The proliferation of the rat intestinal mucosal IEC-6 cell line requires polyamines, whose synthesis is catalyzed by the enzyme ornithine decarboxylase (ODC). ODC inhibition leads to polyamine depletion, as well as inhibition of both cell proliferation and apoptosis by regulating gene expression. The NF-κB transcription factor regulates genes involved in apoptotic, immune, and inflammatory responses. In the present study we tested the hypothesis that NF-κB is activated following ODC inhibition. We found that the inhibition of ODC by α-difluoromethylornithine (DFMO) resulted in a ~50% decrease in intracellular putrescine levels within 1 h. NF-κB is activated by DFMO through the degradation of the inhibitor protein IκBα that sequesters NF-κB in the cytoplasm. The DFMO-induced NF-κB complexes contain the p65 and p50 members of the Rel protein family. DFMO-induced NF-κB activation was accompanied by the translocation of p65 from the cytoplasm into the nucleus. DFMO selectively inhibited a gene reporter construct dependent on the κB site present in the HLA-B7 gene. In contrast, DFMO had no effect on a gene reporter construct dependent on the κB site present in the interleukin-8 gene. Thus, we report that ODC inhibition activates the NF-κB transcription factor, which may mediate the altered physiological state of intestinal cells that occurs following polyamine depletion.

The polymamines, spermidine and spermine, and their precursor putrescine are intimately required for cell growth and proliferation (1). Intracellular polyamine levels are highly regulated by ornithine decarboxylase (ODC), which catalyzes the first rate-limiting step in polyamine biosynthesis. Therefore, specific inhibitors of ODC such as α-difluoromethylornithine (DFMO) have been used to define the role of polyamines in cellular processes. The role of polyamines in the growth and repair of gastrointestinal mucosa has been examined extensively in the cultured intestinal epithelial cell line IEC-6, a nontransformed line derived from adult rat crypt cells. Previous studies have established that inhibition of ODC activity in IEC-6 cells leads to alterations in gene expression (2). Although polyamines are critical for optimal cell growth, excessive accumulation may interfere directly with normal cell function. Polyamines have been implicated recently in the control of the apoptotic response. For example, polyamine depletion by DFMO treatment in IEC-6 cells delays the onset of apoptosis by tumor necrosis factor-α and the DNA topoisomerase inhibitor camptothecin (3). In contrast, in cells overexpressing ODC excessive accumulation of polyamines triggers apoptosis (4, 5).

Nuclear factor κB (NF-κB) is an inducible and ubiquitously expressed transcription factor. NF-κB is a central regulator of the transcription of genes involved in cell survival, as well as genes involved in cell adhesion, immune and inflammatory responses, differentiation, and growth (6–10). Active NF-κB complexes are dimers of various combinations of the Rel/NF-κB family of polypeptides, which includes p50, p65, c-Rel, v-Rel, RelA (p65), and RelB (reviewed in Refs. 11 and 12). NF-κB is sequestered in the cytoplasm by binding to inhibitory IκB proteins, which block the nuclear localization sequences of NF-κB. NF-κB is activated by a variety of stimuli, including phorbol esters, cytokines (IL-1, interferon-α/β, and tumor necrosis factor), lipopolysaccharide, double-stranded RNA, bacteria, and viral transactivators. NF-κB-inducing stimuli promote dissociation of the inactive NF-κB/IκB complexes via the serine phosphorylation and degradation of IκB. These events lead to the unmasking of the nuclear localization sequence of NF-κB, thereby allowing NF-κB to enter the nucleus and bind κB-regulatory elements. In this study we tested the hypothesis that NF-κB is activated following inhibition of ODC in intestinal cells. We found that inhibition of ODC by DFMO treatment results in depletion of cellular putrescine levels by 1 h. The depletion of putrescine levels was accompanied by rapid induction of NF-κB activation as determined by its presence in κB-dependent DNA-protein complexes. Several distinct complexes were detected that differ in Rel protein composition. In response to polyamine depletion, NF-κB translocated from the cytoplasm into the nucleus. The DFMO-induced NF-κB/IκB complexes selectively inhibited κB-dependent reporter constructs. Thus, ODC inhibition by DFMO depletes cellular polyamine levels and activates the NF-κB transcription factor, which may repress the expression of important cellular genes in intestinal cells. The regulation of gene expression mediated by NF-κB activation may manifest itself in alterations in cell physiology, such as the intrinsic resistance of polyamine-depleted cells to apoptosis.
ODC Inhibition Activates NF-κB

MATERIALS AND METHODS

Cells—The normal rat intestinal epithelial IEC-6 cell line (13) was obtained from the American Type Culture Collection (CRL-1592). IEC-6 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 5% dialyzed fetal bovine serum, 10 μg of insulin/ml and 0.05 mg of gentamicin/ml (sDMEM). Stock cultures were subcultured once a week at 1:20, and sDMEM was replenished three times weekly. For experiments, the cells were plated at 4 × 10^5 cells/cm^2 in sDMEM containing 5 mM d, l-difluoromethylornithine (DFMO). This dose of DFMO markedly inhibits ODC activity (95%) and entirely depletes putrescine and spermidine from IEC-6 cells by 6 and 48 h, respectively (2). In addition, this dose partially (60%) depletes spermine by 4 days. Control cultures received no DFMO. In some experiments 10 μM putrescine was added simultaneously with DFMO to demonstrate that exogenous polyamines can prevent the effects of DFMO. The inhibition of growth and migration resulting from polyamine depletion in IEC-6 cells can be prevented by adding 5 μM spermine or 10 μM putrescine to DFMO-containing medium (2). Thus, the effects of DFMO treatment are caused by the absence of polyamines and not by DFMO itself.

Nuclear Extracts and Gel Shift Assays—For preparation of nuclear extracts, the cultures were washed with ice-cold phosphate-buffered saline and harvested with a rubber policeman. Nuclei were extracted with buffer (20 mM Tris-HCl, pH 7.85, 250 mM sucrose, 0.4 mM KCl, 1.1 mM MgCl₂, 5 mM β-mercaptoethanol, 1 mM NaF, 1 mM Na₃VO₄, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml soybean trypsin inhibitor, 5 μg/ml leupeptin and 1.75 μg/ml benzamidine), and extracts were frozen and stored at −80 °C (14). For EMSA, the nuclear extracts were incubated with a 32P-labeled NF-κB probe (5′-AGTTGAGGGACCTTTCCACGG-3′) derived from an NF-κB binding sequence in the immunoglobulin gene promoter (15). To define the presence of specific Rel proteins, nuclear extracts were preincubated with a 1:25 dilution of anti-Rel antibodies at 25 °C for 20 min and then subjected to EMSA. The gels were quantitated by PhosphorImager autoradiography (Molecular Dynamics).

Assay of Intracellular Putrescine—In brief, IEC-6 cells were rinsed three times with ice-cold PBS, 0.5% perchloric acid was added, and the cells were frozen at −80 °C. The extracts were subjected to dansylation, and the intracellular level of putrescine in the range from 0.3 to 10 nmol/mg protein was determined by HPLC as described previously (2). The protein concentrations in extracts were determined by the Bradford method.

Immunocytochemistry—IEC-6 cells were rinsed with PBS without Ca²⁺ or Mg²⁺ (PBS−), fixed for 10 min in 4% paraformaldehyde in PBS−, permeabilized for 5 min in 0.2% Triton X-100, and blocked with 3% bovine serum albumin in PBS−. The cells were successively stained at room temperature for 1 h with rabbit polyclonal antibody to p50 or p65 (p50 or p65 Abs: Santa Cruz Biotechnology, Santa Cruz, CA) and fluorescein isothiocyanate-conjugated anti-rabbit IgG. The cells were washed extensively after each incubation with PBS− and mounted with VectaShield. The Images captured on a Bio-Rad MRC-1024 LaserSharp confocal laser scanning microscope were processed using Adobe Photoshop.

IκBα Degradation—At various times after DFMO treatment, 1 × 10⁸ cells were lysed directly in Laemmli buffer, and equivalent amounts of protein were subjected to SDS-polyacrylamide gel electrophoresis. The proteins were transferred to polyvinylidene difluoride membranes, immunoblotted with specific affinity-purified rabbit anti-IκBα antibody, and visualized by chemiluminescence with the ECL reagent (Amer sham Pharmacia Biotech).

Transcriptional Assays—IEC-6 cells were transfected by electroporation with either the pUXCAT promoter-less chloramphenicol acetyltransferase (CAT) construct; the pUXCAT 3XHLAκB construct, which contains three tandemly repeated copies of the NF-κB site from the HLA-B7 gene; or pUXCAT 3XIL8κB, which contains three tandemly repeated copies of the NF-κB site from the IL-8 gene (16). At 24–48 h after transfection, the cells were transfected with DFMO for the indicated time or with both putrescine and DFMO and assessed for CAT activity. Acetylated and unacetylated [14C]chloramphenicol were separated by thin layer chromatography, and the radioactivity was measured by PhosphorImager autoradiography.

Statistics—All data are expressed as the means ± S.E. from representative experiments. All experiments were repeated three times, in triplicate. Analysis of variance and appropriate post hoc testing determined the significance of the differences between means. Values of p < 0.05 were regarded as significant.

RESULTS

Polyamine Depletion Induces the Activation of NF-κB DNA Binding Activity—NF-κB proteins are present in the cytoplasm as latent transcription factors. To determine whether polyamine depletion induced NF-κB activation, IEC-6 cells were treated with DFMO in the presence or absence of putrescine. Nuclear extracts were prepared from the treated cells and incubated with a labeled κB oligonucleotide probe, and the resultant DNA-protein complexes were analyzed by EMSA. Nuclear extracts from untreated IEC-6 cells show little detectable constitutive binding to a consensus κB oligonucleotide probe. However, DFMO induced κB binding within 1 h (noted by the arrows in Fig. IA). κB binding persisted for 24 h after DFMO treatment (Fig. IA). The DFMO-induced NF-κB complexes at all times examined specifically reflected the effects of ODC inhibition and blockage of polyamine synthesis because their induction was prevented by the addition of putrescine.

To determine whether the binding to the κB probe was specific, nuclear extracts prepared from DFMO-treated cells were incubated in the presence of a 50-fold excess of unlabeled κB or an unrelated oligonucleotide probe. No DNA binding to the κB probe was detected in the presence of excess unlabeled κB oligonucleotide, and binding was competed for by an excess of unrelated sis-inducible element oligonucleotide probe corresponding to a STAT-dependent DNA element (Fig. IB). Taken together these results indicate that the binding to the κB probe was specific.

As shown in Fig. 1, two distinct complexes were found to bind the κB probe. These DFMO-induced complexes were detectable within 1 h of DFMO treatment and persisted for at least 24 h. Because active NF-κB complexes are dimers of various combinations of the Rel/NF-κB family of polypeptides, we defined the
ODC Inhibition Activates NF-κB

Effects of Polyamine Depletion on the Distribution of p65—In unstimulated cells, NF-κB is localized in the cytoplasm because of the binding of inhibitory IκB proteins, which block the nuclear localization sequences present in NF-κB proteins. Upon stimulation, the inactive NF-κB/IκB complexes dissociate, the nuclear localization sequences of NF-κB proteins are unmasked, and NF-κB complexes enter the nucleus. As illustrated in Fig. 3, p65 (RelA) is distributed diffusely in the cytoplasm of control IEC-6 cells with no nuclear staining. After 1 h of treatment with DFMO, there was a dramatic redistribution of p65 into the nucleus, so that p65 was nearly exclusively in the nucleus with little cytoplasmic staining (Fig. 3). The translocation of p65 into the nucleus induced by DFMO treatment persisted for at least 5 h and specifically reflected the effects of ODC inhibition and blockage of polyamine synthesis because nuclear translocation was prevented by the addition of putrescine. Thus, p65 was present nearly exclusively in the cytoplasm of IEC-6 cells incubated for 5 h with DFMO in the presence of putrescine and resembled the distribution in control, untreated IEC-6 cells. The immunofluorescent studies on p65 confirmed the findings obtained by gel shift analysis, i.e. p65 translocates into the nucleus of IEC-6 cells upon DFMO addition.

Polyamine Depletion Promotes the Degradation of IκBα—The activity of NF-κB is tightly controlled by inhibitory IκB proteins that bind to NF-κB complexes and sequester NF-κB in the cytoplasm (11, 12). Viruses, cytokines, lipopolysaccharides, and other stimulating agents promote NF-κB activation by the serine phosphorylation and subsequent degradation of IκB. To determine whether NF-κB activation observed after DFMO treatment reflects IκBα degradation, IκBα levels were determined at various times after DFMO addition by immunoblotting with anti-IκBα antisera. As shown in Fig. 4, DFMO induced a progressive decrease in cellular levels of IκBα, indicating that DFMO induced NF-κB activation by promoting IκBα degradation. IκBα degradation was observable within 1 h of DFMO addition, and a dramatic decrease in IκBα levels was observed at 5 h after treatment. Quantitation of IκBα levels by PhosphorImager analysis of the data demonstrated a 15% decrease in IκBα levels at 1 h and a 70% decrease at 5 h. The kinetics of IκBα degradation are consistent with that of NF-κB activation, i.e. a detectable decrease in IκBα at 1 h, at which time NF-κB activation is observed. DFMO-induced degradation of IκB specifically reflected the effects of blockage of polyamine synthesis because it was prevented by the addition of putrescine (Fig. 4).

Effects of Polyamine Depletion on NF-κB-dependent Reporter Constructs—To determine the functional consequences of NF-κB activation induced by inhibition of ODC, we examined the effect of DFMO treatment on the transcriptional activity of CAT reporter constructs driven by NF-κB-dependent promoters. IEC-6 cells were transfected with either the pUXCAT promoter-less CAT construct; the pUXCAT 3XHLA-κB construct, which contains three tandemly repeated copies of the NF-κB site from the HLA-B7 gene; or pUXCAT 3XIL8κB, which contains three tandemly repeated copies of the NF-κB site from the IL-8 gene (16). The transfected cells were treated for either 1 or 24 h with DFMO and then assayed for CAT activity. As shown in Fig. 5A, there was significant basal activity of the promoterless CAT construct as determined by the
formulation of acetylated chloramphenicol. The basal activity of the promoter-less construct was not affected by DFMO treatment. In contrast, DFMO treatment for either 1 or 24 h resulted in a marked decrease (~90% inhibition) in the transcriptional activity of the 3XHLAxB construct, which contains three tandemly repeated copies of the NF-κB site from the HLA-B7 gene. In addition, DFMO treatment had no effect on a reporter construct driven by the NF-κB site from the IL-8 gene (3XIL8xB CAT).

A time course of the effect of DFMO on the CAT reporter activity driven by the HLA κB site demonstrated that the inhibitory effect was first detected at 1 h of DFMO addition (Fig. 5). The inhibitory effect of DFMO was not observed in a CAT reporter activity driven by the IL-8 κB site. The inhibitory effect of DFMO on the HLA κB-dependent promoter persisted for at least 3 days after DFMO addition (data not shown). The decreased transcriptional activity of the HLA κB-dependent reporter construct was specific for polyamine depletion, because putrescine addition blocked the DFMO-induced decrease in CAT reporter activity (Fig. 5B).

DISCUSSION

The polyamines, spermidine and spermine, and their precursor putrescine are found in virtually all cells of higher eukaryotes and are intimately involved in and are required for cell growth and proliferation (1). An important mechanism of action of the polyamines concerns their control of growth-regulated genes. Increased ODC activity occurs concomitantly with increases in the mRNA of several proto-oncogenes in growth-stimulated cells (17). ODC is the enzyme responsible for catalyzing the first rate-limiting step in polyamine biosynthesis. Inhibition of ODC activity by DFMO decreases mRNA levels for the c-fos, c-myc, and c-jun proto-oncogenes in IEC-6 cells (18). In the present report we investigated the potential role of the NF-κB transcription factor family in the regulation of gene expression by polyamines. We report that inhibition of ODC induces the activation of the NF-κB transcription factor as measured by its presence in DNA-binding complexes and its translocation from the cytoplasm into the nucleus. DFMO-induced activation of NF-κB specifically reflected the effects of ODC inhibition and blockage of polyamine synthesis because it was prevented by the addition of putrescine, which is the end product of the reaction catalyzed by ODC.

Distinct DFMO-induced NF-κB complexes were detected that differ in Rel protein composition as well as in their time course of activation. Two DFMO-induced complexes were detectable within 1 h of DFMO treatment and persisted for at least 24 h. The results of supershift analysis with specific Rel antisera indicate that these complexes are comprised of p50 and p65. The dimeric forms of NF-κB differ in their preference for certain κB sites on DNA, transactivation potentials, kinetics of nuclear translocation, and levels of tissue expression (11). The p50 homodimer is generally thought to act as an inhibitor of κB-dependent transcription (19).

Of particular interest was the rapidity of NF-κB activation in response to DFMO. In Fig. 2 we show that in IEC-6 cells putrescine levels decreased by 50% within 1 h of exposure to DFMO and are at undetectable levels by 6 h. These data are nearly identical to those previously reported for IEC-6 cells (2). However, the levels of the polyamines, spermidine and spermine, did not decline at this time. In fact, spermidine does not decline to undetectable levels until 48 h of DFMO addition, and significant spermine remains after 6 days (2). Therefore, these results demonstrate that NF-κB activation is clearly not caused by a general “polyamine depletion.” Instead, we believe that there is only a small pool of free polyamines within the cell. This pool, consisting largely of putrescine, is rapidly depleted by conversion to spermidine upon inhibition of new polyamine synthesis. Because almost all intracellular polyamines must be bound and unavailable for biological processes, a slight decrease in putrescine levels is sensed by the cell and activates a NF-κB-dependent response pathway. This view of maintaining cellular polyamine equilibrium is supported by the often reported finding that ODC activity peaks within 3–4 h of cellular exposure to serum or other growth stimuli (1). It is obvious that cellular polyamines are not free to take part in the proliferative response and that new polyamines must be synthesized.

We next investigated the effect of ODC inhibition on transcriptional activity using κB-dependent reporter gene assays. DFMO selectively inhibited a gene reporter construct dependent on the κB site present in the HLA-B7 gene but had no effect on a construct dependent on the κB site present in the IL-8 gene. The inhibitory effect of DFMO on the κB site present in the HLA-B7 gene was detectable within 1 h and persisted for at least 24 h after DFMO treatment. These results correlate with the time course of DFMO induction of NF-κB activation, activation within 1 h that persists up to 24 h after DFMO addition. This is of particular importance, as noted above, because the p50 homodimer apparently inhibits NF-κB-dependent transcription. Moreover, the results on the inhibitory effect of DFMO on the κB site in the HLA-B7 gene versus the IL-8 gene highlight the selective effects of decreases in polyamine levels.
on the activated NF-κB complexes that bind to κB regulatory elements. A similar selectivity for κB sites has been described for the interferon-induced expression of a CAT reporter construct driven by the κB site in HLA-B7 gene, but interferon had no effect on a construct driven by the κB site in the IL-8 gene (20).

The polyamine spermine activates NF-κB in human breast cancer cells (21, 22). These findings appear to be in conflict with the results reported herein. One possible explanation for this discrepancy is the differential responses of normal cells (IEC-6 cells) versus cancer cells to polyamine effects. Alternatively, both inhibition of ODC activity by DFMO (Fig. 2) and exogenous spermine addition (21) result in depletion in putrescine levels. Because a slight decrease in putrescine levels may be sensed by the cell to activate a NF-κB-dependent response pathway, these apparently contradictory reports may be entirely consistent with one another.

An emerging area of research in intestinal homeostasis and inflammation is the role of NF-κB in regulating intestinal epithelial cell (IEC) gene expression (23, 24). IECs form a single layer of cells that isolate the host from the hostile gut luminal environment. Aside from their classical absorptive and physical barrier roles, an emerging concept views IECs as immunological sentinels of the intestinal mucosa. IECs are capable of responding to a wide array of biologically active agents commonly found in the lumen, including bacterial products, adherent and invasive bacteria, cytokines, and short chain fatty acids. NF-κB regulates the transcription of a number of proinflammatory molecules, including IL-1β, tumor necrosis factor-α, IL-6, IL-8, IL-12, inducible nitric-oxide synthase, ICAM-1, VCAM-1, and major histocompatibility complex class II molecules, involved in acute responses to injury and in chronic intestinal inflammation (23, 24). NF-κB activation has been documented in the intestine of patients with various forms of inflammatory bowel disease, such as Crohn’s disease, ulcerative colitis, and self-limited colitis (25–27). Immunohistochemistry performed on tissue sections isolated from patients with inflammatory bowel disease demonstrates the presence of activated NF-κB in IECs located at the crypts but not at the surface region (26). Thus, it is clearly important that we have found that polyamine depletion of IEC-6 cells results in NF-κB activation.

In many cell types NF-κB plays a protective role against apoptosis, mediated by death signals such as tumor necrosis factor-α and radiation (6–9). Therefore, NF-κB activation may have an impact on intestinal hyperplasia through cell removal by apoptosis in a manner similar to experimental rheumatoid arthritis (28). We report that ODC inhibition by DFMO treatment rapidly induces NF-κB activation. It has been recently shown that apoptosis induced by DNA damaging agents and tumor necrosis factor-α is delayed in polyamine-depleted cells (3). Taken together, these results implicate NF-κB in the protective action of DFMO against apoptotic agents in IEC-6 cells.

In summary, we have shown that polyamine depletion activates the NF-κB transcription factor. NF-κB activation resulted in the inhibition of selective NF-κB-dependent reporter constructs. Therefore, our results show that polyamine depletion in intestinal epithelial cells activates the NF-κB signal transduction pathway, which is a pathway with important physiological consequences.

Acknowledgments—We thank Dr. Nancy Rice for generously providing anti-NF-κB/Rel Abs and Dr. Jan Vilecek for generously providing CAT reporter constructs.

REFERENCES
1. Pegg, A. E., and McCann, P. P. (1982) Am. J. Physiol. 243, C212–C221
2. McCormack, S. A., Viar, M. J., and Johnson, L. R. (1993) Am. J. Physiol. 264, G367–G374
3. Ray, R., Viar, M. J., Yuan, Q., and Johnson, L. R. (2000) Am. J. Physiol. 278, C480–C489
4. Pouline, R., Pelletier, G., and Pegg, A. E. (1995) Biochem. J. 311, 723–727
5. Tobias, K. E., and Kahana, C. (1995) Cell Growth Differ. 6, 1279–1285
6. Beg, A. A., Sha, W. C., Bronson, R. T., Ghosh, S., and Baltimore, D. (1995) Nature 376, 167–170
7. Beg, A. A., and Baltimore, D. (1996) Science 274, 782–784
8. Van Antwerp, D. J., Martin, S. J., Kafri, T., Green, D. R., and Verma, I. M. (1996) Science 274, 787–789
9. Wang, C.-Y., Mayo, M. W., and Baldwin, A. S., Jr. (1996) Science 274, 784–787
10. Baeuerle, P. A. (1998) Cell 729–731
11. Baeuerle, P. A., and Baltimore, D. (1996) Cell 87, 13–20
12. Thanos, D., and Maniatis, T. (1995) Cell 80, 529–532
13. Quaroni, A., Wands, J., Trexler, R. L., and Isselbacher, K. J. (1988) J. Cell Biol. 106, 248–265
14. Yang, C. H., Shi, W., Basu, L., Murti, A., Constantinescu, S. N., Blatt, L., Croze, E., Mullersmann, J. E., and Pfeffer, L. M. (1996) J. Biol. Chem. 271, 8057–8061
15. Yang, C. H., Murti, A., and Pfeffer, L. M. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 5568–5572
16. Oliviera, I. C., Mukaida, N., Matsushiam, K., and Vilcek, J. (1994) Mol. Cell. Biol. 14, 5300–5308
17. Celano, P., Baylin, S. B., and Casero, R. A. (1989) J. Biol. Chem. 264, 8922–8927
18. Wang, J.-Y., McCormack, S. A., Viar, M. J., Wang, H., Tzen, C.-Y., Strober, W., and Johnson, L. R. (1993) Am. J. Physiol. 265, G331–G338
19. Schmitz, M. L., and Baeuerle, P. A. (1991) EMBO J. 10, 3805–3817
20. Yang, C. H., Murti, A., Pfeffer, S. R., Kim J. G., Donner, D. B., and Pfeffer, L. M. (2001) J. Biol. Chem. 276, 8927–8936
21. Shah, N., Thomas, T., Shirahata, A., Sigal, L. H., and Thomas, T. J. (1999) Biochemistry 38, 14763–14774
22. Shah, N., Thomas, T. J., Lewis, J. S., Kringe, C. M., Shirahata, A., Gelinas, C., and Thomas, T. (2001) Oncogene 20, 1715–1729
23. Schmid, R. M., and Adler, G. (2000) Gastroenterology 118, 1208–1228
24. Jobin, C., and Sartor, R. B. (2000) Am. J. Physiol. 278, C451–C462
25. Neurath, M., Pettersson, S., Meyer Zum Buschenfelde, K.-H., and Strober, W. (1996) Nat. Med. 2, 999–1004
26. Rogler, G., Brandt, K., Vogl, D., Pape, S., Hofmeister, R., Andus, T., Keuselch, R., Baeuerle, P. A., Scholerich, J., and Gross, V. (1998) Gastroenterology 115, 357–369
27. Schreiber, S., Nikolaus, S., and Hampe, J. (1998) Gut 42, 477–484
28. Miagkov, A. V., Kovalenko, D. V., Brown, C. E., Didsbury, J. R., Cogswell, J. P., Stimpson, S. A., Baldwin, A. S., and Makarov, S. S. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 13859–13864
