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# Effects of Butyrate and Glucocorticoids on γ- to β-Globin Gene Switching in Somatic Cell Hybrids

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**Butyrate and its analogs have been shown to induce fetal hemoglobin in humans and primates and in erythroid cell cultures. To obtain insights concerning the cellular mechanisms of butyrate action, we analyzed the effects of butyrate on human globin gene expression in hybrids produced by fusing mouse erythroleukemia cells (MEL) with human fetal erythroid cells (HFE). These hybrids initially express human fetal hemoglobin but subsequently switch to adult globin expression after several weeks in culture. We found that α-aminobutyric acid, a butyrate analog which does not induce terminal maturation, strikingly delays the rate of the γ- to β-globin gene (γ-to-β) switch in the HFE × MEL hybrids. The effect of butyrate on globin expression is transient, with the result that the delay of globin gene switching requires the continuous presence of this compound in culture. Furthermore, butyrate fails to induce fetal hemoglobin expression in hybrids which have switched, suggesting that the effect of this compound on γ-globin expression is due to inhibition of γ gene silencing rather than to induction of γ gene transcription. Since in other cellular systems, glucocorticoids antagonize the action of butyrate, the effect of dexamethasone on the γ-to-β switch in HFE × MEL hybrids was examined. Dexamethasone strikingly accelerated the γ-to-β switch, and its effect was irreversible. The effects of dexamethasone and butyrate on the γ-to-β switch of the HFE × MEL hybrids appear to be codominant. These results indicate that steroids can have a direct effect on globin gene switching in erythroid cells.**

The human β-globin locus consists of five globin genes: an embryonic gene (ε), two fetal genes ($^{G}$γ and $^{A}$γ) and two adult genes (δ and β), arranged 5′-ε, $^{G}$γ, $^{A}$γ, δ, β-3′, which is the order of their expression during development. The control of developmental switching of these genes has been investigated by several approaches, including observations in vivo, in vitro expression systems, analyses of globin gene expression in transgenic mice, and studies in cell culture (for a review, see reference 27). We have been studying globin gene switching in somatic cell hybrids produced by fusion of murine erythroleukemia (MEL) cell lines with human fetal erythroblasts (HFE) (20). These hybrids primarily produce fetal globin; however, after several weeks in culture they switch to predominantly adult globin expression, thus providing an ex vivo system for studying the cellular control of switching. We have previously used this system to test whether factors such as time or number of divisions can influence the rate of γ- to β-globin gene (γ-to-β) switching (36). During these studies, we discovered that factors in the culture media can have a striking effect in the rate of γ-to-β switching. When the HFE × MEL hybrids were grown in medium containing 15% fetal calf serum (FCS), the γ-to-β switch took place after 20 to 40 weeks. Growth of the hybrids in serum-free medium (SFM) greatly increased the rate of switching, indicating that the serum contains factors which either induce γ-globin expression or prevent γ gene silencing (36).

Butyrate and its analogs are well-established inducers or repressors of differentiation in various cellular systems (2, 4, 15, 16, 17, 25, 26, 28, 32, 33). The mechanism whereby these compounds affect gene expression remains unresolved; however, it is assumed that effects on gene expression may be mediated by histone hyperacetylation because of the inhibition of histone deacetylase (17, 31). Butyrate has also been shown to modulate the developmental expression of globin genes in vivo or in culture. Thus, infusion of butyrate delays the γ-to-β switch in sheep fetuses (24), and it induces fetal hemoglobin in adult primates (7, 8) and in a proportion of patients with β chain hemoglobinopathies (23). Although butyrate response elements have been identified in the promoters of other genes inducible by butyrate (3, 12, 13, 14, 30), such elements have not yet been identified in the promoters of human globin genes.

In the experiments described in this paper, we used the somatic cell hybrid system to obtain insights on the cellular mechanisms of γ-globin gene induction by butyrate. We found that α-amino butyric acid (α-ABA), a butyrate analog which does not induce terminal differentiation, can profoundly retard the γ-to-β switch in hybrids and can totally inhibit the acceleration of γ-to-β switching caused by serum starvation. The effect of butyrate on the γ-to-β switch is transient, and it lasts as long as the cells are exposed to the compound. In contrast to the profound inhibition of the γ-to-β switch in hybrids undergoing switching, α-ABA cannot induce γ-globin expression in hybrids which have already switched, suggesting that butyrate cannot reactivate γ-globin genes which have been silenced. These results suggest that butyrate modulates γ-globin expression by inhibiting γ gene silencing rather than by directly inducing γ gene expression. We further found that dexamethasone, a compound known to act as a butyrate antagonist in other cellular systems, produces a striking acceleration of the rate of γ-to-β switch in HFE × MEL hybrid cells. The action of dexamethasone is partially counteracted by α-ABA.

## MATERIALS AND METHODS

**Origin of hybrids.** First-trimester human fetal liver erythroid cells were fused with HPRT$^{-}$ APRT$^{-}$ MEL585 (9) as previously described (20, 36). Selection of cells containing human chromosome 11 was achieved by direct immunoadherence to petri dishes coated with a monoclonal antibody to 53.6 antigen as described elsewhere (20). Periodic enrichment for chromosome 11 by immuno-

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adherence was necessary, since continuously cultured hybrids segregate human chromosomes.

**Growth media.** Cells were grown in Iscove's modified Dulbecco's medium (IMDM; GIBCO, New York, N.Y.) with either 20 or 0.5% (by volume) FCS (HyClone, Logan, Utah), or in SFM (Excell 300, with L-glutamine; JRH Biosciences, Lenexa, Kans.). In order to remove lipids and steroid hormones, some serum was incubated with two changes of charcoal (1 ml/10 mg; Norit-A; Fisher Scientific, Fair Lawn, N.J.) for 1 h at room temperature and with periodic shaking. After a final centrifugation at 8,000 rpm (Sorvall RC-5B; Du Pont Instruments, Hoffman Estates, Ill.) for 20 min, the serum was filter sterilized (7).

**α-ABA and dexamethasone.** L-α-ABA (Sigma, St. Louis, Mo.) was added in culture at a concentration of 70 mM. This compound is relatively nontoxic to the cells. The concentration of 70 mM is close to that used by other investigators in studies showing induction of embryonic chicken ε-globin gene expression in transfected MEL cells (14). To decide on the concentration of dexamethasone, a range of concentrations for dexamethasone sodium phosphate (Lymphomed, Deerfield, Ill.) was tested for effects on cell viability by culturing the cells for 7 days and measuring percentages of dead cells. Cell death increased with increasing amounts of dexamethasone as follows: at 10 μM, 3.0%; at 50 μM, 3.4%; at 100 μM, 27.3%; and at 150 μM, 50%. The 50 μM concentration was chosen for our experiments.

**Induction.** Hybrid cell populations were enriched for 53.6 positive cells (70% or greater) and then induced with medium containing 2% (by volume) of dimethyl sulfoxide (DMSO) (Sigma) for 4 days.

**Assessment of globin expression.** To detect globin protein, cytocentrifuge smears of induced cells were fixed in methanol, labeled with anti-γ-globin and anti-β-globin monoclonal antibodies, and then counterstained with a second antibody [goat $F(ab)_2$ anti-mouse fluorescein isothiocyanate-conjugated immunoglobulin G; Du Pont, Wilmington, Del.] (22). The frequency of γ- and β-globin-positive cells was determined by cell counting using a Zeiss fluorescence microscope. Extensive studies in our laboratory have shown that there is an excellent correlation between the ratio of γ-positive (γ) to γ-positive plus β-positive (γ+β) cells, as determined by this method and the γ/γ+β mRNA ratio determined by RNase protection.

**RNase protection.** RNA was prepared essentially by the method described by Chomczynski and Sacchi (6). Antisense RNA probes were synthesized from linearized DNA templates using either SP6 or T7 polymerase. Template DNAs included the following plasmids: (i) $pT7A\gamma^m$ (protects 170 nucleotides of human $A\gamma^m$; gift of Q. Li), (ii) $pT7\beta^m$ (protects 206 nucleotides of human $B^m$; gift of T. Enver [11]), and (iii) pSP6 Moα (protects 128 nucleotides of murine α [1]). A total of $10^6$ cpm of each probe was hybridized to 2 μg of RNA essentially as described by Melton et al. (18).

## RESULTS

**α-ABA inhibits the γ-to-β switch in HFE × MEL hybrids.** The HFE × MEL hybrids initially express predominantly γ-globin but after 4 to 5 months in culture switch to predominantly β-globin expression. Hybrid aliquots which had been cryopreserved before the onset of the γ-to-β switch were used in this study. Because addition of sodium butyrate to the culture medium induces terminal differentiation of MEL cells (15, 16, 21), we used α-ABA instead of sodium butyrate; α-ABA is a compound which does not, in our experience, trigger terminal differentiation. Cells were grown in the presence of 20% FCS or 20% charcoal-absorbed FCS. Charcoal absorption has been previously shown to remove from the serum unknown elements which induce fetal hemoglobin in adult burst-forming unit-erythroid (BFUe) cultures (7). Periodically, cells were panned to ensure a high proportion of chromosome 11-containing cells, aliquots were induced with DMSO, and globin expression was assessed by immunofluorescence staining of fixed hybrid cell preparations.

Figure 1 shows the results of two experiments in which the changes in γ-globin expression (ratio of the number of γ-globin-expressing cells to the total number of human globin-expressing cells) were monitored over time in culture. At the beginning of the experiment, 98% of the cells of the hybrid expressed γ-globin. Cells grown in 20% FCS or charcoal-absorbed FCS showed a gradual decrease in γ-globin expression, and by 126 days in culture 43 to 48% of human globin-producing cells expressed γ-globin. In contrast, cells grown in the presence of α-ABA maintained high levels of γ-globin and, at 126 culture days, 82 to 90% of the human globin-producing cells contained γ-globin. In one experiment, observations were extended to day 182. At this time point, the proportion of γ-globin-expressing cells was 11% in the control hybrid and 93% in the hybrid grown in the presence of α-ABA, indicating that α-ABA prevents the γ-to-β switch normally occurring in HFE × MEL hybrids.

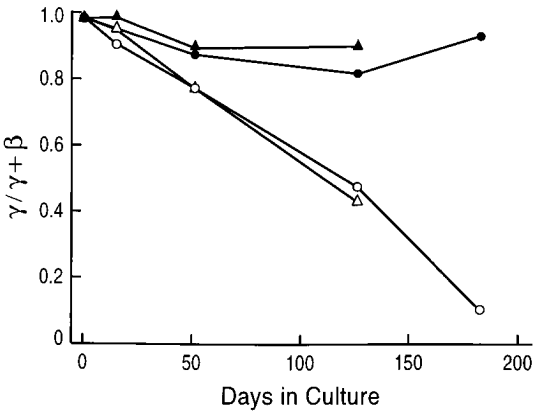


FIG. 1. α-ABA inhibits the γ-to-β switch in HFE × MEL hybrids. Human globin expression in a hybrid produced by fusing MEL cells with HFE. Triangles, data from cells grown in IMDM containing 20% FCS; circles, data from cells grown in IMDM containing 20% charcoal-stripped FCS; ● and ▲, cells grown in the presence of 70 mM α-ABA; ○ and △, cell grown in the absence of α-ABA. Notice that cells grown in the presence of α-ABA consistently maintained high levels of γ-globin expression relative to cells grown in the absence of α-ABA.

**Butyrate inhibits acceleration of the γ-to-β switch by serum deprivation.** We have previously shown that serum deprivation accelerates γ-to-β switching in HFE × MEL hybrids (36). To test whether α-ABA can overcome the effect of serum deprivation, hybrid cell populations expressing γ-globin in a high proportion of cells were divided into three portions which were concurrently cultured either in FCS, in a commercially available SFM, or with 70 mM α-ABA in SFM. Periodically, aliquots of cells were panned, induced with DMSO in the presence of 20% FCS, and assessed by immunofluorescence. Figure 2 shows the ratio of γ-globin-expressing cells over total cells expressing human globin as a function of time. Five independent experiments were conducted using frozen aliquots of the same hybrid. At the beginning of these experiments, 79 to 98% of the cells expressed human γ-globin. Cells grown in 20% serum showed a gradual decrease in the frequency of γ-globin-expressing cells (Fig. 2A), while cells grown in SFM showed a rapid decrease in the frequency of γ-globin-expressing cells (Fig. 2B). In contrast, the rate of switching was significantly retarded in cells grown in SFM with 70 mM α-ABA. Thus, the addition of α-ABA totally inhibited the acceleration of γ-to-β switching caused by serum deprivation. Similar results were obtained by RNase protection (Fig. 3A).

**The effect of butyrate on γ-globin expression is transient.** To test whether treatment of hybrids with α-ABA had a lasting effect on γ-gene expression even after α-ABA was removed, hybrid cells were grown in the presence of SFM with 70 mM α-ABA for 34 days. At this time point, they were analyzed for human globin expression. The cells were subsequently divided into two aliquots; one aliquot of cells was placed in IMDM containing 0.5% FCS, and one aliquot was continued in SFM in the presence of 70 mM α-ABA (Fig. 4A). After an additional 28 days in culture, the cells were panned and analyzed for globin expression. In the hybrid cells grown in SFM plus α-ABA for the initial 34 days, 87% of the human globin-expressing cells expressed γ-globin. In the aliquot grown in the

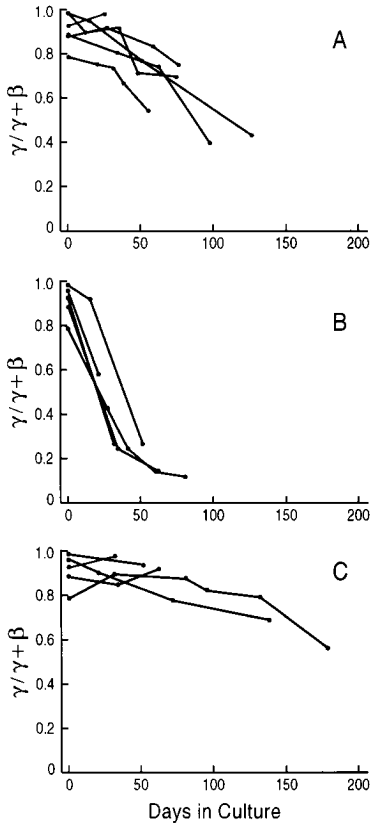


FIG. 2. Butyrate inhibits the acceleration of the γ-to-β switch by serum deprivation. (A) Data from experiments in which HFE × MEL hybrids were grown in IMDM containing 20% FCS; (B) data from concurrent experiments in which hybrids were grown in Excell 300 SFM; (C) data from concurrent experiments in which hybrids were grown in Excell 300 SFM containing 70 mM α-ABA. Note that in panel B, cells switch to β-globin expression more rapidly than in panel A. Note also that the addition of α-ABA to the Excell 300 SFM results in a rate of switching that is lower than that seen with cells grown in IMDM with 20% FCS, indicating that the action of α-ABA does not require serum.

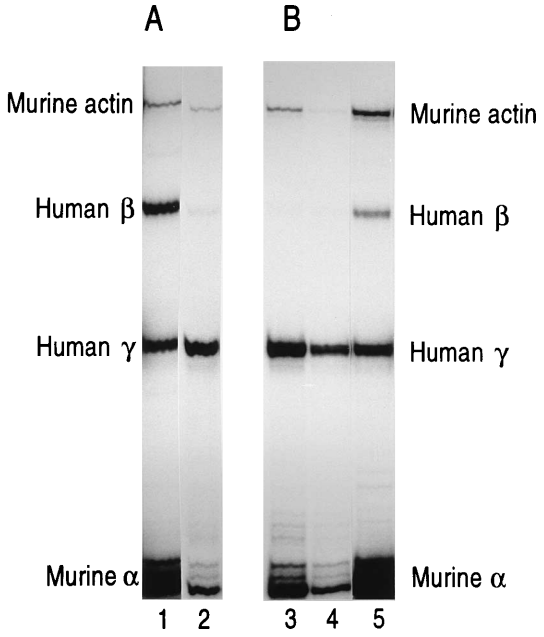


FIG. 3. Analysis of murine and human globin mRNAs by RNase protection. (A) HFE × MEL hybrids grown either in Excell 300 SFM (lane 1) or Excell SFM containing 70 mM α-ABA (lane 2) for 44 days. Note that the level of β-globin expression is significantly higher in the cells grown in the absence of α-ABA in the medium. (B) HFE × MEL hybrid grown in either IMDM containing 20% FCS (lane 4) or IMDM containing 20% FCS with dexamethasone (lane 5) for 27 days. Lane 3 shows the phenotype of the cells at day zero. Note that even after the short exposure of 27 days, the cells grown in the presence of dexamethasone have begun to express β-globin.

presence of α-ABA for an additional 28 days, 89% of the human globin-expressing cells expressed γ-globin. In contrast, in the aliquot grown for 28 days in the absence of α-ABA, only 14% of the hemoglobin-expressing cells contained γ-globin, suggesting that α-ABA has only a transient effect on γ-globin gene expression (Fig. 4A).

**Butyrate cannot reactivate silent γ-globin genes.** To test whether butyrate can reactivate silent γ-globin genes, hybrids which had switched following culture either in SFM or in IMDM with 0.5% FCS were cultured in the presence of α-ABA and 20% FCS for 28 days. No reactivation of γ-globin expression was observed when these hybrid cells were stained with anti-human γ-globin fluorescent antibodies at the end of this period (Fig. 4B and C). Thus, a hybrid which contained 21% γ-positive cells following culture for 34 days in the absence of butyrate displayed about 2% γ-positive cells after it was cultured for an additional 28 days in the presence of butyrate (Fig. 4B). In another experiment, a hybrid contained about 2% γ-positive cells following culture for 34 days in the absence of butyrate. This hybrid was subsequently cultured for an additional 28 days in the presence of butyrate; the frequency of γ-positive cells at this point was less than 1% (Fig. 4C). These data suggest that α-ABA cannot reactivate silenced γ-globin genes.

**Dexamethasone accelerates the rate of switching in HFE × MEL hybrids.** Butyrate and glucocorticoids have antagonistic effects in a number of cell lines. Thus, butyrate prevents gene induction by glucocorticoids in glioma and hepatoma cells and inhibits activation by glucocorticoids of murine mammary tumor virus genes in other cells lines, whereas dexamethasone blocks the butyrate-induced differentiation of rat islet cells (2, 25, 32, 33). Since α-ABA decreases the rate of globin gene switching in the hybrids, we wished to test whether glucocorticoids would have the opposite effect, i.e., acceleration of the γ-to-β switch.

Dexamethasone sodium phosphate, which is an analog of glucocorticoid steroids that is easily used in tissue culture, was applied. Since glucocorticoids interfere with the induction of MEL cells, for DMSO induction all dexamethasone-containing medium was removed and replaced with fresh medium containing DMSO. Figure 5 shows the changes in the proportion of γ-globin expression over time for cultures with or without 50 μM dexamethasone. In contrast to control cultures, the hybrids grown in the presence of dexamethasone had a striking acceleration in the rate of γ-to-β switching, suggesting that steroid hormones (or cellular changes induced by them) can affect globin gene expression. Similar results were obtained by RNase protection (Fig. 3B). To test whether the effect of dexamethasone was concentration dependent, a hybrid grown in IMDM plus 20% FCS was used. Aliquots were obtained at day 66 of culture, when the hybrid was near the middle of the γ-to-β switch. These aliquots were exposed to 1, 10, and 50 μM dexamethasone for 31 days. At the end of this period (at day 97), the frequency of γ-chain-positive cells in the hybrid grown without dexamethasone was 32%. The frequencies of γ-positive cells were 28, 18, and 13% in hybrids grown in the presence of 1, 10, and 50 μM dexamethasone, respectively, indicating

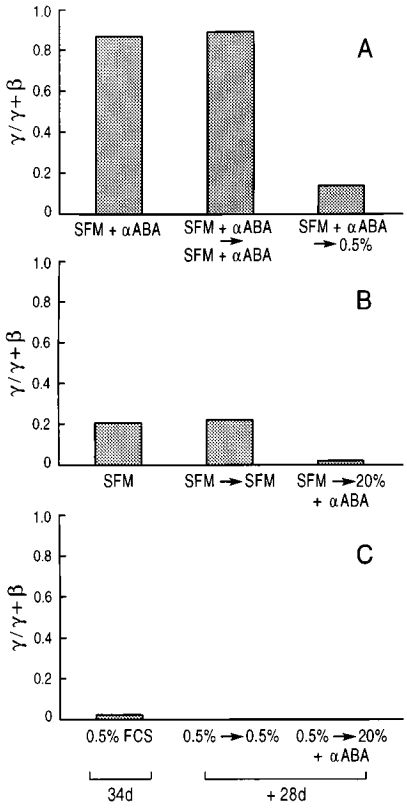


FIG. 4. HFE × MEL hybrids were grown for 34 days in one medium and then grown for an additional 28 days in the same or another medium. (A) Hybrid cells first grown in Excell 300 SFM with 70 mM α-ABA and then divided into two aliquots, one of which was continued in Excell 300 SFM with α-ABA, while the second was changed to IMDM with 0.5% FCS. Note that hybrid cells removed from α-ABA have switched rapidly to predominant β-globin expression. (B) Hybrid cells first grown in Excell 300 SFM, in which they switched to predominant β-globin expression and then divided into two aliquots, one of which continued in Excell 300 SFM while the second continued in IMDM with 20% FCS at 70 mM α-ABA. Note that the addition of 20% FCS and α-ABA did not cause a reappearance of γ-globin expression. Instead, the cells became even more predominantly β-globin expressing. (C) Hybrid cells first grown in IMDM containing 0.5% FCS, in which they switched to predominantly β-globin expression and then divided into two aliquots, one of which continued in IMDM containing 0.5% FCS while the second continued in IMDM containing 20% FCS and 70 mM α-ABA. Note that there is no reappearance of γ-globin expression.

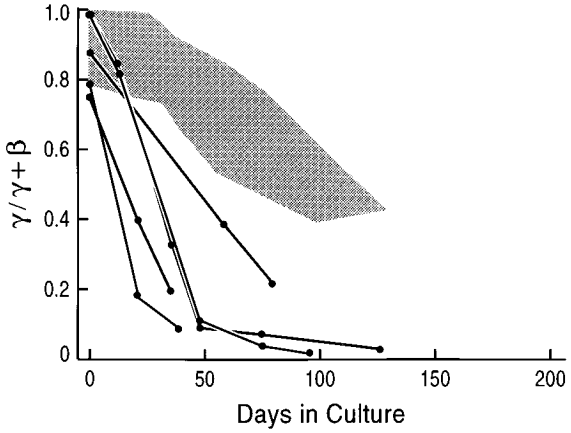


FIG. 5. Dexamethasone accelerates the rate of switching in HFE × MEL hybrids. Note the rapid switch to predominantly β-globin expression in cells grown in IMDM containing 20% FCS and 50 μM dexamethasone (lines) relative to the rate seen in IMDM containing 20% FCS alone (shadowed area).

## DISCUSSION

Butyrate and its analogs, α-ABA and phenylbutyrate, are known inducers of fetal globin gene expression. Initial evidence for this property of butyrate was obtained by Ginder and coworkers (5, 14) with chickens. In this species, there is only one switch from embryonic to definitive globin expression; embryonic and adult globins are limited to the primitive and definitive stages of erythropoiesis, respectively. These investigators showed that anemic chickens expressed embryonic globin genes in the adult stage of development when they were treated with sodium butyrate and 5-azacytidine, whereas either compound alone could not produce this phenotype. Subsequently, it was found that sodium butyrate or α-ABA can induce fetal hemoglobin production in cultures of erythroid progenitors from adult primates, normal persons, and individuals with sickle cell disease (7, 8). Butyrate has also been shown to inhibit the γ-to-β switch in sheep fetuses (24). Currently, butyrate or phenylbutyrate is administered to patients with sickle cell disease or homozygous β-thalassemia in clinical trials aimed at assessing whether induction of fetal hemoglobin by these compounds has therapeutic potential (10, 23).

The hybrid cell system that we have used represents fairly that the degree of the acceleration of the γ-to-β switch relates to the concentration of dexamethasone in the culture.

**The effects of α-ABA and dexamethasone are codominant.** Since α-ABA and dexamethasone displayed opposing effects on γ-globin expression in HFE × MEL hybrids, it was of interest to test whether the effect of either compound was dominant over the other. A hybrid cell population was divided into three aliquots concurrently grown in SFM alone, in 70 mM α-ABA, or in 70 mM α-ABA–50 μM dexamethasone. As shown in Fig. 6, the cell populations grown in α-ABA maintained high levels of γ-globin expression throughout the experiment, while the population grown in SFM switched rapidly. The population grown in the presence of both α-ABA and dexamethasone showed a biphasic curve of γ-globin expression, i.e., an initial decrease of γ-globin and a subsequent stabilization at a level of approximately 50%. We interpret this finding as providing indirect evidence that the effects of the two compounds are codominant.

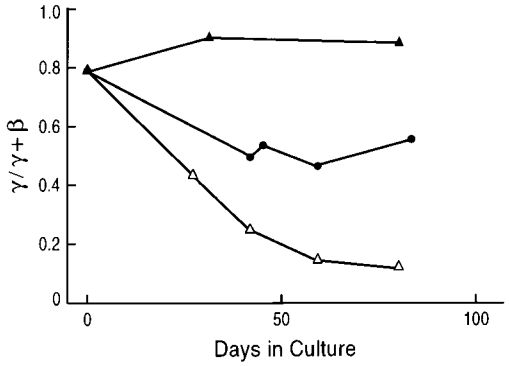


FIG. 6. The effects of α-ABA and dexamethasone are codominant. Data are from hybrids grown in Excell 300 SFM (△), in Excell 300 SFM containing 70 mM α-ABA (▲), and in Excell 300 SFM containing both 70 mM α-ABA and 50 μM dexamethasone (●). Note that the simultaneous presence of both agents results in an initial decrease in α-globin expression which is followed by the prevention of further switching.

well the situation of globin gene switching in vivo. These hybrids are produced by fusing MEL cells with human hemopoietic or nonhemopoietic cells. It has been previously shown that hybrids produced by fusing MEL cells with adult erythroblasts, fibroblasts, or lymphoblasts express only adult human globins (29, 34). When lymphoid cells from persons with hereditary persistence of fetal hemoglobin due either to γ gene promoter mutations or to δ- and β gene deletions are fused with MEL cells, human fetal hemoglobin is produced in the hybrids (21). When erythroid cells from fetuses are fused with MEL cells, the HFE × MEL hybrids produce predominantly human fetal hemoglobin; however, after several weeks in culture, they switch to adult human globin production (20). The rate of switching differs from hybrid to hybrid, but it is highly reproducible for each hybrid cell line (20, 36). This property allows us to use the HFE × MEL hybrids to test the effect of agents such as butyrate on globin gene switching. Frozen cell aliquots from the preswitched period of a hybrid with a known rate of switching are used. When these aliquots are cultured, they display a very predictable course of γ-to-β switching. Therefore, an aliquot can be split in several parts, each subculture can be submitted to various manipulations, and effects on globin gene switching can be assessed by comparing rates of switching with those of the unmanipulated control. As we show here, when this approach is used for the analysis of the effects of butyrate, it is found that this compound substantially decreases the rate of γ-to-β switching. This phenotype is reminiscent of the situation in fetal sheep, in which infusion of butyrate substantially decreases the rate of the γ-to-β switch (24). Globin gene switching in HFE × MEL hybrids is associated with a decrease in the frequency of γ-globin-expressing cells and an increase in the frequency of β-globin-expressing cells (20, 36). The main action of butyrate in this ex vivo switching system is to decrease the rate of appearance of β-globin-expressing cells. Thus, it appears that butyrate decreases the probability that a cell committed to the expression of the fetal hemoglobin program will change its phenotype.

The mechanism whereby butyrate affects γ-globin gene expression remains unknown. A central question is whether butyrate activates γ-globin gene expression de novo. This question was addressed in the studies of somatic cell hybrids described here. We found that while butyrate has a profound effect on the rate of switching of cells which already express fetal hemoglobin, it fails to influence γ-globin expression in cells which have already switched. Thus, addition of this compound in cultures of hybrid cells that have already switched failed to reactivate fetal globin production even after prolonged culture of these cells in the presence of the compound. These results suggest that butyrate may act by preventing the silencing of active γ genes rather than by reactivating γ genes which have already been silenced. A similar conclusion was reached with studies of transgenic mice carrying various types of human β-globin locus constructs (19).

Another question addressed in the present study is whether the effect of butyrate on the program of globin-expressing cells is permanent or transient. This question cannot be addressed with studies of animals treated with butyrate or with studies of the effect of butyrate in erythroid cell cultures, because the erythroid progenitor cells or mature erythroblasts which are exposed to butyrate in vivo or in culture are destined to differentiate. Therefore, any effect of this compound will appear to be transient. Only if butyrate can alter the globin gene program at the level of pluripotent stem cells will the effects not be transient in vivo. In contrast to the differentiating primary progenitor cells, the hybrid cell system has unlimited proliferative capacity. Furthermore, the butyrate analog used in our experiments does not induce downstream differentiation. If the butyrate had a permanent effect on the globin gene program, cells that had their phenotype permanently altered by butyrate would be expected to continue producing progeny expressing the altered phenotype after the butyrate was removed from the culture. In contrast, we found that the inhibitory effect of butyrate on γ-globin gene switching lasts as long as this compound is present in culture. When butyrate is removed from the cultures, γ gene silencing and the γ-to-β switching of the hybrids resume their course.

Experiments done with a number of cell lines have shown that butyrates and glucocorticoids have antagonistic effects (2, 4, 17, 25, 28, 32, 33). Since α-ABA decreases the rate of globin gene switching in HFE × MEL hybrids, we wished to test whether glucocorticoids would have the opposite effect, i.e., acceleration of γ-to-β switching. Indeed, it was found that hybrids grown in the presence of dexamethasone switched rapidly and that this switch was irreversible. We further observed that simultaneous presence of both α-ABA and dexamethasone resulted in rates of switching which were intermediate between those produced separately by each compound. These results generate questions on the role of steroids in globin gene switching. Previous studies have shown that adrenalectomy of sheep fetuses significantly delays the completion of γ-to-β switching (35). When cortisol replacement treatment is given to these fetuses, the rate of switching returns to normal, indicating that lack of cortisol is the cause of the delay in switching (35). The rate of switching is not accelerated when fetuses are exposed to cortisol before switching starts, suggesting that the erythroid cells respond to cortisol only during a specific time period of development. These observations suggest that steroids have a physiological role on switching. Our findings in somatic cell hybrids further show that this role is not indirect (i.e., through action of steroids on other hormonal systems or on accessory cells) but is a direct effect on cells of the erythroid lineage. It is of interest to investigate which sequences in the β-globin locus are responsible for the effects of steroids on the developmental expression of globin genes and whether these sequences are related to those responsible for the effects of butyrate.

## ACKNOWLEDGMENTS

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