether combined immunoblockade of TNF- α and IL-1 β provides similar or more protection against colitis-associated extra-intestinal thrombosis than IL-1 β blockade alone. The results of these experiments indicate that nearly complete protection against thrombosis is provided when both cytokines are immunoneutralized. This finding suggests that the two cytokines are mediating distinct effects on the coagulation cascade and/or platelet function to elicit the extra-intestinal thrombosis response to DSS-induced colonic inflammation.

Our finding that immunoblockade of TNF- α and IL-1 β largely abolishes the extra-intestinal thrombosis associated with experimental IBD suggests that treatment of IBD patients with

antibodies against TNF- α and/or IL-1 β may reduce the incidence of thromboembolism in these patients. While TNF- α neutralizing antibodies are now routinely and effectively used in the treatment of IBD and other chronic inflammatory diseases (e.g., rheumatoid arthritis), there is evidence for higher incidence of thromboembolic events in patients receiving this treatment.(24–26) The enhanced thrombogenicity associated with TNF- α antibody treatment has been attributed to the production of “antiphospholipid” antibodies, which are directed against key components of the coagulation/anticoagulation cascade such as prothrombin, protein C, protein S, and factor XII.(27) The resultant antigen-antibody complexes that are generated can bind and activate several cells that contribute to hemostasis, including endothelial cells, monocytes and platelets.(27) Evidence that IL-1 β blockade can confer its anti-inflammatory actions while protecting against autoimmunity(27,28) suggests that this cytokine may be a more appropriate therapeutic target for reducing thrombosis in IBD.

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Figure 1. Effects of DSS-induced colonic inflammation on light/dye-induced thrombus formation in arterioles and venules of mouse cremaster muscle. $n=7$ in all experimental groups. * $p < 0.05$ vs. control WT.

Figure 2. TNF- α concentration in plasma, colon and skeletal muscle (quadriceps) of control (n=5) and DSS-treated WT mice (n=5). * $p < 0.01$ vs. WT-Control.

Figure 3.

Effects of intrascrotal administration of TNF- α (0.1, 0.5 or 2.5 $\mu\text{g}/\text{mouse}$) dissolved in 0.2 ml of normal saline on thrombus formation (4 hrs after injection) in cremaster muscle arterioles. The data are compared to the responses of combined administration of 0.5 $\mu\text{g}/\text{mouse}$ of TNF- α and 1.0 $\mu\text{g}/\text{kg}$ of IL-1 β with 0.2 ml of normal saline. control WT ($n=7$), all TNF- α administration groups ($n=5$) and TNF- α + IL-1 β administration group ($n=5$). * $p < 0.05$ vs. WT-Control.

Figure 4.

Effects of TNF receptor deficiency (TNFr^{-/-}) on light/dye-induced thrombus formation in cremaster arterioles of DSS treated mice. WT-Control (n=7), WT-DSS (n=7), and TNFr^{-/-}-DSS mice (n=9). **p* < 0.05 vs. WT-Control. #*p* < 0.05 vs. WT-DSS.

Figure 5. Effects of anti-TNF- α antibody treatment, either alone or in combination with an anti-IL-1 β antibody, on DSS colitis-enhanced, light/dye induced thrombus formation in cremaster muscle arterioles. WT-Controls (n=7), WT-DSS (n=7), WT-DSS mice with TNF- α immunoneutralization (n=6), WT-DSS mice with TNF- α + IL-1 β immunoneutralization (n=7). * $p < 0.05$ vs. WT-Control. # $p < 0.05$ vs. WT-DSS.