Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 7907–7911, August 1994
Developmental Biology

Antisense oligonucleotide inhibition of acetylcholinesterase gene expression induces progenitor cell expansion and suppresses hematopoietic apoptosis *ex vivo*

HERMONA SOREQ*, DEBORAH PATINKIN*, EFRAT LEV-LEHMAN*, MIRTA GRIFMAN*, DALIA GINZBERG*, FRITZ ECKSTEIN†, AND HAIM ZAKUT‡

*Department of Biological Chemistry, The Life Sciences Institute, The Hebrew University of Jerusalem, Jerusalem 91904, Israel; †The Max Planck Institute for Experimental Medicine, Gottingen D-37075, Germany; and ‡Department of Obstetrics and Gynecology, The Sackler Faculty of Medicine, Tel Aviv University, The Edith Wolfson Medical Center, Holon 58100, Israel

Communicated by A. A. Moscona, April 13, 1994

ABSTRACT **To examine the role of acetylcholinesterase (EC 3.1.1.7) in hematopoietic cell proliferation and differentiation, we administered a 15-mer phosphorothioate oligonucleotide, antisense to the corresponding *ACHE* gene (AS-ACHE), to primary mouse bone marrow cultures. Within 2 hr of AS-ACHE addition to the culture, ACHE mRNA levels dropped by ≈90%, as compared with those in cells treated with the "sense" oligomer, S-ACHE. Four days after AS-ACHE treatment, ACHE mRNA increased to levels 10-fold higher than in S-ACHE cultures or in fresh bone marrow. At this later time point, differential PCR display revealed significant differences between cellular mRNA transcripts in bone marrow and those in AS-ACHE- or S-ACHE-treated cultures. These oligonucleotide-triggered effects underlay considerable alterations at the cellular level: AS-ACHE but not S-ACHE increased cell counts, reflecting enhanced proliferation. In the presence of erythropoietin it also enhanced colony counts, reflecting expansion of progenitors. AS-ACHE further suppressed apoptosis-related fragmentation of cellular DNA in the progeny cells, and it diverted hematopoiesis toward production of primitive blasts and macrophages in a dose-dependent manner promoted by erythropoietin. These findings suggest that the hematopoietic role of acetylcholinesterase, anticipated to be inverse to the observed antisense effects, is to reduce proliferation of the multipotent stem cells committed to erythropoiesis and megakaryocytopoiesis and macrophage production and to promote apoptosis in their progeny. Moreover, these findings may explain the tumorigenic association of perturbations in *ACHE* gene expression with leukemia.**

In light of the established role of acetylcholinesterase (acetylcholine acetylhydrolase; AcChoEase, EC 3.1.1.7) in termination of neurotransmission (1), the hematopoietic expression of this enzyme (2, 3) has presented an enigma. Accumulated evidence associates AcChoEase with growth of several cell types (4, 5), including hematopoietic cells (2). When expressed in cultured erythroleukemic cells, AcChoEase was shown to arrest growth (3). Moreover, exposure to AcChoEase-inhibitory insecticides increases the risk of leukemia (6). In addition, the human *ACHE* gene (7) is subject to incomplete somatic amplification (2) and frequent mutability (8) in several proliferation disorders in which hematopoietic apoptosis (9) is apparently suppressed. Furthermore, the AcChoEase gene maps to the 7q22 chromosomal location (10), which is frequently observed to be broken in leukemias (1). To examine the basis of these phenomena, we employed primary murine bone marrow cultures as an *ex vivo* system for modulating hematopoietic proliferation and development in nontransformed cells. In such cultures interleukin 3 (IL-3) induces expansion of a fraction of the existing pluripotent stem cells into multipotent progenitors, which can differentiate into megakaryocyte colony-forming units (CFU-MK) composed of granulocytes, megakaryocytes, and macrophages (11, 12); addition of erythropoietin and transferrin to IL-3 and longer incubation times induce CFU-GEMM colonies, which contain granulocytes, erythroid cells, megakaryocytes, and macrophages (13). This implies that colony counts reflect expansion and survival of progenitors that have given rise to progeny, whereas cell numbers reflect proliferation rates (9), and differential cell compositions demonstrate which cell lineages developed and which were programmed to die (9). Interference with expression of hematopoietically important genes by antisense oligonucleotides (AS-oligos) (14) can conceivably alter any or all of the characteristics of these cultures, and we have previously shown that AS-oligos targeted to cdc kinases (11) and to the *ACHE*-related gene *BCHE* (1, 2), which encodes butyrylcholinesterase (EC 3.1.1.8), impair megakaryocytopoiesis in CFU-MK (11, 12). More recently we found that the *ACHE* AS-oligo AS-ACHE, but not the sense oligo S-ACHE or the *BCHE* AS-oligo AS-BCHE, caused transient selective destruction of ACHE mRNA *in vivo*, accompanied by a relative increase in the fraction of myeloid cells 12 days after treatment (15). This could reflect expansion of progenitors, which would first be evident by an increase in the faster-developing myeloid cell fraction. Additionally or alternatively, it could be due to enhanced myeloidogenesis or suppressed erythropoiesis. To distinguish among these possibilities, and to investigate the function of the *ACHE* gene in hematopoiesis, we administered AS-ACHE *ex vivo* and examined its effects on gene expression, expansion of progenitors, and differential cell composition of CFU-MK and CFU-GEMM colonies.

MATERIALS AND METHODS

Primary bone marrow cells (1×10^5 per ml) from 8- to 12-week old C3H/HeJ mice were grown as described (11, 12) for 4 days in IL-3-supplemented serum-free medium (CFU-MK cultures) or for 8 days with the addition to the above medium of 2.8×10^{-4} M human transferrin (Boehringer Mannheim) and erythropoietin (Epo; 1000 units/mg protein; Terry Fox Laboratories, Vancouver) at 2 units/ml

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: AcChoEase, acetylcholinesterase; oligo, oligonucleotide; AS-ACHE and S-ACHE, antisense and sense AcChoEase gene oligos; AS-BCHE, antisense butyrylcholinesterase gene oligo; CFU-MK, megakaryocyte colony-forming units; CFU-GEMM, granulocyte/erythroid cell/megakaryocyte/macrophage colony-forming units; IL-3, interleukin 3; Epo, erythropoietin; RT, reverse transcription.

(CFU-GEMM cultures). Liquid cultures were essentially as described (11) with modifications noted under Fig. 1.

AS-ACHE (5′-CTGCGGGGGCCTCAT-3′) (7, 15) and the complementary S-ACHE oligo were synthesized either in fully phosphorothioated forms or with three 3′-terminal internucleotidic phosphorothioates, as noted. AS-BCHE was as described (12, 15) except that phosphorothioate modifications were introduced only at the three 3′-terminal internucleotidic bonds to reduce nonspecific toxicity. Oligos were melted for 5 min at 65°C prior to their addition to the cultures.

mRNA encoding erythroid transcription factor GATA-1 was quantified by reverse transcription (RT)-PCR mRNA as detailed (15), using the following primers (nucleotides 262–284 and 763–785, GenBank (accession no. X15763): GATA-1, 262 (+), 5′-AAGCTGAGGCCTACAGACACTCC-3′; GATA-1, 785 (−), 5′-CCGGTTCTGACCATTCATCTTGT-3′. PCR conditions were denaturation at 94°C for 1 min (first step, 3 min), annealing at 65°C for 1 min, and elongation at 72°C for 1 min. For semiquantitative comparison of mRNA levels, samples were removed every two cycles. Therefore, a difference of ≈4-fold in GATA-1 cDNA occurred between samples.

RESULTS

The *ex vivo* effects of AS-ACHE were first tested in liquid cultures without Epo. RT-PCR amplification of total cellular RNA revealed reduction of ≈90% in the two hematopoietically expressed alternative ACHE mRNA forms (16, 17) in cultures treated for 2 hr with AS-ACHE, as compared with RNA from S-ACHE cultures (Fig. 1*A*). Four days after AS-ACHE treatment, ACHE mRNA increased in AS-ACHE cultures to levels at least 10-fold higher than in S-ACHE cultures and also higher than in fresh bone marrow (Fig. 1*A*). As an additional control, we monitored the levels of actin mRNA (15); we found them to be essentially similar at 2 hr and at 4 days. This indicated that AS-ACHE affected specific events that determined the level of ACHE mRNA but were not relevant to the processing of actin mRNA. To examine whether the delayed increase in ACHE mRNA in AS-ACHE-treated cultures was only one example of a wider change in transcription pattern, we employed the approach of differential PCR display (18). This analysis, the relevant part of which is presented in Fig. 1*B*, revealed a considerable difference between fresh bone marrow and each of the oligo-treated cell cultures in the pattern of cDNAs amplified when an arbitrarily chosen PCR primer and an oligo(dT)-based primer (Fig. 1*B*) were used. In particular, several PCR fragments were produced from the RNA preparations from cultured cells that were absent from the lane derived from fresh bone marrow RNA. Also, several other PCR products that were common to the bone marrow and the AS-ACHE sample were missing from the S-ACHE lane (Fig. 1*B*). Other PCR products were not produced from the mRNA of bone marrow or of S-ACHE cultures, yet appeared among the fragments from the AS-ACHE preparation (data not shown).

The cellular effects of these oligo treatments were evaluated by counting cell numbers and determining colony counts and compositions. In CFU-MK cultures grown for 4 days, AS-ACHE, but not S-ACHE, increased cell numbers but reduced colony counts (Fig. 2 *A* and *B*). This indicated poorer expansion of CFU-MK progenitors but greater proliferative potential for their progeny under AS-ACHE treatment than was seen in untreated cultures. In CFU-GEMM cultures grown for 8 days, AS-ACHE, but not S-ACHE, increased colony counts 5-fold and cell numbers 2-fold, with a peak effect at 12 μM compared with untreated cultures ($P < 0.001$) (Fig. 2 *C* and *D*), demonstrating dose-dependent expansion and/or survival of progenitors. Thus, the expansion of progenitors, but not the general proliferative effect of AS-ACHE, appeared to depend on Epo.

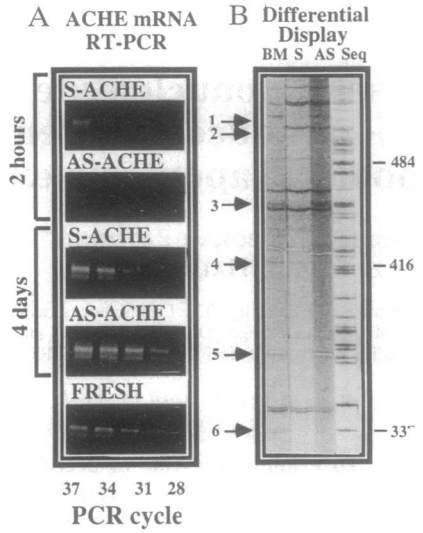

FIG. 1. Transient destruction of ACHE mRNAs and subsequent transcriptional changes under AS-ACHE treatment. (*A*) Quantification of ACHE mRNA levels by RT-PCR. RT-PCR was performed as detailed elsewhere (15), with 100-ng samples of total cellular RNA from cells in liquid cultures to which 2.5 μM AS-ACHE or S-ACHE (15), protected by phosphorothioates in the three 3′-terminal positions, had been added at initiation of culture. RNA from fresh bone marrow served for comparison. The employed primers, selective for the mouse ACHE mRNA subtypes expressed in hematopoietic cells (16), were mAc 1361 (+), 5′-CCGGGTCTATGCCTACATCTTTGAA-3′, where 1361 is the nucleotide number (GenBank accession no. M99492) common to all ACHE mRNA forms, and mAc 240 (−), 5′-AAGGAAGAAGAGGAGGGACAGGGCTAAG-3′, where 240 is the nucleotide number in the EH exon included in hematopoietic mouse ACHE mRNAs. This pair of PCR primers can amplify two alternative ACHE cDNA fragments, 457 and 573 bp in length, representing two modes of alternative splicing that occur in bone marrow ACHE mRNAs (16, 17). RNA was extracted after 2 hr or 4 days in culture, or from fresh bone marrow as detailed (15), with the addition of microcarrier (Molecular Research Center, Cincinnati). (*B*) Differential PCR display of mRNA transcripts from oligo-treated cultures. Differential amplification of total cellular RNAs from 4-day treated cultures or fresh bone marrow, as in *A* (above) was performed with 0.2-μg samples essentially according to Liang and Pardee (18), using 1 mM dNTPs and the primers for PCR reactions: R_{15} (+), 5′-AGAGTGCAGGCCATG-3′, and $dT_{11}AN$ (−), 5′-TTTTTTTTTTTTAN-3′. [α-^{33}P]dATP (Amersham) was used for labeling. Exposure was for 2 days at −70°C, for 10% of reaction mixtures per lane and in parallel to a sequence ladder [sequence M13mp18, with the standard primer included in the Sequenase 2.0 kit (United States Biochemical)] (length in nucleotides is on the right). Numbered arrows denote PCR products conspicuous in AS-ACHE culture (lane AS; band 3), in both cell cultures, including the S-ACHE-treated (lane S; bands 2 and 4), or in bone marrow (BM) and AS-ACHE cultures (bands 1, 5, and 6).

The specificity of the enhancement of CFU-GEMM colony formation by AS-ACHE, observed under Epo stimulation, was further demonstrated by treating cultures with AS-BCHE, which blocked expression (12) of the closely related yet distinct (2) *BCHE* gene, assumed to serve as a "backup" for *ACHE* (1). AS-BCHE failed to increase colony counts to a significant degree in CFU-GEMM or CFU-MK cultures ($P < 0.05$, not shown), consistent with a dominant role for AcCho-Ease as compared with butyrylcholinesterase in myeloid, erythroid, and megakaryocyte development (15).

To determine whether the enhanced expansion of CFU-GEMM progenitors was associated with abrogation of apoptosis in the progeny cells, we evaluated yields and integrity of DNA (19) after addition of AS-ACHE to the cultures. AS-ACHE-treated CFU-GEMM cultures yielded at least 5-fold higher amounts of DNA than S-ACHE-treated or nontreated control CFU-GEMM cultures, confirming that

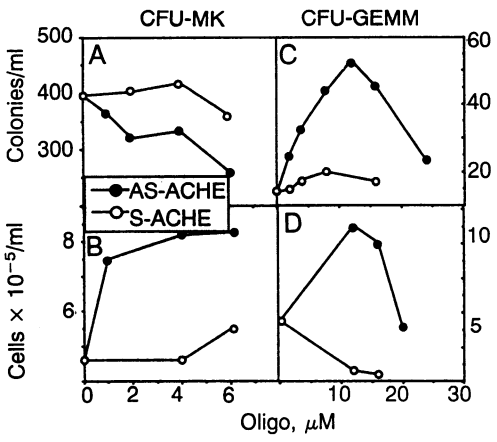

FIG. 2. Increases in colony counts and cell numbers effected by AS-ACHE oligos. Cultures were grown as detailed in *Materials and Methods*. One of eight and five reproducible experiments, respectively, is presented for colony counts (*A* and *C*) and cell numbers (*B* and *D*) after treatment with fully phosphorothioated oligos. ●, AS-ACHE; ○, S-ACHE.

AS-ACHE relieves the *ex vivo* suppression of proliferation (Fig. 3*A*). When equal quantities of these DNA preparations were electrophoresed, blotted, and hybridized with a ^{32}P-labeled mouse genomic DNA probe, DNA from AS-ACHE-treated cells displayed patterns similar to those of native bone marrow DNA, whereas DNA from untreated and S-ACHE control cultures demonstrated conspicuous fragmentation, characteristic of apoptosis (19, 20) (Fig. 3*B*). As the abrogation of apoptosis was limited to AS-ACHE, the only oligo capable of inducing targeted destruction of hematopoietic ACHE mRNA *ex vivo* (Fig. 1*A*) and *in vivo* (15), this suggested that ACHE mRNA expression limits proliferation by directing erythropoietic cells toward apoptosis. Further studies will be required to decipher the mechanism of this effect.

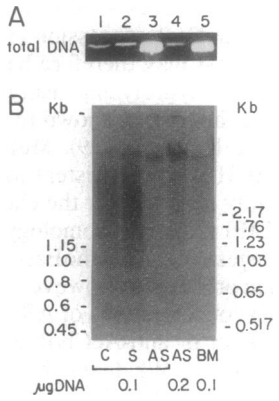

FIG. 3. AS-ACHE suppresses hematopoietic apoptosis *ex vivo*. (*A*) AS-ACHE treatment increases DNA yields. Cells were picked and collected in cold TBS (136 mM NaCl/2.6 mM KCl/25 mM Tris·HCl, pH 7.4), centrifuged (1500 × *g*, 10 min), and lysed (1 hr, 37°C) with 0.5 ml of 100 mM EDTA/10 mM Tris·HCl, pH 8.0, containing RNase A at 20 μg/ml and 0.5% SDS. After treatment with proteinase K (100 μg/ml, 3 hr, 50°C) and extraction with phenol/chloroform, DNA was precipitated at −20°C with 28 μl of 4 M NaCl and 1 ml of 96% (vol/vol) ethanol, washed with 70% (vol/vol) ethanol, air-dried, and dissolved in 20 μl of 1 mM EDTA/10 mM Tris·HCl, pH 7.4. Samples (10 μl) were denatured (65°C, 15 min) in 2 μl of loading buffer (50 mM EDTA, pH 8.0/15% Ficoll/0.25% bromophenol blue). DNA was extracted from 1 × 10^5 IL-3- + Epo-induced cells cultured for 8 days in the absence (lane 1) or presence of 16 μM totally phosphorothioated S-ACHE (lane 2) or AS-ACHE (lane 3). Total amounts of extracted DNA from each culture and 0.1 μg (lane 4) and 1.0 μg (lane 5) of bone marrow DNA were electrophoresed (1.5% agarose, 1.5 hr, 60 V) in the presence of ethidium bromide and UV-photographed. (*B*) AS-ACHE circumvents DNA fragmentation in cultured bone marrow cells. The noted quantities of DNA from nontreated control (C) cultures or those treated with S-ACHE (S) or AS-ACHE (AS) or bone marrow DNA (BM) were electrophoresed as in *A*. To achieve adequate sensitivity of detection, the DNA was hybridized with total mouse DNA, labeled with ^{32}P. Autoradiographic exposure was for 3 days. Molecular size markers (Boehringer Mannheim) were electrophoresed in parallel (right). A ladder of oligonucleosomes (sized on the left), reflecting apoptosis, appeared in the control cultures and in those treated with S-ACHE, but not in fresh bone marrow (BM) or in 0.1- and 0.2-μg DNA samples from AS-ACHE-treated cultures.

Macrophages from AS-ACHE-treated CFU-GEMM cultures appeared smaller, less vacuolated, and with unblebbed cytoplasm and less condensed nuclei than those in control cultures, as expected from cells in which apoptosis had been suppressed (19). They were also aggregated into large clusters (Fig. 4 *A* and *B*), suggesting modifications in their cell adhesion properties. In CFU-MK cultures AS-ACHE, but not AS-BCHE (Table 1), induced the accumulation of relatively primitive, small (≈5 μm in diameter; Fig. 4*D*) blast cells and macrophages, with corresponding decreases in early and late megakaryocytes. The blast cells were, in large part, immunoreactive with antibodies to both glycophorin and glycoprotein IIb/IIIa (ref. 21; results not shown), suggesting that these were committed promegakaryocytes

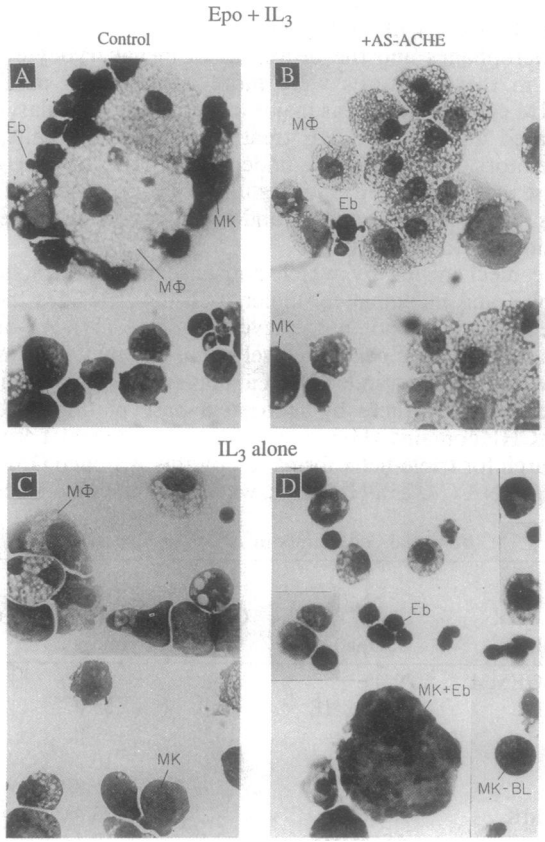

FIG. 4. AS-ACHE enhancement of macrophage production in the presence of Epo + IL-3 and of blast formation with IL-3 alone. Colonies were harvested, stained, and analyzed as described for Table 1. (×630.) (*A*) In the presence of IL-3 + Epo, characteristic CFU-GEMM culture colonies include numerous erythroblasts (Eb), a few apparently apoptotic macrophages (MΦ), and megakaryocytes (MK). (*B*) AS-ACHE (16 μM, totally phosphorothioated) -treated CFU-GEMM cultures with many MΦs, early MKs, and a single Eb. (*C*) In the presence of IL-3 alone, CFU-MK colonies include primarily MKs and MΦs. (*D*) AS-ACHE-treated (2.5 μM, totally phosphorothioated) CFU-MK cultures display erythroblast formation and MK-associated blasts (MK-BL).

and/or erythroblasts (22). This notion was confirmed by RT-PCR tests in which the mRNA encoding transcription factor GATA-1, specific for promegakaryocytes and erythroblasts (22), was amplified. A 10-fold increase in GATA-1 mRNA occurred in liquid CFU-MK cultures treated with AS-ACHE, but not with S-ACHE, demonstrating enhanced erythropoiesis and megakaryocytopoiesis (data not shown). Neither AS-BCHE nor AS-ACHE promoted growth in the absence of both Epo and IL-3.

It is generally accepted that erythrocytes and megakaryocytes share a common multipotent stem cell progenitor (22) and that this progenitor cell expresses both GATA-1 and AcChoEase at levels higher than those in myeloid cells (22, 23). This is in line with the higher levels of ACHE mRNA (Fig. 1*A*) and GATA-1 mRNA in CFU-MK cells 4 days after the addition of AS-ACHE, when progeny blast cells accumulate in these cultures (Fig. 4*D*).

To further examine the sequence and lineage specificity of the oligo effects, differential cell compositions were determined. Dose-dependent increases in macrophage and granulocyte fractions and corresponding decreases in megakaryocyte and erythroid fractions were found in CFU-GEMM cultures after treatment with AS-ACHE, and to a much lower extent with AS-BCHE (Table 1). In view of the increase in cell and colony counts (Fig. 2 *C* and *D*), this indicated largely unchanged total numbers of erythrocytes and megakaryocytes. In untreated CFU-MK cultures no erythrocytes developed, in line with the absence of Epo. Both the increases in macrophages and the decreases in megakaryocyte fractions in these cultures were more limited than in CFU-GEMM cultures, whereas major fractions of early blast cells appeared under AS-ACHE treatment (Table 1). Thus, the progenitors that expanded under AS-ACHE in CFU-MK cultures differentiated successfully into myeloid cell lineages, while their erythroid and megakaryocyte progenies remained in the blast stage.

The mechanisms by which antisense inhibition acts are complex and incompletely understood (24). Besides the intended, specific action at the level of mRNA (14), AS-oligos may also act at the genomic level by insertion into the major groove of double-stranded DNA at polypurine·polypyrimidine sequences, held there by Hoogstein base pairing (25). As AS-ACHE contains a G_5 homooligomer, we thought it prudent to search for triple-helix formation by this AS-oligo (15) with *ACHE* DNA (24, 25). However, we failed to find evidence for such hybrids, either with S-ACHE oligomers or with plasmid DNA. This made genomic intervention unlikely and strengthened the proposal that the targeted destruction of ACHE mRNA, similar to the *in vivo* situation (15) was the cause of the *ex vivo* hematopoietic effects of AS-ACHE.

DISCUSSION

We found that a 15-mer AS-oligo targeted to the *ACHE* gene induces transcriptional changes, expansion of progenitors, and proliferation of nontransformed primary bone marrow cells and suppresses apoptosis in their progeny. Increased proliferation was reported to follow treatment of a human endothelial cell line with an AS-oligo preventing the synthesis of interleukin 1 (26). Survival/differentiation-linked effects were more recently reported for the apoptosis-related *bcl-2* gene (27). To the best of our knowledge, however, our experimental system is unique in the capacity to induce expansion of primary progenitor cells *ex vivo*. This foreshadows further use of the differential PCR display approach (Fig. 1; ref. 18) to identify and clone genes whose transcripts are modified under suppression of hematopoietic apoptosis.

At present, we cannot exclude the possibility that both the stem cell expansion and the apoptotic effects described in this report may be due to an indirect interaction of the AS-ACHE oligo with as-yet-undefined protein(s) in the treated cells (28). However, whether exhibiting a direct antisense effect or acting indirectly, this AS-oligo reduces ACHE mRNA levels at 2 hr and thus disrupts AcChoEase expression. The apoptotic consequences of this process may therefore be connected to the role of AcChoEase in hydrolyzing acetylcholine. Indeed, acetylcholine agonists have been shown to enhance cell division in nonneuronal cell cultures (29). Moreover, the effects observed for AS-ACHE are consistent with the arrest of hematopoiesis recently observed for the cholinergic leukemia inhibitory factor (LIF) (30). The homology with *Drosophila* neurotactin (31) suggests that AcChoEase affects apoptosis through cell adhesion properties. However, the aggregation of macrophages at high concentrations of AS-ACHE (Fig. 4), as well as under the effect of anti-AcChoEase antibodies (32), weakens this latter theory. Whatever the molecular basis of the action of AcChoEase, our findings suggest that AcChoEase enhances hematopoietic apoptosis (9) while reducing the capacity of multipotent stem cells committed to myeloid, erythroid, and megakaryocyte lineages to expand.

Table 1. Differential cell counts in AS-oligo-treated cultures

Culture	Oligo	Conc., μM	% of total cells: Macrophages	Granulocytes	Megakaryocytes (early/late)	Erythroid cells (early/late)	Early blasts	Cells counted
CFU-GEMM	None	—	8.7	9.3	16.2/6.6	24.2/35.1	0.0	1383
	AS-ACHE	4.0	35.0	4.0	11.9/4.9	20.6/22.7	0.0	1274
		16.0	61.0	12.4	8.0/3.8	9.1/5.8	0.0	1197
	AS-BCHE	5.0	10.2	4.1	11.6/8.3	30.7/34.8	0.0	1429
		15.0	15.8	16.8	7.7/7.2	15.5/36.9	0.0	1641
CFU-MK	None	—	20.4	2.2	28.4/42.4	0.0	6.5	627
	AS-ACHE	2.5	19.5	1.0	15.5/11.8	0.0	52.1	1274
		5.0	35.6	5.7	21.3/11.0	0.0	26.4	1662
	AS-BCHE	5.0	27.7	22.4	23.9/25.8	0.0	0.0	1324

Colonies were picked, washed twice in phosphate-buffered saline (PBS), cytocentrifuged (500 × *g*, 5 min), and stained with Gurr's improved Giemsa stain (BDH) for morphological classification (11, 12). For immunocytochemical staining, cytocentrifuged cells were fixed for 10 min in acetone/methanol (1:1, vol/vol), washed twice in PBS, and incubated (60 min, room temperature) with monoclonal antibodies (mAbs) toward fluorescein isothiocyanate (FITC)-labeled human glycophorin (Immunotech, Marseilles, France), selective for small, 5- to 10-μm diameter, blast and erythroid cells, or anti-GPIIb/IIIa (from B. S. Coller, Stony Brook), selective for early and late megakaryocytes (21). In the latter case, fluorescence detection was performed with a second anti-mouse mAb labeled with FITC or Texas red (Amersham). Incubations with mAbs were followed by two washes with PBS with 2.5% fetal calf serum and staining with the Giemsa stain. Immunostained cells were mounted in Entellan (Merck) and fluorescence was observed with a Zeiss Axioplan fluoromicroscope. Macrophages and polymorphonuclear cells (granulocytes), both unreactive with these two antibodies, were distinguished by their vacuolized cytoplasms and multilobed nuclei, respectively. Data represent percent compositions for one of three reproducible experiments for each treatment.

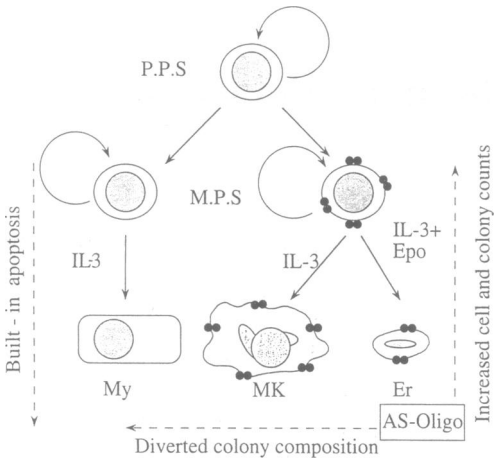

FIG. 5. Antisense prevention of apoptosis as an alternative to hematopoietic differentiation *ex vivo*. Reinforced proliferation of stem cells (circular arrows) or enhanced differentiation (straight solid arrows) of variously committed hematopoietic cells expressing different proteins determine colony and cell counts and their differential composition. Differentiated cells inevitably undergo apoptosis (downward dashed arrow, left). Administration of oligos that interfere with expression of developmentally important genes such as *ACHE* (dashed arrows, right and bottom) may reverse these processes, increasing cell and colony counts (upward arrow), and/or altering cell composition within surviving colonies. In cases where inhibition of expression of these genes does not interfere with myeloid cell formation, development is diverted toward myeloidogenesis (leftward arrow). P.P.S., pluripotent stem cell; M.P.S., multipotent stem cell; Er, erythroid cell; MK, megakaryocyte; My, myeloid cell; and filled circles, cell-surface AcChoEase dimers.

In *ex vivo* primary cell cultures AS-ACHE may be expected to trigger hematopoietic stem cell expansion, as is schematically presented in Fig. 5. Under AS-ACHE treatment, the multipotent stem cell progenitors in CFU-MK cultures continue to proliferate. The hematopoietic balance is diverted toward myeloidogenesis and blast formation, a leukemia-like profile (Fig. 5). The occurrence of myeloid leukemia in farmers exposed to AcChoEase-inhibitory organophosphorus insecticides (6), the pronounced *in vivo* hematopoietic effects of AS-ACHE (15), and the conspicuous *in vivo* production of active macrophages after injection of anti-AcChoEase antibodies into mice (32) may all parallel the *ex vivo* effects of AS-ACHE.

Interestingly, the suppression of apoptosis by AS-ACHE seems to be dominant over induction of differentiation by cytokines. Defects in *ACHE* gene expression, analogous to AS-ACHE inhibition, may therefore interfere with the programmed death of normal hematopoietic cells. This can leave uncontrolled the differing growth-inducing activities of Epo (33), c-*myc* (34), and *bcl2* (30), which may lead to leukemia (6, 35). Under *ex vivo* conditions, AS-ACHE may perhaps be used to improve preparations for bone marrow transplantation.

The AcChoEase protein may mediate apoptotic effects also in the embryonic nervous system, where *ACHE* gene expression peaks immediately after the surge of cell division, during terminal differentiation of fetal neurons (4). This raises the possibility that AcChoEase is involved in the long-delayed programmed cell death of neurons as well (36). Dual involvement in apoptosis and orientation toward cholinergic functioning could thus explain the roles of AcChoEase in the multitude of cell types that express this protein, calling for awareness of the iatrogenic effects of cholinergic drugs and the consequences of careless agricultural use of organophosphorus AcChoEase inhibitors.

The authors are grateful to Dr. B. S. Coller (Stony Brook, NY) for anti-GPIIb/IIIa antibodies and to Dr. D. Glick (Jerusalem) for helpful discussions. This research has been supported by a Bundesministerium für Forschung und Technologie Biotechnology Program Grant (to H.S. and F.E.), the Israel Cancer Research Society (to H.S.), the Israel Health Ministry (to H.Z. and H.S.), and the Israel Ministry of Science and Technology (to H.S. and H.Z.). E.L.-L. is the recipient of a Golda Meir Predoctoral Fellowship.

1. Soreq, H. & Zakut, H. (1993) *Human Cholinesterases and Anticholinesterases* (Academic, San Diego).
2. Lapidot-Lifson, Y., Prody, C. A., Ginzberg, D., Meytes, D., Zakut, H. & Soreq, H. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 4715–4719.
3. Paoletti, F., Mocali, A. & Vannucchi, A. M. (1992) *Blood* **79,** 2873–2879.
4. Layer, P. G., Weikert, T. & Willbold, E. (1992) *Cell Tissue Res.* **268,** 409–418.
5. Zakut, H., Ehrlich, G., Ayalon, A., Prody, C. A., Malinger, G., Seidman, S., Ginzberg, D., Kehlenbach, R. & Soreq, H. (1990) *J. Clin. Invest.* **86,** 900–908.
6. Brown, L. M., Blair, A., Gibson, R., Everett, G. D., Cantor, K. P., Schuman, L. M., Burmeister, L. F., Van Lier, S. F. & Dick, F. (1990) *Cancer Res.* **50,** 6585–6591.
7. Soreq, H., Ben-Aziz, R., Prody, C. A., Seidman, S., Gnatt, A., Neville, L., Lieman-Hurwitz, J., Lev-Lehman, E., Ginzberg, D., Lapidot-Lifson, Y. & Zakut, H. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 9688–9692.
8. Zakut, H., Lapidot-Lifson, Y., Beeri, R., Ballin, A. & Soreq, H. (1992) *Mutat. Res.* **276,** 275–284.
9. Koury, M. J. (1992) *Exp. Hematol.* **20,** 391–394.
10. Ehrlich, G., Viegas-Pequignot, E., Ginzberg, D., Sindel, L., Soreq, H. & Zakut, H. (1992) *Genomics* **13,** 1192–1197.
11. Lapidot-Lifson, Y., Patinkin, D., Prody, C. A., Ehrlich, G., Seidman, S., Ben-Aziz, R., Benseler, F., Eckstein, F., Zakut, H. & Soreq, H. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 579–583.
12. Patinkin, D., Seidman, S., Eckstein, F., Benseler, F., Zakut, H. & Soreq, H. (1990) *Mol. Cell. Biol.* **10,** 6046–6050.
13. Fauser, A. A. & Messner, H. A. (1978) *Blood* **52,** 1243–1248.
14. Stein, C. A. & Cheng, Y.-C. (1993) *Science* **261,** 1004–1012.
15. Lev-Lehman, E., Ginzberg, D., Hornreich, G., Ehrlich, G., Meshorer, A., Eckstein, F., Soreq, H. & Zakut, H. (1993) *Gene Ther.* **1,** 127–135.
16. Li, Y., Camp, S. & Taylor, P. (1993) *J. Biol. Chem.* **268,** 5790–5797.
17. Karpel, R., Ben Aziz-Aloya, R., Sternfeld, M., Ehrlich, G., Ginzberg, D., Tarroni, P., Clementi, F., Zakut, H. & Soreq, H. (1994) *Exp. Cell Res.* **210,** 268–277.
18. Liang, P. & Pardee, A. B. (1992) *Science* **257,** 967–971.
19. Shi, Y., Glynn, J. M., Guilbert, L. J., Cotter, T. G., Bissonnette, R. P. & Green, D. R. (1992) *Science* **257,** 212–214.
20. Okumura, N., Tsuji, K. & Nakahata, T. (1992) *Blood* **80,** 642–650.
21. Coller, B. S., Beer, J. H., Scudder, L. E. & Steinberg, M. H. (1989) *Blood* **74,** 182–192.
22. Visvader, J. E., Elefanty, A. G., Strasser, A. & Adams, J. M. (1992) *EMBO J.* **11,** 4557–4564.
23. Burstein, S. A., Adamson, J. W. & Harker, L. A. (1980) *J. Cell. Physiol.* **103,** 201–208.
24. Nellen, W. & Lichtenstein, C. (1993) *Trends Biochem. Sci.* **18,** 419–423.
25. Strobel, S. A. & Dervan, P. B. (1990) *Science* **249,** 73–75.
26. Maier, J. A. M., Voulalas, P., Roeder, D. & Maciag, T. (1990) *Science* **249,** 1570–1574.
27. Fairbairn, L. J., Cowling, G. J., Reipert, B. M. & Dexter, T. M. (1993) *Cell* **74,** 823–832.
28. Yaswen, P., Stampfer, M. R., Ghosh, K. & Cohen, J. S. (1993) *Antisense Res. Dev.* **3,** 67–77.
29. Ashkenazi, A., Ramachandran, J. & Capon, D. J. (1989) *Nature (London)* **340,** 146–150.
30. Escary, J.-L., Perreau, J., Dumenil, D., Ezine, S. & Brulet, P. (1993) *Nature (London)* **363,** 361–364.
31. de la Escalera, S., Bockamp, E.-O., Moya, F., Piovant, M. & Jimenez, F. (1990) *EMBO J.* **9,** 3593–3601.
32. Brimijoin, S., Moser, V., Hammond, P., Oka, N. & Lennon, V. A. (1993) *Neuroscience* **54,** 201–223.
33. Kelley, L. L., Koury, M. J., Bondurant, M. C., Koury, S. T., Sawyer, S. T. & Wickrema, A. (1993) *Blood* **82,** 2340–2352.
34. Smith, C. A., Williams, G. T., Kingston, R., Jenkinson, E. J. & Owen, J. J. T. (1989) *Nature (London)* **337,** 181–184.
35. Soreq, H., Lapidot-Lifson, Y. & Zakut, H. (1991) *Cancer Cells* **3,** 511–516.
36. Collins, M. K. L. & Lopez Rivas, A. (1993) *Trends Biochem. Sci.* **18,** 307–309.