TNF suppresses acute intestinal inflammation by inducing local glucocorticoid synthesis

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Although tumor necrosis factor (α) (TNF) exerts proinflammatory activities in a variety of diseases, including inflammatory bowel disease, there is increasing evidence for antiinflammatory actions of TNF. In contrast, glucocorticoids (GCs) are steroid hormones that suppress inflammation, at least in part by regulating the expression and action of TNF. We report that TNF induces extraadrenal production of immunoregulatory GCs in the intestinal mucosa during acute intestinal inflammation. The absence of TNF results in a lack of colonic GC synthesis and exacerbation of dextran sodium sulfate–induced colitis. TNF seems to promote local steroidogenesis by directly inducing steroidogenic enzymes in intestinal epithelial cells. Therapeutic administration of TNF induces GC synthesis in oxazolone-induced colitis and ameliorates intestinal inflammation, whereas inhibition of intestinal GC synthesis abrogates the therapeutic effect of TNF. These data show that TNF suppresses the pathogenesis of acute intestinal inflammation by promoting local steroidogenesis.

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Abbreviations used: DSS, dextran sodium sulfate; GC, glucocorticoid; IBD, inflammatory bowel disease; JNK, c-Jun N-terminal kinase; LRH-1, liver receptor homologue–1/NR5A2; MAP, mitogen-activated protein; MPO, myeloperoxidase; TNBS, 2,4,6-trinitrobenzenesulphonic acid; YAMC, young adult mouse colonocyte.

TNF is a well-characterized proinflammatory cytokine with a critical role in the pathogenesis of various inflammatory diseases (Eigler et al., 1997). The importance of TNF in the initiation of inflammatory disorders is particularly obvious in inflammatory bowel disease (IBD), such as Crohn's disease and ulcerative colitis, where unrestrained reactions against luminal antigens and commensal bacteria result in devastating inflammatory responses in the intestinal mucosa. TNF is rapidly induced in the intestinal mucosa upon initial activation of immune cells and seems to be important for the further acceleration of the inflammatory response. Consequently, absence or inhibition of TNF activity ameliorates disease progression in different experimental IBD models and human patients (Neurath et al., 1997; Corazza et al., 1999; Papadakis and Targan, 2000).

Apart from its well-characterized proinflammatory role, there is increasing evidence for various antiinflammatory properties of TNF. For example, although TNF is critical for the initiation of experimental autoimmune encephalomyelitis, it is also involved in the resolution of the disease (Kassiotis and Kollias, 2001). Similar observations have been made in dextran sodium sulfate (DSS)–induced colitis, where the absence or neutralization of TNF leads to an exacerbation of disease (Naito et al., 2003). These antiinflammatory properties of TNF may be attributed at least in part to its apoptosis-modulating activities in immune cells (Zheng et al., 1995). In particular, TNF strongly sensitizes T cells to apoptosis induction, resulting in an accelerated resolution of the inflammatory response (Zhou et al., 1996). Although the antiinflammatory effects of TNF on activated T cells are well described, little is known about its immunosuppressive activity in nonimmune cells.

Glucocorticoids (GCs) are steroid hormones with potent antiinflammatory properties (Riccardi et al., 2002). Thus, a variety of inflammatory disorders are successfully treated with GCs. Endogenous GCs are predominantly produced in the adrenal glands in response to emotional, physical, and immunological stress. Consequently, removal of the adrenal glands or pharmacological inhibition of systemic GC synthesis may result in shock or death after induction of a strong immune response (Gonzalo et al., 1993). Over the last decade, there has

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been accumulating evidence that synthesis of bioactive GCs is not restricted to the adrenal glands but extends to other extraadrenal sources, such as the thymus, brain, skin, and vascular endothelium (Davies and MacKenzie, 2003). Yet, in most situations the function of this extraadrenal GC production is unknown.

We have recently shown, however, that the intestinal mucosa is a potent extraadrenal source of GCs, and that local GCs contribute to the maintenance of intestinal immune homeostasis by controlling the activation of intestinal T lymphocytes (Cima et al., 2004). The major source of intestinal GCs appears to be the proliferating and undifferentiated epithelial cells of the crypts (Cima et al., 2004; Atanasov et al., 2008). Interestingly, the molecular control of adrenal and intestinal GC synthesis seems to be fundamentally different. Although the nuclear receptor and transcription factor steroidogenic factor–1/NR5A1 is essential for the expression of steroid-producing enzymes of the cytochrome P450 family in the adrenal cortex (Parker et al., 2002), it is absent in the intestinal mucosa and seems to be functionally replaced by the close homologue liver receptor homologue–1/NR5A2 (LRH-1; Mueller et al., 2006, 2007). Consequently, LRH-1 deficiency in the intestinal epithelium leads to defective expression of steroidogenic enzymes and intestinal GC synthesis (Mueller et al., 2006). Interestingly, this lack of local GC synthesis also correlates with an increased susceptibility of LRH-1–deficient mice to experimentally induced colitis (Coste et al., 2007). Furthermore, a potentially critical role for LRH-1 and intestinal GC synthesis in the control of IBD is supported by the observation that LRH-1 and steroidogenic enzymes are expressed at reduced levels at sites of intestinal inflammation in patients with Crohn's disease and ulcerative colitis (Coste et al., 2007).

In this study, we aimed at investigating the relationship between acute intestinal inflammation, TNF, and intestinal GC synthesis. We show that experimentally induced colitis promotes up-regulation of steroidogenic enzymes and the synthesis of intestinal GCs. Notably, the induction of GC synthesis does not seem to depend on the inflammation per se but on the type of inflammation. Intestinal GC synthesis was induced by Th1-type inflammation initiated by the hapten 2,4,6-trinitrobenzenesulphonic acid (TNBS) and DSS, but not by the Th2-type inflammation–promoting hapten oxazolone. TNF was found to be a key factor in the regulation of intestinal GC synthesis, as absence of TNF resulted in an almost complete lack of steroidogenic enzyme expression and intestinal GC synthesis. Importantly, DSS-induced colitis in the absence of TNF resulted in reduced intestinal GC synthesis with simultaneous exacerbation of disease, suggesting an antiinflammatory role of TNF-induced GC synthesis in the pathogenesis of DSS colitis. Injection of TNF alone was sufficient to trigger intestinal GC synthesis, and stimulation of intestinal epithelial cells with TNF directly induced the expression of steroidogenic enzymes. Finally, repetitive administration of TNF restored colonic GC synthesis during oxazolone-induced colitis and resulted in an amelioration of all clinical parameters and reduced inflammation. These data define a novel antiinflammatory role of TNF by inducing local GC synthesis in the intestinal mucosa.

RESULTS

DSS colitis–induced expression of steroidogenic enzymes and synthesis of intestinal GCs

To investigate whether the induction of acute intestinal inflammation affects intestinal GC synthesis, we used different well-characterized models of chemically induced colitis. A common feature of chemically induced colitides appears to be an initial disruption of the epithelial cell barrier that promotes increased immune cell exposure to constituents of the luminal microflora. To this date many different mouse models of mucosal inflammation have been characterized, which allows us to investigate various aspects of the pathogenesis operative also in patients with IBD (Strober et al., 2002). We have previously shown that the absence of LRH-1 exacerbates TNBS-induced colitis (Coste et al., 2007), likely because of the lack of colonic GC synthesis. As TNBS colitis is T cell dependent and characterized by a typical (Th1) cytokine pattern, we aimed at investigating intestinal GC synthesis in T cell–dependent and –independent models of colitis, as well as colitis mediated by either a Th1- or Th2-type T cell response.

We initially used the DSS model as a model for T cell–independent intestinal inflammation by feeding animals with 3% DSS in the drinking water. As DSS colitis can also be induced in animals lacking T and B cells (Strober et al., 2002), the adaptive immune system obviously plays a minor role in this model, at least in the acute phase. Inflammation is largely driven by activated nonlymphoid cells, such as macrophages and neutrophils, and associated release of proinflammatory cytokines such as TNF (Dieleman et al., 1994). Hence, induction of acute DSS colitis is particularly useful to study the contribution of innate immune mechanisms. DSS-mediated intestinal inflammation was characterized by extensive epithelial erosion, loss of goblet cells, and strong leukocytic infiltration of the colonic mucosa of diseased animals (Fig. 1, A and B), and was associated with clinical parameters such as weight loss (Fig. 1 C) and colon shortening (Fig. 1 D). Simultaneously, increased expression levels of the Th1-type cytokines TNF and IFN-γ were observed in colonic tissue samples of DSS-treated animals (Fig. 1, E and F).

GC synthesis is critically regulated by the expression of steroidogenic enzymes. In particular, the rate-limiting enzyme P450scc, encoded by the *CYP11A1* gene, converts cholesterol to pregnenolone, and 11β-hydroxylase (P450C11), encoded by the *CYP11B1* gene, converts 11-deoxycorticosterone to active corticosterone. Induction of DSS colitis resulted in a profound and significant increase in the expression of *CYP11A1* (Fig. 1 G) and *CYP11B1* (Fig. 1 H). Furthermore, in ex vivo organ cultures of colonic tissue from DSS-treated mice, we observed a sharp and strong increase in corticosterone synthesis, with peak production at day 7 and a subsequent decrease thereafter (Fig. 1 I). The observed GC synthesis

was bona fide in situ produced, as it was blockable with metyrapone, a potent inhibitor of 11β-hydroxylase and GC synthesis (Sprunt and Hannah, 1968).

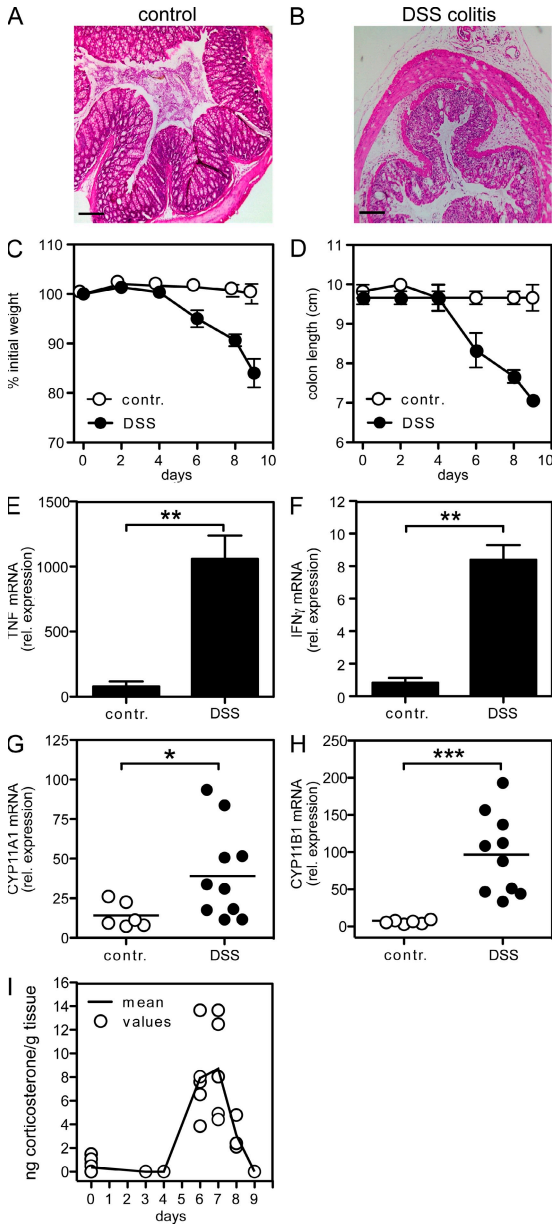

Figure 1. DSS-induced colitis and intestinal GC synthesis. Mice were control treated or colitis was induced by 3% DSS in drinking water. (A and B) Histology of colon from control mice (A) or colitogenic mice (B) at day 9. (C and D) Time course of weight loss (C) and colon length (D) in control- and DSS-treated mice. Means ± SD are shown ($n = 5$ per group). (E and F) Expression of TNF (E) and IFN-γ (F) in control- and DSS-treated mice at day 7. Means ± SD are shown ($n = 5$ per group). (G and H) Expression of the steroidogenic enzymes *CYP11A1* (G) and *CYP11B1* (H) in control-treated and colitogenic mice at day 7. Mean and individual values of 6–10 mice per group are shown. (I) Time course of GC synthesis in colonic organ cultures of DSS-treated mice. Mean values and individual values of two to six mice per time point are shown. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$. Pooled data are from three independent experiments. Bars, 100 µm.

TNBS induces a Th1-type colitis and intestinal GC synthesis

We next assessed intestinal GC synthesis in TNBS-induced colitis representing an acute T cell–dependent model of intestinal inflammation. Rectal administration of the haptenizing agent TNBS resulted in a Th1-mediated colonic inflammation characterized by infiltration of colitogenic $CD4^+$ T cells (Neurath et al., 2000), destruction of the colonic epithelium (Fig. 2 B), rapid weight loss (Fig. 2 C), and shortening of the colon (Fig. 2 D). These parameters were only transiently observed in control-treated animals. In agreement with a Th1 type of inflammation, TNF and IFN-γ expression were increased in diseased but not control animals (Fig. 2, E and F), with undetectable levels of the Th2 cytokine IL-4 (not depicted). Comparable to DSS colitis, we observed a substantial increase in the expression levels of the steroidogenic enzymes *CYP11A1* (Fig. 2 G) and *CYP11B1* (Fig. 2 H), and the induction of metyrapone-blockable corticosterone synthesis (Fig. 2 I). No induction of colonic steroidogenesis was observed in control-treated animals. These data indicate that the induction of experimental colitis results in intestinal GC synthesis as a way to counterbalance colonic inflammation.

Oxazolone-induced Th2-type colitis does not promote intestinal GC synthesis

Various animal models of colitis are characterized with different types of polarized T cell responses. Although TNBS induces a profound Th1 type of inflammation, administration of the haptenizing agent oxazolone results in a profound and acute intestinal inflammation caused by a polarized Th2 T cell response (Strober et al., 2002). Treatment of sensitized mice by rectal application of oxazolone in 50% ethanol resulted in rapid weight loss (Fig. 3 C) and colon shortening (Fig. 3 D) because of massive inflammation of the colonic tissue (Fig. 3 B). This inflammatory response was characterized by a strong increase in the Th2 cytokine IL-4 (Fig. 3 F), whereas no increase in the Th1 cytokines TNF (Fig. 3 E) or IFN-γ (not depicted) was observed. Surprisingly, although TNBS- and oxazolone-induced colitis had a similar kinetic of weight loss and colon shortening, and the overall inflammation-induced tissue destruction was comparable, absolutely no increase in the expression of the steroidogenic enzymes *CYP11A1* and *CYP11B1* was observed in colonic tissue of oxazolone-treated mice (Fig. 3, G and H). Interestingly, we even observed a slight but significant decrease in *CYP11A1* expression in the colonic tissue of oxazolone- versus control-treated animals. In agreement with the absence of induction of the steroidogenic enzymes *CYP11A1* and *CYP11B1*, no increase in intestinal GC synthesis was observed. These data suggest that an acute Th1 type of colonic inflammation promotes intestinal GC synthesis, whereas a Th2 type of response fails to do so.

TNF but not colonic inflammation is required for colitis-induced intestinal GC synthesis

The results described in the previous section demonstrate that intestinal GC synthesis depends on the type of inflammation and not the inflammatory process per se. A major difference

in these animal models was the induction of TNF expression. We thus postulated that TNF may represent an important key factor in the regulation of colonic GC synthesis.

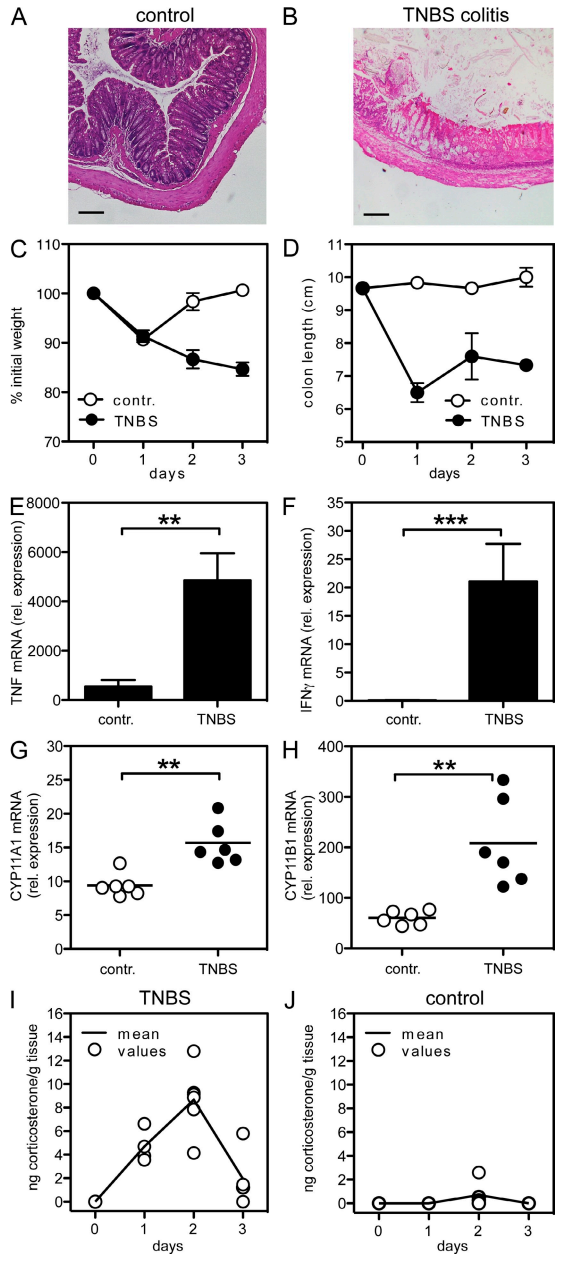

Figure 2. TNBS-induced Th1 colitis and intestinal GC synthesis. (A and B) Histology of control- (A) and TNBS-treated (B) mice at day 3. (C and D) Time course of weight loss (C) and colon length (D) in control- and TNBS-treated mice. Means ± SD are shown ($n = 5$ per group). (E and F) Expression of TNF (E) and IFN-γ (F) in control- and TNBS-treated mice at day 2. Means ± SD are shown ($n = 5$ per group). (G and H) Expression of the steroidogenic enzymes *CYP11A1* (G) and *CYP11B1* (H) in control- and TNBS-treated mice at day 2. Mean and individual values of five to six mice per group are shown. (I and J) Time course of GC synthesis in colonic organ cultures of TNBS- (I) and control-treated mice (J). Mean and individual values of two to six mice per time point are shown. **, $P < 0.01$; ***, $P < 0.005$. Pooled data are from three independent experiments. Bars, 100 µm.

The TNF dependency has been well characterized for TNBS-induced colitis (Neurath et al., 1997; Ebach et al., 2005). Although administration of TNBS into wild-type animals promoted intestinal inflammation (Fig. 2, B and C), TNF-deficient mice did not develop colitis and showed no induction of colonic GC synthesis (Fig. S1). These data support the notion that TNF and associated inflammation may be required for colitis-induced intestinal GC synthesis.

In contrast to the TNBS model, DSS-induced colitis proceeds in a TNF-independent manner (Fig. 4 A; Naito et al., 2003). Interestingly, TNF even seems to have a protective effect in DSS colitis, as absence of TNF leads to an exacerbation and acceleration of the disease. This is illustrated by increased weight loss of TNF-deficient mice (Fig. 4 A). Furthermore, significantly higher levels of myeloperoxidase (MPO) activity were observed in the colonic tissue of TNF-deficient compared with wild-type mice (Fig. 4 B), indicating a stronger infiltration of the affected tissue with neutrophilic granulocytes. The alteration of disease progression was also paralleled by a reduction in IFN-γ expression (Fig. 4 C) and an increase in IL-4 expression (Fig. 4 D), indicative of a shift from a Th1 to a Th2 response.

Remarkably, although inflammation and associated weight loss were accelerated in TNF-deficient mice, colonic steroidogenesis was drastically reduced. Although DSS induced a dramatic increase in the expression of *CYP11A1* and *CYP11B1* and associated colonic GC synthesis in wild-type animals, almost no induction of these steroidogenic enzymes and steroidogenesis was observed in TNF-deficient mice (Fig. 4, E and F), despite the pronounced induction of colonic inflammation. These data indicate that TNF has an important and unique role in the induction of colitis-associated intestinal GC synthesis.

TNF is critical for the induction of intestinal steroidogenesis

To further define the general importance of TNF in the induction of intestinal GC synthesis, we assessed the induction of *CYP11B1* and corticosterone synthesis in wild-type and TNF-deficient mice after injection of anti-CD3ε antibodies, which were previously shown to be a potent inducer of intestinal GC synthesis (Cima et al., 2004; Mueller et al., 2006). As shown in Fig. 5 (A and B), absence of TNF resulted in a significant reduction of *CYP11B1* expression and corticosterone synthesis in colonic organ cultures in response to in vivo T cell activation, demonstrating that TNF represents a more general trigger of extraadrenal GC synthesis in the intestinal epithelium. This was confirmed by i.p. injection of recombinant TNF, which led to a significant increase in *CYP11B1* expression (Fig. 5 D) and intestinal GC synthesis (Fig. 5 C).

TNF seemed to directly target the intestinal epithelium, as treatment of the young adult mouse colonocyte (YAMC) epithelial cell line (Whitehead et al., 1993) resulted in a time-dependent degradation of IκB (Fig. 5 E), indicating TNF responsiveness, and a dose-dependent induction of *CYP11B1* expression (Fig. 5 F). Interestingly, TNF-induced *CYP11B1* expression was blockable by the proteosome and NF-κB activation inhibitor lactacystin but not by c-Jun N-terminal kinase

(JNK) inhibitors or mitogen-activated protein (MAP) kinase inhibitors, suggesting a role for NF-κB activation in TNF-induced steroidogenesis in intestinal epithelial cells (Fig. 5 G).

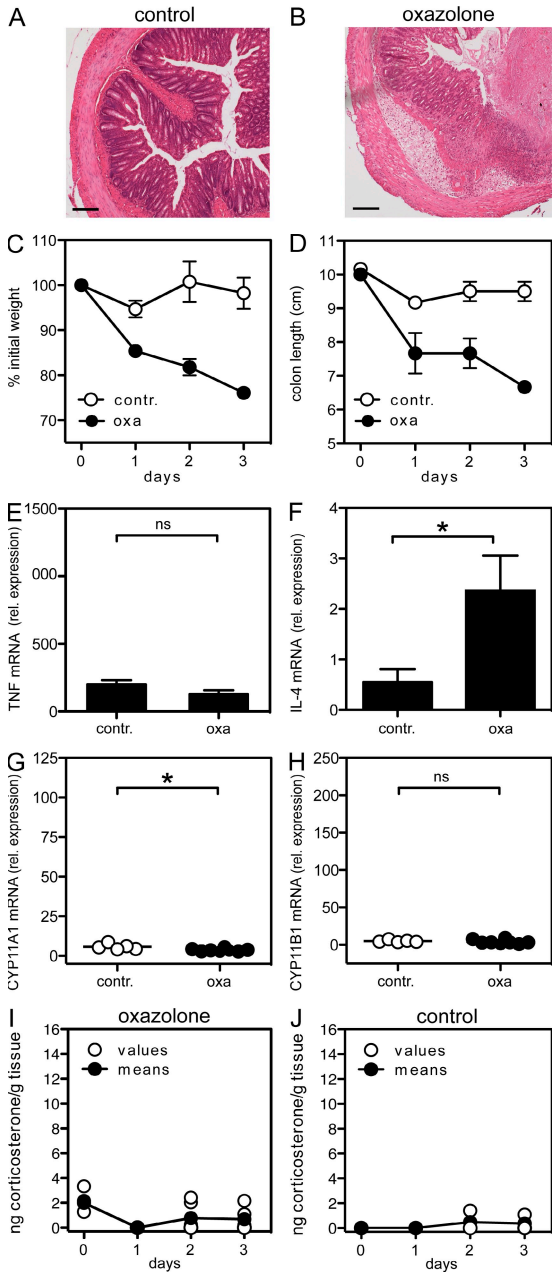

Figure 3. Oxazolone-induced Th2 colitis and intestinal GC synthesis. (A and B) Histology of control- (A) and oxazolone-treated (B) mice at day 3. (C and D) Time course of weight loss (C) and colon length (D) in control- and oxazolone-treated mice. Means ± SD are shown (n = 5 per group). (E and F) Expression of TNF (E) and IL-4 (F) in control- and oxazolone-treated mice at day 2. Means ± SD are shown (n = 5 per group). (G and H) Expression of the steroidogenic enzymes *CYP11A1* (G) and *CYP11B1* (H) in control- and oxazolone-treated mice at day 2. Mean and individual values of five to eight mice per group are shown. (I and J) Time course of GC synthesis in colonic organ cultures of oxazolone- (I) and control-treated mice (J). Mean and individual values of three to five mice per time point are shown. *, $P < 0.05$. Pooled data are from three independent experiments. ns, not significant. Bars, 100 µm.

The TNF-mediated induction of *CYP11A1* and *CYP11B1* mRNA expression could also be reproduced in ex vivo–cultured primary colonic epithelial cells (Fig. 5 H and Fig. S2).

Administration of TNF during oxazolone colitis restores intestinal GC synthesis and ameliorates disease

Our data described in the previous section indicated that TNF is critical for induction of intestinal GCs during experimental

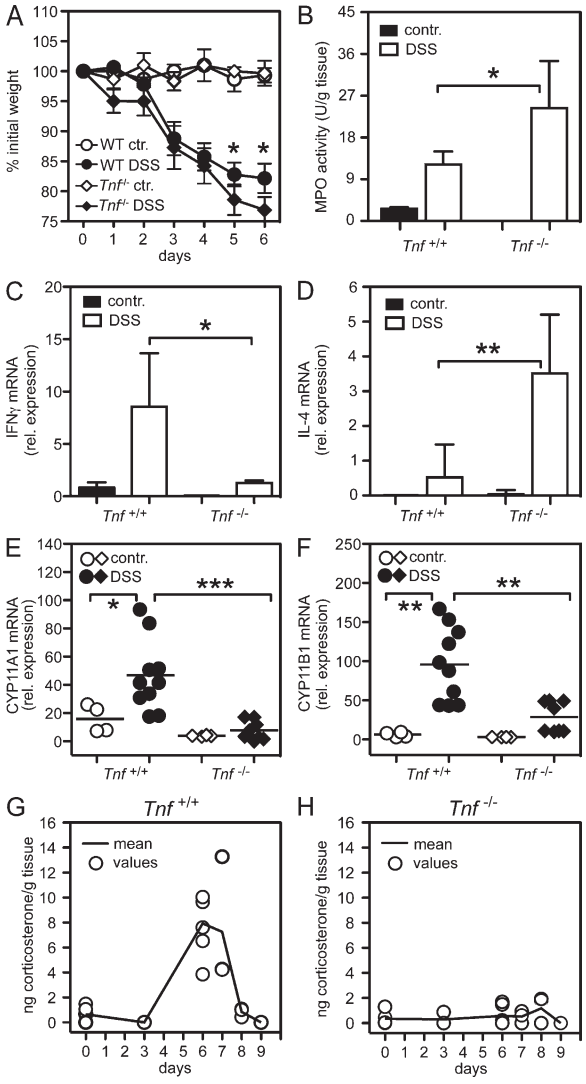

Figure 4. TNF is required for DSS colitis–induced GC synthesis. (A) Kinetic of weight loss in control- or DSS-treated wild-type ($TNF^{+/+}$) or $TNF^{-/-}$ mice. (B) MPO activity in colonic tissue from control- or DSS-treated $TNF^{+/+}$ or $TNF^{-/-}$ mice. (C and D) Expression of IFN-γ (C) and IL-4 (D) in control or colitogenic mice. Means ± SD of five mice per group are shown. (E and F) Expression of *CYP11A1* (E) and *CYP11B1* (F) in colonic tissue from control- or DSS-treated $TNF^{+/+}$ or $TNF^{-/-}$ mice at day 6. Mean and individual values of 4–10 mice per group are shown. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$. (G and H) Kinetic of corticosterone synthesis in colonic tissue from DSS-treated $TNF^{+/+}$ and $TNF^{-/-}$ mice. Mean and individual values of three to four mice per time point are shown. One typical experiment out of two is shown.

colitis. Furthermore, the failure to induce TNF during oxazolone colitis may be the reason for lack of colonic GC synthesis. To test whether administration of TNF could restore colonic GC synthesis, oxazolone colitis was induced and mice were further treated daily with PBS or mouse TNF. Although TNF has been previously described to induce cachexia (Haslett, 1998), TNF injection into control mice did not substantially alter the body weight during the observation time (Fig. 6 D). In marked contrast, however, daily treatment of colitogenic mice with TNF significantly reduced oxazolone-induced body weight loss, suggesting disease amelioration. This finding was further substantiated by reduced signs of inflammation in colonic tissue (Fig. 6, A and B), reduced colitis score (Fig. 6 C), and colon shortening (Fig. 6 E) in TNF-treated colitogenic mice compared with mice receiving oxazolone only. Interestingly, we also observed a strong increase in TNF expression in oxazolone plus TNF–treated mice, whereas only baseline levels were seen in mice treated with oxazolone only (Fig. 6 G). In contrast, TNF treatment substantially reduced IL-4 expression typically seen in the oxazolone-induced Th2–type colitis (Fig. 6 F). In agreement with a critical role of TNF in the induction of colonic GC synthesis, we observed increased *CYP11A1* (Fig. 6 H) and *CYP11B1* (Fig. 6 I) expression, and associated GC synthesis (Fig. 6 J) in colonic tissue from mice treated with TNF or TNF plus oxazolone. Thus, TNF treatment restores colonic GC synthesis and ameliorates oxazolone colitis.

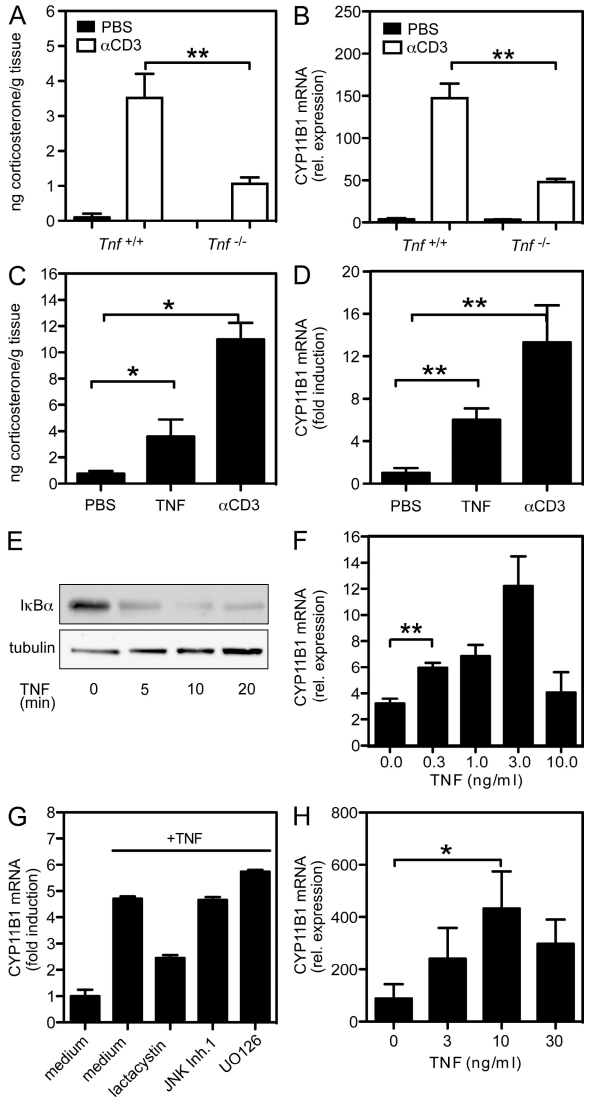

Figure 5. Role of TNF in anti-CD3–induced intestinal GC synthesis. (A) $TNF^{+/+}$ or $TNF^{-/-}$ mice were injected i.p. with PBS control or anti-CD3 antibody, and corticosterone production in colonic organ cultures was assessed. (B) *CYP11B1* expression in colonic tissue from PBS- and anti-CD3–treated $TNF^{+/+}$ or $TNF^{-/-}$ mice. (C) Corticosterone synthesis in colonic organ culture from control-treated (PBS) mice or mice that were injected with recombinant TNF or anti-CD3 antibody. (D) Mice were treated as in C and *CYP11B1* expression was measured in colonic tissue. Means ± SD from five mice per group are shown. *, $P < 0.05$; **, $P < 0.01$. (E) YAMCs were stimulated with TNF for the indicated time points and IκB degradation was assessed by Western blotting. (F) Cells were stimulated with increasing concentrations of TNF for 6 h and *CYP11B1* expression was analyzed. (G) YAMCs were pretreated with medium control, lactacystin, JNK inhibitor 1, or the MAP kinase inhibitor U0126 before stimulation with TNF. *CYP11B1* expression was analyzed after 6 h by real-time PCR. (H) Primary colonic epithelial cells were stimulated for 4 h with TNF, and *CYP11B1* mRNA expression levels were measured by quantitative PCR. One typical experiment out of three is shown.

Inhibition of intestinal GC synthesis abrogates the antiinflammatory effect of TNF

To prove that the therapeutic effect of TNF in oxazolone-induced colitis is mediated via the induction of colonic GC synthesis, we aimed at blocking TNF-induced GC synthesis by metyrapone. Although therapeutic administration of TNF restored colonic GC synthesis, inhibition of 11β-hydroxylase by in vivo treatment with metyrapone resulted in significantly reduced intestinal corticosterone synthesis. The inhibition of TNF-induced colonic GC synthesis by metyrapone was paralleled by increased weight loss and histological colitis score, indicating that inhibition of colonic GC synthesis abrogates the observed antiinflammatory therapeutic effect of TNF in oxazolone-induced colitis (Fig. 7). These findings confirm that the antiinflammatory activity of TNF in oxazolone-induced colitis is mediated via the induction of colonic GC synthesis. A comparable protective effect of therapeutic administration of TNF was also found in the DSS colitis model. TNF-treated mice showed reduced weight loss and neutrophil infiltration, paralleled by increased colonic steroidogenesis (Fig. S3).

DISCUSSION

TNF is an important initiator cytokine of inflammatory responses. Thus, TNF has been identified as a major therapeutic target in different inflammatory diseases. The importance of TNF in IBD has been demonstrated in a variety of colitis models (Neurath et al., 1997; Corazza et al., 1999, 2004). Consequently, neutralization of TNF by antibodies or soluble receptor, or TNF deficiency significantly attenuates the pathogenesis of experimental IBD. Similarly, administration

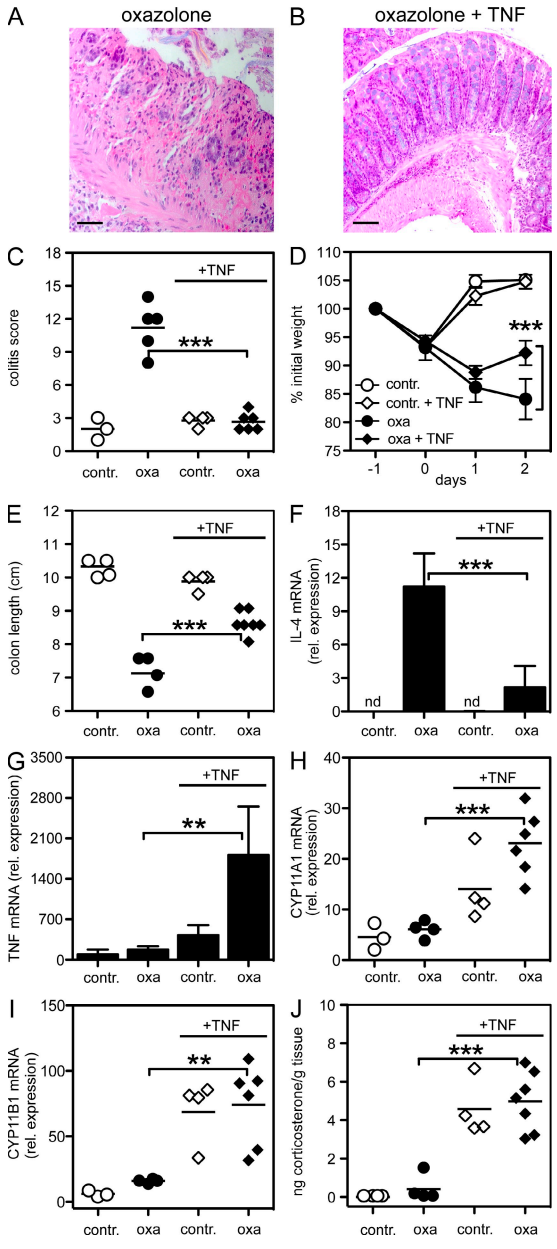

Figure 6. Administration of TNF restores intestinal GC synthesis and protects from oxazolone-induced colitis. Colitis was induced by intrarectal administration of ethanol control or oxazolone. Mice were then treated daily with buffer control or TNF. (A and B) Histology of colonic tissue of mice with oxazolone colitis (A) or oxazolone colitis treated with TNF. (C) Analysis of histological alterations (colitis score) in the colonic tissue of mice treated with oxazolone and/or TNF. (D and E) Time course of weight loss (D) and colon length (E) in control- and oxazolone-treated mice with or without TNF therapy. (F and G) IL-4 (F) and TNF (G) mRNA expression in colonic tissue. (H and I) Expression of the steroidogenic enzymes *CYP11A1* (H) and *CYP11B1* (I). (J) GC synthesis in ex vivo colonic organ cultures. All parameters were measured at day 2. Symbols represent values of individual mice; horizontal bars indicate mean values. In some experiments, means ± SD are shown ($n = 4–7$ mice per group). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$. One typical experiment out of three is shown. Bars, 100 µm.

of humanized anti-TNF antibody (infliximab) has astonishing therapeutic effects in IBD patients (Hanauer and Present, 2003). As chronic inflammation may be one of the underlying causes of cancer, anti-TNF also has preventive effects on the development of colitis-associated colon cancer (Wilson, 2008).

Apart from these well-established proinflammatory activities, there is increasing evidence for antiinflammatory properties of TNF. At least in part, these antiinflammatory effects of TNF may be attributed to apoptosis-inducing and/or apoptosis-modulating activities in immune cells. Although TNF-induced apoptosis in intestinal epithelial cells further compromises the epithelial barrier integrity and accelerates intestinal inflammatory responses by permitting constant stimulation of immune cells with luminal antigens and factors, TNF-induced apoptosis in immune cells may help to terminate and/or control inflammation. Interestingly, differential signals via TNFR1 or 2 are at least in part responsible for this dual role of TNF in inflammatory processes. In line with the differential signaling of TNFRs in immune cells is the observation that TNBS colitis is more severe in TNFR1-deficient mice, whereas it is significantly reduced in TNFR2-deficient animals (Holtmann et al., 2002; Ebach et al., 2005). Similarly, neutralization of TNF (Kojouharoff et al., 1997) or absence of TNF (our own findings; Naito et al., 2003) exacerbates acute DSS-induced colitis. Often, the proinflammatory action of TNF is critical for the initiation of disease, whereas its antiinflammatory function helps to resolve the disease. This dual role of TNF has also been recently demonstrated in the pathogenesis of experimental autoimmune encephalomyelitis (Kassiotis and Kollias, 2001).

In this paper, we report on a so far unrecognized role of TNF in (negatively) controlling acute intestinal immune responses. We identified TNF as a critical factor in the induction of local GC synthesis during the course of experimental colitis. DSS and TNBS, both promoting a Th1-type inflammation including a strong production of TNF, induced a pronounced and transient GC synthesis in the colon. In marked contrast, the Th2 cytokine–dominated inflammation induced by oxazolone failed to induce TNF and intestinal GC synthesis. Remarkably, therapeutic administration of TNF was able to restore colonic GC synthesis and resulted in an overall improvement of all clinical colitis parameters. Critically, inhibition of colonic GC synthesis by metyrapone abrogated the therapeutic effect of TNF, indicating that the antiinflammatory effect of TNF in experimental colitis is mediated via the induction of colonic GC synthesis. Although the present protocol of therapeutic TNF administration only resulted in a partial improvement in oxazolone-induced colitis, these experiments demonstrate that TNF is critical for the induction of intestinal GC synthesis, and that TNF-induced GCs have an antiinflammatory role in the regulation of experimental colitis. More optimized TNF-based treatments or selective engagement of further downstream signaling pathways leading to intestinal GC synthesis may result in even better therapeutic effects. Importantly, although previously antiinflammatory effects of TNF have been attributed to its action on T cells,

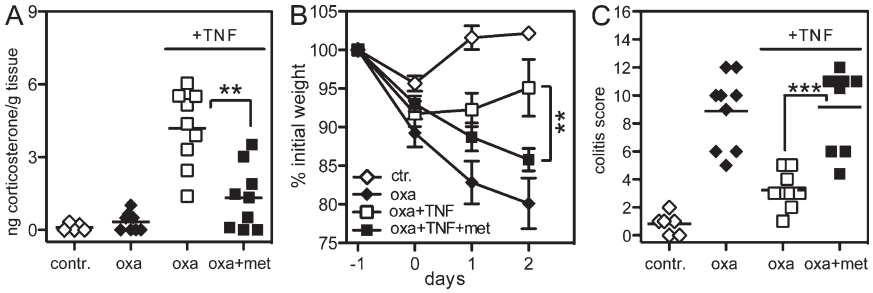

Figure 7. Inhibition of intestinal GC synthesis abrogates the antiinflammatory effect of TNF. Colitis was induced by intrarectal administration of ethanol control or oxazolone. Mice were then treated daily with buffer control or TNF, and intestinal GC synthesis was blocked by administration of metyrapone. (A) Corticosterone synthesis in ex vivo colonic organ cultures. (B) Kinetic of body weight loss. (C) Analysis of histological alterations (colitis score) in colonic tissue of mice treated with ethanol as control, oxazolone alone, oxazolone + TNF, and oxazolone + TNF + metyrapone. Means ± SD are shown (n = 6–9 animals per group). **, $P < 0.01$; ***, $P < 0.005$. One typical experiment out of three is shown.

i.e., modulation of apoptosis sensitivity, we now provide evidence for an alternative pathway via the induction of immunoregulatory GCs in intestinal epithelial cells.

We have previously demonstrated the relevance of intestinal GC synthesis in the regulation of local immune cells. For example, lack of intestinal GC production resulted in stronger activation of virus-specific intestinal T cells (Cima et al., 2004). Colonic GC synthesis likely also contributes to the regulation of IBD. Indirect proof for this idea is seen in DSS colitis, where absence of TNF results in a lack of colonic GC synthesis and exacerbation of disease (Naito et al., 2003). Further support for a role for intestinal GC synthesis in the regulation of IBD comes from a recent report by Coste et al. (2007). We recently identified the nuclear receptor and transcription factor LRH-1 as a critical regulator of intestinal GC synthesis (Mueller et al., 2006, 2007). Consequently, it was observed that LRH-1–deficient mice are more susceptible to DSS- and TNBS-induced colitis, supporting a role of LRH-1–regulated GC synthesis in experimental models of mucosal inflammation (Coste et al., 2007). The transient nature of colitis-induced GC synthesis mentioned above and the failure of intestinal GCs to completely control local inflammation may also be caused by the selective loss of LRH-1–expressing and GC-producing cells in the intestinal crypts. In fact, during IBD the intestinal crypts are characterized by excessive apoptosis, promoting epithelial barrier disruption and access to proinflammatory factors in the lumen, but also impaired epithelial barrier recovery and reduced GC synthesis. It is of interest to note that LRH-1 is not only critically involved in the regulation of intestinal GC synthesis, but is also an important regulator of intestinal crypt cell proliferation and thereby intestinal epithelial cell renewal (Botrugno et al., 2004). Furthermore, GCs promote tight junction formation in intestinal epithelial cells and thereby contribute to the maintenance of intestinal epithelial barrier function. Thus, LRH-1 likely represents a critical regulator of intestinal tissue integrity via the regulation of epithelial cell integrity and intestinal immune homeostasis. Defects in LRH-1–induced epithelial layer integrity and intestinal GC synthesis may further exacerbate the local inflammation and course of the disease by promoting a vicious cycle.

In summary, we provide first-time evidence for a novel antiinflammatory role of TNF in experimental colitis via the local production of immunoregulatory GCs. Although we defined an antiinflammatory role of TNF and local GC synthesis specifically in acute models of intestinal inflammation, it will be interesting to determine whether the same applies also for chronic types of intestinal inflammation, more closely related to Crohn's disease and ulcerative colitis in humans. Nonetheless, the use of the three acute models of experimental colitis strongly support the idea that intestinal GC synthesis is an important negative regulatory feedback mechanism controlling intestinal immune responses and inflammation. Furthermore, they illustrate that TNF has a Janus-like pro- and antiinflammatory activity in the regulation of intestinal inflammation. The identification of critical signaling molecules regulating the local synthesis of antiinflammatory GCs may represent interesting novel targets in the treatment of IBD.

MATERIALS AND METHODS

Cells and reagents

YAMCs (provided by R.H. Whitehead, Vanderbilt University, Nashville, TN) have been described previously (Whitehead et al., 1993). YAMCs were cultured in RPMI 1640 supplemented with 5% FCS, 2 mM L-glutamine, 1 mg/ml insulin, 0.55 mg/ml human transferrin, 0.5 µg/ml sodium selenite, 5 U/ml of mouse recombinant IFN-γ (PeproTech), and 50 µg/ml gentamycin, and were grown at the permissive temperature of 33°C and 5% CO_2. Lactacystin, JNK inhibitor 1, and MAP kinase inhibitor U0126 were obtained from Enzo Life Sciences, Inc. Primary intestinal crypt cells from large and small bowels were isolated as previously described (Sato et al., 2009).

Animals

Animal experiments were performed in compliance with Swiss laws and guidelines, and were approved by the animal experimentation committee of the State of Bern. Wild-type and TNF-deficient (Corazza et al., 2004) C57BL/6 mice were bred and kept in the Central Animal Facility of the Department of Medicine, University of Bern, and group-housed with free access to food and tap water. Mice were used for experiments between 7–11 wk of age.

Mouse models of colitis

DSS-induced colitis. DSS colitis was induced by administration of 3% (wt/vol) DSS (reagent-grade DSS salt; molecular mass = 36–50 kD; MP Biomedicals) in normal drinking water for 5 d, followed by a 4-d normal water consumption.

TNBS-induced colitis. Animals were fasted for 24 h and anesthetized by a subcutaneous injection of xylasine/ketamine upon intrarectal administration of 60 µl TNBS (150 mg/kg; Fluka) dissolved in 50% ethanol. Under the same conditions, control mice received 60 µl of 50% ethanol. Solutions were

administered via a 3.5F catheter equipped with a 1-ml syringe. The tip of the catheter was inserted 4 cm proximal to the anal verge. To ensure distribution of TNBS within the entire colon, animals were held in a vertical position for 30 s after injection.

Oxazolone-induced colitis. Mice were presensitized by epicutanous application of 3% oxazolone (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one; Sigma-Aldrich) in 100% ethanol in a volume of 150 µl at day −7. Animals were fasted and anesthetized as described in the previous section, and received an intrarectal administration of 100 µl of 1% oxazolone in 50% ethanol as described in the previous section. Control mice received 100 µl of 50% ethanol under the same conditions.

Body weight loss and rectal bleeding of all animals were monitored during the experiments. Mice were killed at different time points by CO_2 asphyxiation and blood was sampled. Plasma was obtained by centrifugation at 2,000 *g* for 10 min at 4°C and stored at −80°C until analysis. Large bowels were removed, measured, and snap frozen or fixed in 4% paraformaldehyde for further biochemical and histological analysis. Histological analysis of colonic inflammation was performed as previously described (colitis score) with a minimal score of 0 and a maximum score of 15 (Corazza et al., 1999, 2004). Adrenal glands were collected as positive controls for steroidogenic enzyme expression and corticosterone synthesis.

In vivo administration of anti-CD3 and TNF

In some experiments, intestinal GC synthesis was induced by i.p. injection of 20 µg anti-CD3ε antibody, as described previously (Cima et al., 2004), or 1 µg of recombinant mouse TNF (PreproTech). After 3 h, mice were euthanized and colonic tissue was isolated for analysis of GC synthesis and expression of steroidogenic enzymes.

During oxazolone-induced colitis, PBS control or TNF (1 µg/dose) were administered daily i.p. at days 0.5, 1, and 2 before analysis of GC synthesis and intestinal inflammation. In some experiments, intestinal GC synthesis was blocked by i.p. administration of 11β-hydroxylase and the GC synthesis inhibitor metyrapone (200 mg/kg of body weight; Sigma-Aldrich) twice daily at days –1, 0, 1, and 2.

Detection of corticosterone synthesis in intestinal organ cultures

The entire large intestine was opened longitudinally and cut into 5-mm-long pieces, washed extensively in PBS/2% charcoal-stripped horse serum (SHS), and incubated for 10 min at 4°C in PBS/2% SHS containing 1 mM 1,4-dithiothreitol to remove excess mucus. After an additional washing step, tissue pieces were randomly distributed to 2 wells of a 24-well plate and cultured in 10% charcoal-stripped IMDM in the presence or absence of 200 µg/ml of the GC synthesis inhibitor metyrapone (Sigma-Aldrich). As positive control for GC synthesis, the adrenal glands were cut in half and incubated as described. After 6 h of incubation at 37°C, cell-free supernatant was harvested and corticosterone concentration was measured using a radioimmunoassay. Results are expressed as nanograms of corticosterone per gram of tissue and are shown as the difference of samples cultured without metyrapone and samples cultured with metyrapone (metyrapone-blockable corticosterone synthesis) to correct for variable contamination with serum GCs (Mueller et al., 2006).

Real-time PCR

One third of the entire colons of colitogenic and control animals was homogenized and lysed in TRI-reagent (Sigma-Aldrich) for further RNA isolation. RNA was DNase-treated and reverse transcribed using a high capacity RT kit (Applied Biosystems). RT-PCR was performed in the 7500 Real-Time PCR System (Applied Biosystems) using Quantitect primer assays for the steroidogenic enzymes *CYP11A1* and *CYP11B1*, and the cytokines IL-4, TNF, and IFN-γ. GAPDH or villin were used to normalize expression levels.

In some experiments, YAMCs were pretreated with the proteosome and NF-κB inhibitor lactacystin, JNK inhibitor 1, or the MAP kinase inhibitor U0126 (all at 1 µM) before stimulation with 3 ng/ml TNF and analysis of *CYP11B1* induction.

NF-κB activation assay

YAMCs were incubated for different time points with 3 ng/ml TNF. Total protein lysates were extracted after standard procedures. After blotting, nitrocellulose membranes were incubated overnight at 4°C with a rabbit polyclonal IκBα antibody, washed, and incubated for 1 h at room temperature with a goat anti–rabbit peroxidase conjugate secondary antibody. Equal protein loading was confirmed by detection of α-tubulin.

MPO activity

Neutrophil infiltration in control and colitogenic animals was quantified by measuring the activity of MPO in colonic homogenates, as described previously (Pfister et al., 2000). In brief, 50 mg of tissue was resuspended in 50 mM of potassium phosphate buffer containing 0.5% hexadecyltrimethylammonium bromide, and homogenized subsequently. The mixture was submitted to three cycles of freeze-thawing, followed by sonication for 20 s and centrifugation for 30 min. MPO activity was measured by incubating the supernatants with 20 mg/ml O-dianisidine dihydrochloride and 20 mM hydrogen peroxide. The reaction was terminated by the addition of 2% sodium azide. Optical density was read at 450 nm. Samples were measured in triplicates and are expressed as units per gram of tissue. All reagents were purchased from Sigma-Aldrich.

Statistics

Results are expressed as means ± SEM. Alternatively, values of individual samples and the mean of the group are shown. Differences between groups were analyzed by the unpaired Student's *t* test. $P < 0.05$ was considered statistically significant.

Online supplemental material

Fig. S1 shows that TNF is required for TNBS-induced colonic inflammation as well as GC synthesis. Fig. S2 describes the effect of TNF on expression of steroidogenic enzymes in primary intestinal epithelial cells. Fig. S3 describes the therapeutic effects of TNF in DSS-induced colitis. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20090849/DC1.

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