Butyrate Enhances the In Vitro Anti-SRBC (Sheep Red Blood Cell) Antibody Responses in Murine Splenocytes

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ABSTRACT—Butyrate at concentrations of 200–600 µM markedly enhanced the in vitro antibody productions against sheep red blood cells (SRBC) in murine splenocytes. However, other saturated short-chain fatty acids, including acetate, propionate and valeric acid, and 4-carbon compounds such as butanol, acetoacetate and β- and γ-hydroxybutyrate had no such effects. The presence of butyrate in the early phase of the cell culture was crucial for enhancement of the response. Butyrate also augmented the antibody production in T-cell-depleted splenocytes supplemented with the culture supernatant of concanavalin A (Con A)-stimulated lymphocytes. Interleukin (IL)-2 secreted from splenocytes in response to SRBC was increased by adding butyrate to the culture, but IL-1 secretion was not affected. On the other hand, Con A or lipopolysaccharide-stimulated proliferation of splenocytes was partly depressed by the addition of butyrate, while Con A-induced IL-2 production was not effected. These findings suggest that butyrate may act on T and B cells to promote their differentiation during the process of antibody production.

Keywords: Butyrate, Short-chain fatty acid, Antibody production, Interleukin-1, Interleukin-2

Butyric acid, a straight-chain saturated 4-carbon fatty acid, is produced from non-polysaccharides (dietary fiber) through digestion and fermentation by gastrointestinal bacteria such as Clostridium and Eubacterium, which are ordinarily present in ruminants; and its concentration in human colon is around 20 mmol/kg (1). Ester forms of butyrate are contained in the fatty milk of ruminants and in lacto-products.

Sodium butyrate is known to have a variety of effects on the growth and differentiation of cultured animal cells. When butyrate at millimolar concentrations is added to the culture of cell lines, it inhibits the proliferation, arresting the cell cycle predominantly in the G1 phase and sometimes in the G2 phase (2–5). It also induces a differentiation in human and murine tumor cell lines; that is, increase of membraneous antigens and enzymatic activities (6–10). These effects of butyrate may be associated with a hyperacetylation of histone resulting from an inhibition of histone deacetylase and a change in chromatin structure (11). Butyrate treatment of some cell lines modulates various gene expressions (9, 10, 12). Recently, a promotorm fragment necessary for the butyrate-dependent activation of chicken embryonic globin gene was found in murine erythroleukemia cells (13). Furthermore, butyrate-responsive sequences were defined in the human immunodeficiency virus type I long terminal repeat (14).

During the course of studies on agents affecting IgE synthesis induced by LPS plus IL-4 in murine splenocytes, we incidentally observed that butyrate stimulates IgE production (I. Yamamoto, unpublished data). To our knowledge, there are only a few reports that elucidate the mechanism of action of butyrate on immune responses, where proliferative responses of bovine PBL or human T cells (15, 16) are inhibited and immunoglobulin secretion of bovine PBL (17) and IL-1 production of human monocytes are stimulated by butyrate (16). Here, we examined whether butyrate could modify the antigen-specific antibody production in murine splenocytes in vitro and elucidated its mode of action.

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Abbreviations used are: LPS, lipopolysaccharide; IL, interleukin; PBL, peripheral blood lymphocytes; Con A, concanavalin A; FCS, fetal calf serum; SRBC, sheep red blood cell; 3H-TdR, [6-3H]thymidine; PFC, plaque forming cell; CAS, Con A supernatant; ELISA, enzyme-linked immunosorbent assay; PEC, peritoneal exudate cell; TNP, 2,4,6-trinitrophenyl; BrdU, bromodeoxyuridine.
MATERIALS AND METHODS

Animals
Female BALB/c mice (8–12 weeks) and C3H/HeJ mice (5–6 weeks) were purchased from Charles River Japan, Inc. (Kanagawa) and Japan SLC, Inc. (Shizuoka), respectively.

Reagents
Reagents and monoclonal antibodies used in this study were obtained from the following sources: sodium butyrate, sodium propionate, valeric acid (Tokyo Kasei Kogyo Co., Ltd., Tokyo); sodium acetate and butanol (Ishizu Pharmaceutical Co., Ltd, Osaka); acetoacetic acid lithium salt, \( \beta \)- and \( \gamma \)-hydroxybutyric acid sodium salt, LPS from E. Coli 055:B5, Con A (Sigma, St. Louis, MO, USA); FCS (Gibco BRL, Grand Island, NY, USA); monoclonal anti-Thy-1.2 antibody (Serotec, Oxford, England); low-tox rabbit complement (Cederlane, Ontario, Canada); SRBC (Japan Lamb, Hiroshima); \(^3\)H-TdR (Amersham, Tokyo). RPMI-1640 (ICN Biomedicals, Costa Mesa, CA, USA) was usually supplemented with penicillin G potassium (100 U/ml; Banyu Pharmaceutical Co., Ltd., Tokyo) and streptomycin sulfate (100 \( \mu \)g/ml; Meiji Seika Co., Ltd., Tokyo).

Antibody production
BALB/c mouse spleen cells (8 x 10⁶) were cultured with SRBC (2 x 10⁶) in 1.5 ml RPMI-1640 medium containing 10% FCS using a multidish with 24-wells (Nunc, Roskilde, Denmark) for 5 days at 37°C under 5% CO₂ - 95% air. The number of anti-SRBC PFC was enumerated by the method of Jerne and Nordin (18). The supernatant were continuously collected from the culture, and their IL-2 activities were assayed as described below. In some experiments, T cell-depleted splenocytes (3.5 x 10⁶), prepared by the treatment of splenocytes with anti-Thy-1.2 antibody (1/500) for 30 min at 4°C and low-tox rabbit complement (1/15) for another 40 min at 37°C, were used. Using flow cytometrical analysis, we confirmed that the percentage of residual Thy-1.2⁺ cells was less than 3%. To reconstitute the anti-SRBC PFC response of T cell-depleted splenocytes, CAS (see below) was added to it to neutralize any remaining Con A activity. This preparation was stored at -70°C until use.

IL-2 assay
The IL-2 activity of culture supernatants was measured by a Mouse Interleukin-2 ELISA Kit (Becton Dickinson Labware, Bedford, MA, USA) or bioassay using a IL-2-dependent cell line, CTLL-2, as described before (19). Briefly, appropriately or serially twofold diluted culture supernatants were added to the culture of CTLL-2 cells (4 x 10³) for 24 hr in 5% FCS supplemented RPMI-1640 medium using a 96-well, flat-bottomed microculture plate. Cultures were pulsed with \(^3\)H-TdR (9.25 kBq/well) for the last 6 hr, and the radioactivity incorporated into CTLL-2 cells was determined.

IL-1 production and IL-1 assay
PEC were collected by peritoneal lavage with cold PBS in mice that were injected i.p. with 1 ml of 3% thioglycolate medium (Difco Laboratories, Detroit, MI, USA) 4 days before. They were cultured in 5% FCS supplemented RPMI-1640 medium at a concentration of 1 x 10⁶ /ml/well using a 24-well multidish. After 4 hr incubation to allow for adherence of macrophages, the wells were washed to remove nonadherent cells. Then, the macrophage monolayer was treated with SRBC (6.7 x 10⁵/well), TNP-LPS (5 mg/ml) or LPS (5 pg/ml) for 24 or 48 hr. Supernatants were collected and dialyzed against PBS. Their IL-1 activity was assayed as follows: thymocytes (3 x 10⁶) from C3H/HeJ mice were cultured for 48 hr with diluted IL-1 supernatants and Con A (1 \( \mu \)g/ml) in 0.2 ml of 5% FCS supplemented RPMI-1640 medium using a 96-well flat-bottomed plate. Cultures were pulsed with 10 \( \mu \)M BrdU (Yamasa Shoyu Co., Ltd., Chiba) for another 18 hr. The cell proliferation was determined by quantifying BrdU contents incorporated into cells using ELISA specific for BrdU.

BrdU ELISA
This non-isotope method for the proliferation assay was originally developed in our laboratory (20). After the culture, plates were centrifuged at 1,500 rpm for 5 min at 20°C and supernatants were discarded. Then, cells were fixed to the plate by adding 0.2 ml of methanol-acetic acid (3:1), and the supernatant was removed after the centrifugation. This procedure was repeated again. The wells were washed with PBS twice and treated with 0.1 ml of 0.0025% trypsin for 20 min at 37°C. After washing with PBS, they were incubated with 0.2 ml of 95% formamide-0.15 M sodium citrate for 45 min at 70°C. After the wells were washed with PBS three times, 0.1 ml rabbit anti-BrdU antibody prepared in our laboratory was added to each. After the incubation for 1 hr at 37°C, the wells were
washed with 0.2% Tween 20-PBS and PBS, and 0.1 ml of peroxidase conjugated goat anti-rabbit IgG antibody (Cappel, West Chester, PA, USA) was added to each, followed by incubation for another 60 min at 37°C. The wells were washed again and 0.1 ml of substrate solution (2.5 mM 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (Sigma) in 0.1 M citric acid buffer (pH 4.0) with 0.17% H₂O₂) was added to each. After the reaction for 30 min at room temperature, the OD at 405 nm was measured by an ELISA autoreader (Sanko Junyaku Co., Ltd., Tokyo). The BrdU incorporation was calculated as the value of each well subtracted by the value obtained from the assay of cells cultured without BrdU.

Mitogen-induced proliferation and IL-2 production

BALB/c mice splenocytes (2 x 10⁶) were cultured for 44 hr in the presence of Con A or LPS in 0.2 ml of 5% FCS containing RPMI-1640 medium using a 96-well, flat-bottomed plate. After the addition of BrdU (5 μM), they

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**Fig. 1.** Effect of butyrate on the anti-SRBC antibody production. Splenocytes (8 x 10⁶/well) were cultured with varying concentrations of butyrate in the presence of SRBC (2 x 10⁶/well). After 5 days, the number of anti-SRBC PFC (closed circle) and the cell viabilities (open circle) were determined. Data are presented as the mean±S.D. of triplicate sets of a typical experiment. A significant difference from the control value analyzed by Student's t-test is indicated as * (P<0.05) and ** (P<0.01).

**Fig. 2.** Effects of short-chain fatty acids and 4-carbon compounds on the anti-SRBC antibody production. Splenocytes (8 x 10⁶/well) were cultured with various compounds in the presence of SRBC (2 x 10⁶/well). After 5 days, the number of anti-SRBC PFC was determined. Data are presented as the mean±S.D. of triplicate sets of a typical experiment. A significant difference from the control value analyzed by Student's t-test is indicated as * (P<0.05) and ** (P<0.01).
were cultured for another 6 hr, and the BrdU incorporation was measured by BrdU ELISA. IL-2 production was conducted as follows: splenocytes (5 x 10^6) were cultured for 24 hr with Con A in 1 ml of 5% FCS–RPMI-1640 and the supernatants were collected. Their IL-2 activity was determined by CTLL-2 bioassay.

Statistical analyses
The results are expressed as the mean±S.D. of triplicate sets of a typical experiment in several independent studies. Statistical significance was analyzed by Student’s t-test.

RESULTS
Effect of butyrate on the anti-SRBC antibody production of splenocytes
First of all, the effect of butyrate on the antibody production against a T cell-dependent antigen, SRBC, was examined. Anti-SRBC PFC of murine splenocytes were apparent on day 3 of the culture, reached maximum by day 5, and gradually disappeared. Their cell viabilities were usually reduced to under 50% within a day, which was maintained with little change throughout the culture period. Figure 1 shows that butyrate at concentrations of 200–600 μM significantly augmented the anti-SRBC antibody production without affecting the cell-viability. The maximal augmentation was observed by the addition of butyrate at 400 μM. Although the data are not shown here, butyrate was cytotoxic at concentrations more than 900 μM. Next, we investigated whether other saturated short-chain fatty acids have stimulatory effects on the anti-SRBC PFC response like those of butyrate. A moderate and statistically significant increase of anti-SRBC PFC was observed by propionate (C3) and valeric acid (C5), which were less active compared to that observed by butyrate (Fig. 2A). Acetate (C2) did not affect the response. In addition, effects of 4-carbon compounds including ketones was studied. Only β-hydroxybutyrate showed a little stimulation of this response, but buthanol, acetoacetate or γ-hydroxybutyrate did not (Fig. 2B).

Kinetics of the stimulatory effect of butyrate on the anti-SRBC antibody production
Next, we studied the stage of this culture at which butyrate stimulated the antibody production against SRBC. In Fig. 3A, the anti-SRBC PFC was increased by the addition of butyrate at the concentration of 400 μM within 2 days after the initiation of the culture, but not by its addition thereafter. In a subsequent experiment, we found that this response was still successfully increased even though butyrate, which was added at the culture initiation, was removed on day 2 of the culture, whereas butyrate-treatment of the cells only for the first day did not stimulate the anti-SRBC PFC (Fig. 3B). These results demonstrate that butyrate must be present in an early stage of the culture for stimulation of the antibody production.

Effect of butyrate on the anti-SRBC antibody production of T cell-depleted splenocytes
To characterize the cellular target of butyrate, we examined whether butyrate would augment the anti-SRBC antibody production by T cell-depleted splenocytes. The splenocytes in which T cells were eliminated by the treatment with anti-Thy-1.2 antibody plus complement needed a supplement of CAS to induce the PFC response against SRBC. Butyrate at the concentration of 400 μM elevated the anti-SRBC PFC response of T cell-depleted splenocytes, which was insufficiently induced by the addition of 5% CAS, to the same level of response as that of untreated or complement-treated splenocytes cultured with butyrate (Table 1). Addition of 10% CAS supported the anti-SRBC PFC of T cell-depleted splenocytes at a higher level than that of untreated or complement-treated splenocytes, which was also stimulated by butyrate. It is suggested that butyrate exhibits the stimulatory effect on the antibody production in a T cell-independent manner.
Effect of butyrate on the IL-2 production in anti-SRBC antibody production

The IL-2 activity of culture supernatants in the course of induction of anti-SRBC PFC in the presence or absence of butyrate was measured. As shown in Fig. 4, IL-2 production in the anti-SRBC PFC response was maximum on day 3 and subsequently decreased. Butyrate significantly increased the IL-2 level secreted in the supernatants of days 2–4 during the culture. Using ELISA specific for IL-2, we ascertained the elevated IL-2 level by butyrate on day 3 (control: 1.1 U/ml, +400 μM butyrate: 3.5 U/ml). These findings suggest that butyrate also stimulates a T cell function in terms of IL-2 production.

Effect of butyrate on the IL-1 production of PEC stimulated with SRBC

Next, we examined the IL-1 production of PEC elicited by thioglicollate when they were stimulated with SRBC in the presence or absence of butyrate. Table 2 shows that butyrate does not change the IL-1 level secreted in the supernatant 24 hr or 48 hr after the stimulation. LPS or TNP-LPS-induced IL-1 production was not affected by butyrate, either.

Effect of butyrate on the Con A- and LPS-induced proliferation

It is well known that butyrate inhibits the proliferation of cultured cell lines arresting the cell cycle in the G1 or G2 phase (3–6). Here, we confirmed that butyrate suppresses the mitogen-induced proliferations of splenocytes. Con A- and LPS-induced proliferations were reduced by the addition of butyrate at concentrations of 200 and 400 μM, and butyrate appeared more susceptible to the response induced by Con A than that by LPS (Fig. 5). Furthermore, the effect of butyrate on the IL-2 production stimulated with Con A was studied. As shown in Table 3, butyrate at the concentration of 400 μM did not change the IL-2 secretion induced by optimum or suboptimum concentration of Con A.

Table 1. Effect of butyrate on the anti-SRBC antibody production of T cell-depleted splenocytes

| Cell treatment       | Butyrate (400 μM) | Anti-SRBC PFC/culture | % of control response |
|----------------------|-------------------|-----------------------|-----------------------|
| None                 | −                 | 290±31                | 100                   |
| + Complement (C.)    | 5893±820          | 2032                  |                       |
| + Anti-Thy-1.2 + C.  | 310±115           | 100                   |                       |
| + Anti-Thy-1.2 + C.  | 11620±1807        | 3748                  |                       |
| + Anti-Thy-1.2 + C.  | 65±9              | 100                   |                       |
| + Anti-Thy-1.2 + C.  | 1033±181          | 16266                 |                       |
| + Anti-Thy-1.2 + C.  | 27193±9918        | 2632                  |                       |
| + Anti-Thy-1.2 + C.  | 37±6              |                       |                       |

None- or complement-treated splenocytes (8 × 10⁶/well) or anti-Thy-1.2 plus complement-treated (3.5 × 10⁶) were cultured with or without butyrate (400 μM) in the presence of SRBC (2 × 10⁴). After 5 days, the number of anti-SRBC PFC was determined. Data are presented as the mean±S.D. of triplicate sets of a typical experiment.

Table 2. Effect of butyrate on the IL-1 production of PEC stimulated with SRBC, LPS or TNP-LPS

| Stimuli       | Butyrate (μM) | IL-1 activity, BrdU uptake (OD₄₉₀) |
|---------------|---------------|-----------------------------------|
|               | Culture time  |
|               | 24 hr         | 48 hr                             |
| SRBC          | 0             | 0.455±0.026                       | 0.449±0.027                  |
|               | 400           | 0.461±0.018                       | 0.444±0.054                  |
| LPS           | 0             | 0.710±0.217                       | 0.565±0.044                  |
|               | 400           | 0.670±0.053                       | 0.633±0.046                  |
| TNP-LPS       | 0             | 0.547±0.090                       | 0.693±0.079                  |
|               | 400           | 0.696±0.061                       | 0.674±0.108                  |

Thioglycollate-elicited PEC (2 × 10⁶/well) were cultured with or without butyrate in the presence of SRBC (6.7 × 10⁴/well), LPS (5 μg/ml) or TNP-LPS (5 μg/ml). After 24 or 48 hr, the culture supernatants were collected and dialyzed. Their IL-1 activity was measured by the ability to stimulate the Con A-induced proliferation of C3H/HeJ mouse thymocytes. Data are presented as the mean±S.D. of triplicate sets of a typical experiment.

Fig. 4. Effect of butyrate on the IL-2 secretion in supernatants of anti-SRBC antibody production. Splenocytes (8 × 10⁶/well) were cultured with (closed circle) or without (open circle) butyrate (400 μM) in the presence of SRBC (2 × 10⁴). Culture supernatants collected on the indicated day were diluted to 1/32, and their IL-2 activity was tested by the ability to induce CTLL-2 proliferations. Data are presented as the mean±S.D. of triplicate sets of a typical experiment. A significant difference from the control value analyzed by Student’s t-test is indicated as * (P < 0.05) and ** (P < 0.01).
DISCUSSION

In this paper, we studied the effect of butyrate on several immune responses, mainly an antibody production against SRBC in murine splenocytes. Butyrate at concentrations of 200–600 µM showed a pronounced stimulatory effect on the anti-SRBC PFC response without any cytotoxicity. Such a significant stimulation of anti-SRBC antibody production was specific for butyrate among other saturated short-chain fatty acids or 4-carbon compounds that we have studied, although propionate, valeric acid and β-hydroxybutyrate were very slightly stimulatory. In this point, our data agree with those of Nonnecke et al. (17). They reported that pokeweed mitogen-induced IgM synthesis of bovine PBL was enhanced by butyrate but not by acetate, acetoacetate or β-hydroxybutyrate.

During the process of the antibody producing cell development, direct interactions among T cells, B cells and macrophages and their indirect interactions via cytokines are necessary. In an in vitro system, antibody producing cells appeared 3 days after the antigen stimulation, reached maximum on day 5 and disappeared thereafter. Butyrate was found to be necessary on days 1–3, an early phase of the culture, to increase the number of PFC. Moreover, it elevated the IL-2 production on days 2–4, which reached its peak on day 3. Therefore, the mechanism by which butyrate augments antibody production is considered partly to involve an increase in IL-2 secretion from T cells. There are three populations of murine helper T cells: Th1 that produces IL-2, IL-3 and IFN-γ; Th2 that produces IL-4 and 5; and Th0 that secretes cytokines which are produced by both Th1 and Th2 (21, 22). Butyrate might activate Th1 or Th0 and enhance the IL-2 production, which leads to the increase of antibody production. However, the Con A-induced IL-2 production was not enhanced by butyrate. Con A is a strong inducer of IL-2. In general, the IL-2 level of culture supernatants stimulated with Con A is much higher than that stimulated with a specific antigen such as SRBC, and might not be susceptible to a potentiator. For this reason,
the enhancement of IL-2 production by butyrate was observed in SRBC-stimulated splenocytes but not in the Con A-stimulated ones.

The T cell-depleted splenocytes can differentiate into PFC in response to a T cell-dependent antigen, SRBC, if CAS is added as a T cell-replacing factor. Butyrate increased the number of anti-SRBC PFC in T cell-depleted splenocytes supplemented with CAS as well as that of complement-treated or non-treated cells, suggesting that butyrate might act on B cells in the absence of T cells and accelerate the B cell differentiation. By flow cytometrical analysis, it was confirmed that the percentage of Thy-1.2+ cells remaining in the T cell-depleted cells was under 3.0%. However, we cannot exclude the possibility that a remaining small portion of Thy-1.2+ cells may be recovered by the addition of CAS plus butyrate and are involved in the stimulation of the anti-SRBC PFC response. Thus, we are currently investigating the direct effect of butyrate on the antibody production of murine splenic purified B cells stimulated with T cell-independent antigen, TNP-LPS and the differentiation of pre-B cell lines. Although the data were not shown here, butyrate did not induce the anti-SRBC PFC when CAS was not added to the T cell-depleted culture. It suggests that butyrate might not be a polyclonal activator of B cells. Thus, in the antibody production, butyrate seems to affect not only T cell function, stimulating IL-2 production, but also B cells and macrophages. Here, we found that butyrate at the concentration of 400 μM did not show a significant effect on the IL-1 production of PEC stimulated with SRBC. Eftimiaidi et al. reported that butyrate at concentrations of 1,500–2,000 μM stimulated the IL-1 secretion of human monocytes (16). We observed that butyrate at higher concentrations than 900 μM was toxic for lymphocytes and have not studied the effect of butyrate at millimolar concentrations. They measured IL-1 spontaneously produced without stimuli using ELISA. Since our experimental conditions such as concentrations of butyrate, stimuli to induce IL-1 and cell sources were different from theirs, an inconsistent result might be obtained.

Several papers reported that butyrate inhibited the proliferation of various cell lines (2–5). The mitogen-induced proliferations of bovine and human PBL were also suppressed by butyrate at the concentrations of 250 μM and higher than 750 μM, respectively (15, 16). We confirmed the suppressive effect of butyrate on the proliferation of murine splenocytes. As was expected, butyrate at 200 and 400 μM inhibited the Con A- or LPS-induced proliferation. It is considered that butyrate may interfere with the proliferation, arresting the cell cycle at a certain stage, and accelerate the differentiation to produce antibodies through acting on T cells and B cells.

The molecular mechanism of butyrate-induced growth arrest in the G1 phase of the cell cycle was studied in Swiss 3T3 cells, murine fibroblast cells (12). It described that butyrate reduced the mRNA level of growth-associated genes, c-myc, p53 and thymidine kinase, and stimulated that of aP2, which is preferentially expressed during the differentiation of pre-adipocytes into mature adipocytes and is not expressed in actively proliferating fibroblasts (23). The decreased mRNA level of c-myc and p53 was demonstrated using other cell lines (24, 25). Recent reports have suggested that butyrate modulates gene expressions through specific promoter regions (13, 14). Whether or not these changes in gene expressions is associated with the augmentation of antibody production by butyrate remains to be elucidated.

Our results suggest that butyrate may be an agent causing the differentiation of both T and B cells in the process of antibody production. One of its mechanisms for enhancing the antibody production is the increase of IL-2 secretion. Butyrate also appears to stimulate the differentiation, acting on B cells. The increase of anti-SRBC PFC is regarded as an expansion of SRBC-specific B cell clones. It is considered that butyrate may stimulate the clonal expansion specific for SRBC, not as a polyclonal activator, together with its action on T cells to augment IL-2 production. However, the mechanism for how butyrate promotes the differentiation of B cells committed to a specific antigen remains unclear. It is possible that butyrate may regulate some events that occur in the intracellular signal transduction or gene expressions associated with the differentiation in B cells stimulated with the antigen. Further investigations to elucidate the direct effect of butyrate on B cell differentiation are in progress.

REFERENCES

1 Macfarlane GT and Cummings JH: The colonic flora, fermentation, and large bowel digestive function: The large intestine. In Physiology, Pathophysiology, and Disease, Edited by Phillips SF, Pemberton JH and Shorter RG, pp 51–92, Raven Press Ltd, New York (1991)

2 Fallon RJ and Cox RP: Cell cycle analysis of sodium butyrate and hydroxyurea, inducers of ectopic hormone production in Hela cells. J Cell Physiol 100, 251–262 (1979)

3 Xue S and Rao PN: Sodium butyrate blocks Hela cells preferentially in early G1 phase of the cell cycle. J Cell Sci 51, 163–171 (1981)

4 Yamada K and Kimura G: Formation of proliferative tetraploid cells after treatment of diploid cells with sodium butyrate in rat 3Y1 fibroblasts. J Cell Physiol 122, 59–63 (1985)

5 Yamada K, Ohtsu M, Sugano M and Kimura G: Effects of butyrate on cell cycle progression and polyploidization of various types of mammalian cells. Biosci Biotech Biochem 56, 1261–1265 (1992)

6 Tsao D, Morita A, Bella A Jr, Luu P and Kim YS: Differential
effects of sodium butyrate, dimethyl sulfoxide and retinoic acid on membrane-associated antigen, enzymes and glycoproteins of human rectal adenocarcinoma cells. Cancer Res 42, 1052 - 1058 (1982)

7 Abe M and Kufe DW: Sodium butyrate induction of milk-related antigens in human MCF-7 breast carcinoma cells. Cancer Res 44, 4574 - 4577 (1984)

8 Sutherland J, Mannoni P, Rosa F, Huyat D, Turner AR and Fellous M: Induction of the expression of HLA class I antigens on K562 by interferons and sodium butyrate. Hum Immunol 12, 65 - 73 (1985)

9 Pan C-H, Sartwell AD and Chou JY: Transcriptional regulation and the effects of sodium butyrate and glycosylation on catalytic activity of human germ cell alkaline phosphatase. Cancer Res 51, 2058 - 2062 (1991)

10 deFazio A, Chiew Y-E, Donoghue C, Lee CSL and Sutherland RL: Effect of sodium butyrate on estrogen receptor and epidermal growth factor receptor gene expression in human breast cancer cell lines. J Biol Chem 267, 18008 - 18012 (1992)

11 Kruh J: Effects of sodium butyrate, a new pharmacological agent, on cells in culture. Mol Cell Biochem 42, 65 - 82 (1982)

12 Toscani A, Soprano DR and Soprano KJ: Molecular analysis of sodium butyrate-induced growth arrest. Oncogene Res 3, 223 - 238 (1988)

13 Glauber JG, Wandersee NJ, Little JA and Ginder GD: 5'-Flanking sequences mediate butyrate stimulation of embryonic globin gene expression in adult erythroid cells. Mol Cell Biol 11, 4690 - 4697 (1991)

14 Bohan CA, Robinson RA, Luciw PA and Srinivasan A: Mutational analysis of sodium butyrate inducible elements in the human immunodeficiency virus type 1 long terminal repeat. Virology 172, 573 - 583 (1989)

15 Franklin ST, Young JW and Nonnecke BJ: Effects of ketones, acetate, butyrate, and glucose on bovine lymphocyte proliferation. J Dairy Sci 74, 2507 - 2514 (1991)

16 Eftimiadi C, Stashnko P, Tonetti M, Mangiante PE, Massara R, Zupo S and Ferrari M: Divergent effect of the anaerobic bacteria by-product butyric acid on the immune response: suppression of T-lymphocyte proliferation and stimulation of interleukin-1 beta production. Oral Microbiol Immunol 6, 17 - 23 (1991)

17 Nonnecke BJ, Franklin ST and Young JW: Effects of ketones, acetate and glucose on in vitro immunoglobulin secretion by bovine lymphocytes. J Dairy Sci 75, 982 - 990 (1992)

18 Jerne NK and Nordin AA: Plaque formation in agar by single antibody-producing cells. Science 140, 405 (1963)

19 Fujiwara M, Mitsui K and Yamamoto I: Inhibition of proliferative responses and interleukin 2 productions by salazosulfapyridine and its metabolites. Jpn J Pharmacol 54, 121 - 131 (1990)

20 Iida S, Mitoh S, Mutoh N and Yamamoto I: Evaluation of the proliferative response of lymphocytes by BrdU-ELISA. J Immunol Methods (in press)

21 Mosmann TR, Cherwinski H, Bond MW, Giedlin MA and Coffman RL: Two types of immune helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 136, 2348 - 2357 (1986)

22 Firestein GS, Roeder WD, Laxer JA, Townsend KS, Weaver CT, Hon JT, Linton J, Torbett BE and Glasebrook AL: A new murine CD4+ T cells subset with an unrestricted cytokine profile. J Immunol 143, 518 - 525 (1989)

23 Chapman AB, Knight DM, Dieckmann BS and Ringold GM: Analysis of gene expression during differentiation of adipogenic cells in culture and hormonal control of the developmental program. J Biol Chem 259, 15548 - 15555 (1984)

24 Souleimani A and Asselin C: Regulation of c-myc expression by sodium butyrate in the colon carcinoma cell line Caco-2. FEBS Lett 236, 45 - 50 (1993)

25 Gope R and Gope ML: Effect of sodium butyrate on the expression of retinoblastoma (RBI) and p53 gene and phosphorylation of retinoblastoma protein in human colon tumor cell line HT29. Cell Mol Biol 39, 589 - 597 (1993)