Inhibition of Interferon γ Signaling by the Short Chain Fatty Acid Butyrate

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Abstract

The short chain fatty acid butyrate promotes proliferation and survival of normal epithelial cells, but induces G1 or G2-M arrest in transformed cells, which is coupled to differentiation and apoptosis. Local administration of butyrate has been shown to ameliorate inflammation in ulcerative colitis; however, the precise mechanism of its anti-inflammatory activity is not known. IFN-γ is one of the principle cytokines secreted by lamina propria cells in inflamed mucosa and elevated levels of the transcription factor required for IFN-γ signaling, STAT1 (signal transducer and activator of transcription 1), are present in the colonic mucosa of patients with ulcerative colitis and Crohn’s disease. Here we report that butyrate is a strong inhibitor of signaling by IFN-γ. We demonstrated that this short chain fatty acid inhibits IFN-γ-induced tyrosine and serine phosphorylation of STAT1. IFN-γ-induced JAK2 activation was inhibited by butyrate, implicating JAK2 as a target of butyrate action. Accordingly, STAT1 nuclear translocation and its DNA binding were completely inhibited in butyrate-treated cells. Transient transfection experiments using a reporter gene construct containing eight GAS sites (γ-activated sites) revealed that butyrate inhibits IFN-γ-induced, STAT1-dependent, transcriptional activation. Proinflammatory cytokines, including IFN-γ, play an important role in the pathogenesis of inflammatory bowel disease, and abnormal activity of STAT1 is associated with human malignancies and intestinal inflammatory diseases. Thus, our data suggest that butyrate negatively regulates mucosal inflammation through the inhibition of IFN-γ/STAT1 signaling.

Introduction

Butyrate is a short chain fatty acid produced by bacterial fermentation of fiber and acts as a physiological regulator of homeostasis of colonic epithelial cells by regulating the balance among proliferation, differentiation, and apoptosis (1, 2). Butyrate is a survival factor for normal colonic epithelial cells (3), but induces cell cycle arrest, differentiation, and apoptosis in transformed epithelial cells (4–8). The mechanism regulating the switch between butyrate-induced proliferation and apoptosis is not understood. We have recently demonstrated that the acquisition of a k-ras mutation, a common genetic abnormality in colon cancer, is sufficient to sensitize colonic epithelial cells to butyrate-induced apoptosis.1

In addition to inducing G1 or G2-M cell cycle arrest, coupled to differentiation and apoptosis, butyrate has been shown to interfere with the activity of NF-κB (9–13). NF-κB is a transcription factor, induced by several proinflammatory cytokines, including TNF and Interleukin-1 (IL-1; 14). The activity of NF-κB is frequently deregulated during transformation of epithelial cells (15, 16), and in inflammatory bowel diseases (IBDs), such as ulcerative colitis (UC) and Crohn’s disease (12, 17, 18). The ability of nonsteroidal anti-inflammatory drugs (NSAIDs) and butyrate to inhibit NF-κB activity underlies the well-documented ability of these agents to exert anti-inflammatory as well as chemopreventive activity (10, 12, 19–25).

Another proinflammatory cytokine that is strongly up-regulated in IBD mucosa and that is secreted by lamina propria cells is IFN-γ (26–29). IFN-γ is not known to signal through NF-κB, but instead exerts its action through activation of the latent cytoplasmic transcription factor STAT1 (signal transducer and activator of transcription 1; 30). To better define the mechanism whereby butyrate suppresses inflammation, we examined the effect of butyrate on signaling by IFN-γ.

Binding of IFN-γ to the type II IFN receptor initiates signaling through transphosphorylation and activation of the Janus kinases JAK1/JAK2 (30). Exposure of cells to IFN-γ induces tyrosine (Y701) and serine (S727) phosphorylation of STAT1, which results in STAT1 dimerization, its nuclear translocation, DNA binding, and stimulation of expression of the IFN-responsive genes (31, 32). The JAK1/JAK2-mediated tyrosine phosphorylation of STAT1 is important for STAT1 dimerization, its translocation to the nucleus, DNA binding, and

activation of transcription (32). Serine phosphorylation of STAT1 is required for its full transcriptional activity (31), but the kinase responsible for the phosphorylation of STAT1 on S727 remains elusive.

Substantial levels of STAT1, as well as the other STAT family members (STAT3, STAT5, and STAT6), were detected in the colonic mucosa of patients with UC and Crohn’s disease (33). Significantly, STAT1 has been shown to be activated (i.e., phosphorylated) in UC patients and in pouchitis (34, 35). Abnormal activity of STAT1 has also been shown to accompany cellular transformation. The expression of STAT1 has been reported to be reduced (36–39) or constitutively activated in tumors (40–42), pointing to a complex role of STAT1 in tumorigenesis. It is noteworthy that patients with UC and Crohn’s disease are at increased risk to develop colorectal cancer (43, 44).

Here we report that butyrate inhibits signaling by IFN-γ. We showed that butyrate inhibits activation of STAT1, a transcription factor required for signaling by IFNs, through inhibition of its tyrosine and serine phosphorylation, nuclear translocation, and DNA binding activity. We suggest that this activity of butyrate is an important factor that underlies both its anti-inflammatory and chemopreventive activities.

Results

Butyrate Inhibits STAT1 DNA Binding Activity

The expression of STAT1 is frequently deregulated in intestinal inflammatory disease, such as UC (33–35), and the short chain fatty acid butyrate has been recognized as an effective treatment for multiple types of colitis (12, 22, 23). We therefore examined whether butyrate modulates STAT1 DNA binding activity in HCT116 and Hke-3 colon carcinoma cell lines by performing electromobility shift assays (EMSAs). HCT116 cells harbor a k-ras mutation and Hke-3 cells were derived from the HCT116 cell line by the targeted deletion of the mutant k-ras allele (45). We performed EMSA using a radiolabeled oligonucleotide comprising a STAT1 consensus DNA binding site (CAT GTT ATG CAT ATT CCT GTA AGT G). Nuclear proteins were isolated from HCT116 and Hke-3 cells that were either left untreated (0) or were treated with IFN-γ for 30 min alone or after pretreatment with sodium butyrate (Na-Bu; 3 mM) for 16 h. As shown in Fig. 1A, we did not detect STAT1 DNA binding activity in untreated cells, and treatment of cells with butyrate alone did not affect STAT1 DNA binding activity. IFN-γ induced STAT1 DNA binding in both cell lines, but consistently with higher efficiency in Hke-3 cells (Fig. 1A). Pretreatment of cells with butyrate, at concentrations that are readily attainable in the colonic lumen in vivo (46), inhibited IFN-γ induced STAT1 DNA binding activity in both cell lines. The specificity of DNA binding was confirmed by elimination of DNA binding with a 200-fold molar excess of the unlabeled wild-type oligonucleotide, but not an oligonucleotide containing a mutant STAT1 DNA binding site (Fig. 1B). The identity of the specific band was further confirmed by preincubating the nuclear extracts with STAT1 or STAT5 antibodies. As shown in Fig. 1B, specific binding was abolished by STAT1, but not the STAT5 antibodies. The specific band did not contain STAT3 (data not shown), excluding the formation of STAT1/STAT3 heterodimers by IFN-γ. We demonstrated that the levels of STAT1 were not reduced in butyrate-treated cells (see Fig. 4), excluding the possibility that butyrate abrogates STAT1 DNA binding through the inhibition of STAT1 expression.

These results demonstrate that in colon cancer cells, IFN-γ induces the formation of STAT1 homo- and heterodimers and that butyrate prevents binding of STAT1 homodimers to their specific DNA binding element.

Butyrate Inhibits STAT1 Nuclear Translocation

To determine the mechanism whereby butyrate inhibits STAT1 DNA binding activity, we examined whether butyrate inhibits STAT1 DNA binding by interfering with STAT1 nuclear translocation. Hke-3 cells were grown on chamber slides, serum starved overnight and treated with IFN-γ (50 ng/ml) for 30 or 60 min alone, or after precubication of cells with...
3 mM butyrate for 16 h. Cells were fixed in methanol/acetic acid, as described in “Materials and Methods,” and incubated with anti-STAT1 antibody for 1 h at 37°C and a secondary anti-mouse antibody conjugated to FITC for 45 min at 37°C. As shown in Fig. 2, STAT1 was localized exclusively to the cytoplasm in untreated cells (Fig. 2A) and butyrate did not alter subcellular localization of STAT1 (Fig. 2D). In contrast, treatment of cells with IFN-γ for 30 min induced a complete nuclear translocation of STAT1 which persisted for at least 60 min (Fig. 2, B and C). Pretreatment of cells with butyrate for 16 h completely inhibited IFN-γ-induced nuclear translocation of STAT1 (Fig. 2, E and F).

These results demonstrate that butyrate inhibits IFN-γ-induced translocation of STAT1 from the cytoplasm to the nucleus and thereby abrogates STAT1 DNA binding activity.

Butyrate Inhibits JAK2 Activation and Tyrosine and Serine Phosphorylation of STAT1

The signal for the nuclear translocation of STAT1 is its phosphorylation on tyrosine 701. We therefore tested whether butyrate interferes with nuclear translocation and subsequent DNA binding of STAT1 through inhibition of STAT1 tyrosine phosphorylation. HT29 colon cancer cells, which have wt k-ras and are highly responsive to treatment with IFN-γ, were treated with IFN-γ (50 ng/ml) for 15 min alone or after preincubation with 3 mM butyrate for 16 h. Cells were stained by a phosphospecific STAT1 antibody (anti STAT1 Y-701, Upstate Biotechnology, Charlottesville, VA), which recognizes activated STAT1, as described in “Materials and Methods.” Using this antibody, we detected a faint background staining in untreated cells as well as in butyrate-treated cells (Fig. 3, A and C). In contrast, treatment of cells with IFN-γ resulted in a distinct nuclear staining of cells for STAT1-Y701 (panel B). Pretreatment of HT29 cells with 3 mM butyrate for 16 h prevented IFN-γ-induced phosphorylation of STAT1 on Y701 (panel D).

Due to the presence of the background staining for STAT1-Y701 in untreated cells, we further examined the activation of STAT1 in Hke-3 cells and its modulation by butyrate by performing immunoblotting. Hke-3 cells were either left untreated, or were treated with IFN-γ for 15 or 30 min alone or after preincubation with butyrate for 16 h and cell lysates were examined for the expression of p-STAT1-Y701 by immunoblotting. Untreated cells did not have detectable levels of activated STAT1 and, consistent with results shown in Fig. 3, IFN-γ induced rapid tyrosine phosphorylation of STAT1, which was inhibited by butyrate by >95% (Fig. 4). Pretreatment of cells with butyrate for 1 h was not effective in inhibiting phosphorylation of STAT1, whereas 5 h pretreatment was sufficient to inhibit ~70% of STAT1 activity (data not shown). As we demonstrated that 16 h pretreatment with butyrate nearly completely inhibits STAT1 tyrosine phosphorylation, we used this time point in all our subsequent experiments.

IFN-γ is known to induce both tyrosine and serine phosphorylation of STAT1 and both modifications are required for the optimal activity of STAT1. To examine whether butyrate modulates also serine phosphorylation of STAT1, we examined the levels of STAT1 serine phosphorylation by immunoblotting using an anti STAT1-S727 antibody (Upstate Biotechnology). In contrast to STAT1 tyrosine phosphorylation, we detected low basal level of STAT1 phosphorylation on serine 727 in untreated Hke-3 cells, which was further increased by IFN-γ. Butyrate did not inhibit basal serine phosphorylation of STAT1, but it markedly inhibited the IFN-γ-induced increase in serine phosphorylation of STAT1 (Fig. 4), which may contribute to decreased IFN-induced, STAT1-dependent, transcripational activity in butyrate-treated cells (see below).

While the kinase responsible for serine phosphorylation remains elusive, JAK2 has been shown to phosphorylate STAT1 on tyrosine 701. We therefore tested whether butyrate inhibits STAT1 tyrosine phosphorylation through inhibition of JAK2. Cells were treated with IFN-γ, butyrate, or the combination of agents as described above, and JAK2 activity was assessed by using an antibody specific for JAK2 phosphorylated on Y1007 and Y1008 (Upstate Biotechnology). We detected low levels of activated JAK2 in untreated untreated...
cells (Fig. 4) and treatment of cells with IFN-γ further enhanced JAK2 phosphorylation. Densitometric analysis of the data demonstrated that butyrate significantly inhibited both constitutive and IFN-γ-induced JAK2 phosphorylation. This result suggests that at least in part, inhibition of JAK2 activation is responsible for the inhibition of tyrosine phosphorylation of STAT1 by butyrate. Fig. 4 also shows that the levels of total STAT1 and Rac1 (loading control) were not significantly modulated by treatment of cells with butyrate.

This result demonstrates that butyrate inhibits STAT1 nuclear translocation and its subsequent DNA binding through the inhibition of STAT1 phosphorylation on tyrosine 701 and implicates JAK2 as a target of butyrate action.

Butyrate Inhibits STAT1-Driven Transcriptional Activity

Phosphorylation of STAT1 on tyrosine and serine residues is required for its full transcriptional activation (31). Because we demonstrated that both serine and tyrosine phosphorylation of STAT1 is inhibited by butyrate, we examined whether butyrate inhibits transcriptional activity of STAT1. We used a reporter gene construct containing eight GAS (γ activated binding sites) and tested whether butyrate inhibits IFN-γ-driven transcription activity. Hke-3 cells were transiently transfected with the 8XGAS-LUC plasmid and were either left untreated or were treated simultaneously with IFN-γ alone, butyrate, or the combination of IFN-γ and butyrate for 24 h.

Transcriptional activity of the 8XGAS-LUC reporter plasmid was low in untreated cells and was assigned the value 1 (Fig. 5, and inset). Treatment of cells with IFN-γ significantly induced the promoter activity (50- to 120-fold increase, Fig. 5). Butyrate markedly inhibited both the basal activity of the reporter gene (see the inset, Fig. 5), as well as IFN-γ-induced transcriptional activation of the STAT1-dependent promoter (Fig. 5). Thus, although butyrate does not inhibit STAT1 expression (Fig. 4), it interferes with the expression of STAT1-regulated genes through inhibition of JAK2 activation and STAT1 phosphorylation.

**FIGURE 3.** Butyrate inhibits IFN-γ-induced tyrosine phosphorylation of STAT1. HT29 cells were either left untreated or were treated with IFN-γ alone (50 ng/ml) for 10 min or after preincubation with Na-Bu (3 mM) for 16 h as indicated. Immunofluorescence was performed as described in “Materials and Methods” using a phosphospecific STAT1 antibody, which recognizes STAT1 phosphorylated on tyrosine 701 (Upstate Biotechnology).

**Discussion**

IFNs are pleiotropic cytokines that mediate the anti-viral response, inhibit proliferation, and participate in immune surveillance and tumor suppression (47–49). In addition, IFN-γ has been shown to play a major role in the pathophysiology of several intestinal inflammatory diseases, including UC, pouchitis, and Crohn’s disease (26–29, 50).

Here we report that butyrate, at concentrations readily attainable in the colonic lumen in vivo (46), inhibits signaling by IFN-γ. We demonstrated that butyrate inhibits IFN-γ-induced tyrosine and serine phosphorylation of STAT1, which resulted in the inhibition of STAT1 nuclear translocation and DNA binding activity, and impaired STAT1-driven transcriptional activity. These data strongly suggest that butyrate interferes with the expression of STAT1-dependent genes.

Targeted disruption of STAT1 in mice has revealed that STAT1 is an obligatory transcription factor for signaling by IFNs (30). Abnormal activity of STAT1 has been demonstrated in human malignancies (39, 51) and in IBDs, such as UC and
Crohn’s disease, which show constitutive activation of several members of the STAT family, including STAT1 (33, 34). JAKs are important upstream activators of STAT1 and endogenous JAK inhibitors, such as JAB (JAK binding protein) and SOCS1 (suppressor of cytokine signaling 1), play a negative regulatory role in intestinal inflammation by down-regulating STAT activity (33). In addition, treatment of IBD patients with glucocorticoids resulted in decreased activation of STAT1 in intestinal mucosa (34), suggesting that glucocorticoids inhibit intestinal inflammation at least in part through the inhibition of STAT1 activation.

Because IFN-γ is one of the main cytokines produced at sites of inflammation in IBD, we propose that the ability of butyrate to inhibit signaling by IFN-γ is an important mechanism whereby butyrate exerts its anti-inflammatory activity in UC patients. Our data demonstrate that butyrate inhibits IFN signaling at the level of phosphorylation of the main transcription factor in the IFN signaling cascade, STAT1, which may contribute to the ability of butyrate to decrease inflammation, improve clinical recovery, and to restore the absorption of electrolytes in UC patients (19, 22, 23, 52). Our data demonstrate that butyrate inhibits STAT1 activation at least in part through the inhibition of JAK2 phosphorylation. However, we have not tested the effect of butyrate on JAK1 activation and/or IFN-γ receptor phosphorylation, which could further modulate IFN/STAT signaling.

![Figure 4](image1.png) Butyrate inhibits STAT1 phosphorylation on tyrosine 701 and serine 727. Cell lysates were isolated from Hke-3 cells that were either left untreated or were treated with 3 mM Na-Bu for 16 h, IFN-γ for 15 or 30 min alone, or after preincubation with Na-Bu for 16 h. The levels of pSTAT1-Y701, p-STAT1-S727, p-JAK2, total STAT1, and Rac1 were detected by Western blot analysis. Densitometry was performed to quantitate the changes in IFN- and butyrate-treated cells. NSP, nonspecific band.

![Figure 5](image2.png) Butyrate inhibits STAT1 transcriptional activity. Hke-3 cells were transfected with a reporter plasmid containing eight GAS sites (pGL3-8GAS-LUC) and treated with Na-Bu (3 mM), IFN-γ (1 or 100 ng/ml), or the combination of IFN-γ and butyrate for 24 h. The results are expressed as the ratio between the activity of the reported plasmid in treated and untreated cells. Columns, mean fold increase over untreated cells calculated from four independent experiments; bars, SE. The inset is shown to demonstrate the effect of butyrate on the basal STAT1 activity.
Transforming growth factor β (TGF-β) is an important negative regulator of mucosal inflammation and also acts as a negative regulator of cell proliferation (53). However, patients with IBD display marked overexpression of SMAD7, a negative regulator of TGF-β signaling (54). IFN-γ-STAT1 signaling has been shown to inhibit TGF-β/SMAD signaling through activation of SMAD7 expression (55). Thus, it is possible that butyrate, through the inhibition of STAT1 signaling, negatively regulates SMAD7 expression and thereby restores TGF-β signaling in inflamed and/or transformed tissue.

Dysplastic lesions associated with UC mucosa have been suggested to be precursors of carcinoma formation (56–58). Accordingly, UC patients have an increased risk of developing colon cancer. The frequent cycles of epithelial injury and regeneration associated with chronic inflammation in IBD increase the likelihood of cellular transformation; however, the molecular pathways leading to colon cancer due to chronic inflammation are less understood than the classical Apc-initiated tumorigenesis. The IFN-responsive gene IFN-1-8U has been shown to be significantly overexpressed in both UC-associated colon cancers and in inflamed colonic mucosa (59). Although its expression is related to the severity of the disease, its function is still unknown. Nevertheless, the ability of butyrate to inhibit the basal expression of the IFN-1-8U gene in SW620 cells (data not shown) may underlie both its anti-inflammatory and chemopreventive activities. Furthermore, butyrate has been shown to inhibit IFN-γ-induced expression of the IL-18 binding protein (IL-18BP) (60). Evidence suggests that IL-18 is an inhibitor of tumor growth (61, 62) and that IL-18BP neutralizes IL-18 biological activity (63). The fact that butyrate enhances bioactivity of IL-18 through inhibition of the IL-18BP synthesis is consistent with the tumor suppressive/chemopreventive potential of butyrate.

We recently performed cDNA microarray analysis in the SW620 colon cancer cell line to compare their response to butyrate and the pharmacological chemopreventive agent, sulindac (64). Analysis of these data revealed that both butyrate and sulindac inhibit the basal expression of several IFN-dependent genes (data not shown). Sulindac has recently been shown to inhibit activation of STAT3 (65); however, its effects on STAT1 activity have not been examined. These data suggest that butyrate and sulindac may inhibit constitutive activation of STAT1 and STAT3, often present in tumors.

Several cytokines, in addition to IFN-γ, signal through STAT1 activation, such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), IL-6, and colony-stimulating factor 1 (CSF-1), which play an important role in both inflammation and colon cancer. For example, intact EGF receptor (EGFR) signaling has been shown to be required for the development of Apc-initiated tumors (66). STAT1 and STAT3 are directly phosphorylated by EGFR, the expression of which is up-regulated in colon cancers (67) and activated by bioactive lipids, such as PGE2 (68). Therefore, our data suggest that the short chain fatty acid butyrate could interfere with the activity of several cytokines, in addition to IFN-γ, which signal through STAT1.

In summary, we demonstrated that the short chain fatty acid butyrate inhibits signaling by IFN-γ through the inhibition of STAT1 activation. Our results advance the understanding of the anti-inflammatory effect of butyrate in intestinal inflammatory diseases such as UC, and strongly suggest that components of the JAK/STAT signaling pathway may represent novel molecular targets for the action of chemopreventive agents in colon cancer.

**Materials and Methods**

**Cells and Western Blot Analysis**

The HCT116 colorectal carcinoma cell line, its clonal derivative Hke-3 that lack the mutant k-ras allele, and HT29 cells were cultured under standard conditions in MEM, supplemented with 10% FCS and antibiotics. Western blot analysis was performed using standard procedures. Briefly, 50 μg of total cell lysates were separated by 10% SDS polyacrylamide gels and transferred to nitrocellulose membrane. The membrane was incubated with antibodies for 3 h at room temperature and enhanced chemiluminescence (Amersham, Piscataway, NJ) was used for visualization of immune complexes.

**Electromobility Shift Assay**

Cells were pelleted, washed 3 times with PBS, and solubilized with buffer A (20 mM HEPES, 10 mM KCl, 1 mM EDTA, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 0.1 mM NaN₃, 0.2% NP40, 10% glycerol, and 1 μg/ml of each aprotinin, pepstatin, and leupeptin). The lysates were centrifuged and nuclear proteins extracted with buffer B (20 mM HEPES, 350 mM NaCl, 10 mM KCl, 1 mM EDTA, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 0.1 mM NaN₃, 20% glycerol, and 1 μg/ml of each aprotinin, pepstatin, and leupeptin). The extracts were centrifuged and supernatants quickly frozen at −80°C. Five micrograms of nuclear proteins were incubated with a radiolabeled oligonucleotide (50,000 cpm) comprising a STAT1 binding site (CAT GTT AGT CAT ATT CCT GTA AGT G). One microgram of poly(dI-dC) was used as a nonspecific DNA competitor in each reaction. The specificity of binding was controlled by the addition of an excess of the unlabeled wt oligonucleotide or the oligonucleotide with a mutated STAT1 binding site (CAT GTT ATG CAT ATT CCT GTA AGT G) (200-fold molar excess). The identity of the proteins bound to the radiolabeled probe was determined by preincubating nuclear proteins for 10 min on ice with antibodies specific for STAT1, STAT3, or STAT5 (Upstate Biotechnology). DNA/protein complexes were separated on 6% non-denaturing gels in 0.25× TBE and their mobility detected by autoradiography.

**Immunofluorescence**

Cells were grown on chamber slides, serum starved for 16 h, and were either treated with IFN-γ for 30 or 60 min alone, or after preincubation with 3 mM Na-Bu for 16 h. Cells were fixed in ice-cold methanol-acetic acid solution (95:5 v/v) for 20 min at −20°C. Incubation with anti-STAT1 (Upstate Biotechnology) or anti-phospho-STAT1 (Y701, Upstate Biotechnology) antibodies was performed for 1 h at 37°C. Slides were washed with PBS and incubated with a secondary anti-rabbit antibody conjugated to FITC for 45 min at 37°C. Samples were examined with a fluorescent microscope and photographs taken utilizing a SPOT CCD camera and analyzed by SPOT software.
Transient Transfections and Reporter Genes Assay

To examine the effect of butyrate on STAT1-dependent transcriptional activity, we used plasmid 8XGAS-p36LUC which was kindly provided to us by Dr. C. Glass. It contains eight GAS (gamma activated sequence) sites cloned upstream of the luciferase reporter gene. Cells were transfected with 1 µg of DNA/six-well plate using the Ca phosphate method. Transfection efficiency was normalized by cotransfection with pTK-renilla (Dual-luciferase reporter assay system, Promega, Madison, WI). Cells were either left untreated or were treated with IFN-γ (1 or 100 ng/ml) alone or in the presence of 3 mM butyrate. The basal and IFN-γ inducible transcriptional activity was determined 48 h after transfection (24 h after treatment).

Results are expressed as the ratio between the activity of the reporter plasmid in treated and untreated cells.

References


Inhibition of Interferon-γ Signaling by Butyrate

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Inhibition of Interferon γ Signaling by the Short Chain Fatty Acid Butyrate Inhibition of Interferon γ Signaling by the Short Chain Fatty Acid Butyrate

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