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The antisense *bcl-2–IgH* transcript is an optimal target for synthetic oligonucleotides

(antisense RNA/hybrid RNA/apoptosis/*bcl-2* regulation/antitumor)

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ABSTRACT **In most human follicular B cell lymphomas the *bcl-2* gene is up-regulated as a result of the t(14;18) chromosomal translocation generating a hybrid *bcl-2–IgH* mRNA. Recently, we have identified in t(14;18)-positive cells a *bcl-2–IgH* mRNA in the antisense orientation, putatively responsible for the overexpression of *bcl-2*. Herein we show that this chimeric antisense transcript is an optimal target for synthetic oligodeoxynucleotides (ODNs). A variety of sense-oriented oligonucleotides have been designed that target the antisense transcript in regions endowed with a sequence specificity presumably restricted to an individual cell line (the *bcl-2–IgH* fusion regions) or extended to all t(14;18) cells (the ectopic *bcl-2* segment upstream from the major breakpoint region and the *IgH* segment). All sense-oriented ODNs complementary to the antisense transcript induced an early strong inhibition of cell growth and a late fulminant cell death. As expected, the activity of ODNs targeting the fusion region was restricted to each individual cell line, whereas the activity of all ODNs targeting the common *bcl-2* and *IgH* segments was extended to all t(14;18) cell lines tested. These sense ODNs were not effective in untranslocated cell lines. Antisense-oriented ODNs, complementary to the *bcl-2–IgH* mRNA, and control ODNs (scrambled, inverted, or mismatched) were biologically ineffective. The selectivity and efficacy of all sense ODNs tested provide support for the development of therapeutic ODNs targeting the *bcl-2–IgH* antisense transcript expressed in human follicular lymphomas.**

The antisense approach offers a new way of overcoming the lack of specificity of conventional cancer chemotherapeutic agents (1, 2). Indeed, several *in vitro* studies as well as experiments in nude mice transplanted with human tumors demonstrate that antisense oligodeoxynucleotide (ODN) targeting genes associated with neoplastic transformation or progression are effective against tumor cells but not against normal cells (3–7). Tumor-specific molecular targets for ODNs include fusion sequences arising from chromosomal translocations (8, 9). In our laboratory, the 14;18 (q32;21) chromosome translocation, present in most human follicular B cell lymphomas, has been selected for ODN targeting (10).

Human follicular lymphomas are characterized in 70–80% of the cases by the molecular rearrangement of the 3′ noncoding region of the antiapoptotic gene *bcl-2* with the immunoglobulin heavy chain locus (*bcl-2–IgH*), due to the t(14;18) chromosomal translocation (11, 12). As a result, these lymphomas express elevated levels of both the *bcl-2–IgH* chimeric transcript and BCL-2 protein (13, 14), which confers a survival advantage associated with neoplastic transformation (15). It should be noticed that so far the reason for the overexpression of *bcl-2–IgH* mRNA is not totally clear.

In recent years we have investigated the response of follicular lymphoma cells to ODNs, including those targeting N region insertions (stretches of DNA inserted during *bcl-2–IgH* recombination) whose sequence is unique to each individual lymphoma (16). These studies have unexpectedly led us to identify in these lymphomas a hybrid *bcl-2–IgH* transcript in the antisense orientation (17), originating in the *IgH* locus and encompassing the N region and part of the 3′ untranslated region of the *bcl-2* gene. This transcript has a certain relationship with another antisense transcript previously identified in Burkitt lymphomas starting in the μ switch region of the *IgH* locus and spanning the c-*myc* gene (18).

Endogenous antisense RNAs have been identified for some eukaryotic genes, such as chicken and mouse *IGFII* (19, 20), *Xenopus* and human *FGF-2* (21, 22), human *WT1* (23), c-*myc* and n-*myc* (18, 24), and p53 (25). They appear to regulate the expression of the corresponding sense gene by promoting its degradation (26). However, in the case of the *bcl-2–IgH* antisense RNA, peculiar to t(14;18)-positive lymphomas, it is thought that it contributes to *bcl-2* overexpression and, therefore, to oncogenicity, although direct evidence to support this hypothesis is lacking. We have also hypothesized that this antisense transcript might act by functionally inactivating negative regulatory regions, such as the adenine- and uracil-rich degradation consensus elements (27, 28), which we have identified in the 3′ untranslated region of the *bcl-2* portion of the *bcl-2–IgH* mRNA (17). On this assumption, the *bcl-2–IgH* antisense RNA might be an optimal cancer-specific target for ODNs, especially because the antisense transcripts are generally present in cells at a low copy number due to their rapid degradation (29).

In a number of lymphoma cell lines, we have targeted this unique tumor-specific *bcl-2–IgH* antisense RNA by sense-oriented ODNs, in the N region, the discrete molecular structure endowed with an individual nucleotide sequence for each cell line, or in the other regions shared by the t(14;18) cells. In all cases, the ODN treatment induced massive cell death by possibly lowering *bcl-2* expression despite the presence of other oncogenic alterations in the cell lines treated (30).

MATERIALS AND METHODS

Cell Lines. The human follicular B cell lymphoma lines [DOHH2 (31), SU-DHL-4 (16), and K422 (32) carrying the t(14;18) chromosomal translocation, obtained as indicated (17)] and the human t(14;18)-negative cell lines [Burkitt lymphoma Raji, T cell leukemias, Jurkat, Molt-3, and LCL, erythroleukemia K562, and promyelocytic leukemia HL-60], all mycoplasma-free, were grown in RPMI 1640 medium

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Abbreviations: ODN, oligodeoxynucleotide; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling.

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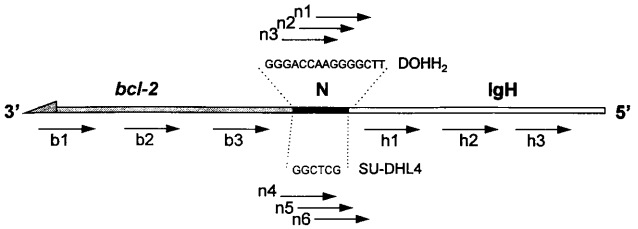

FIG. 1. Schematic structure of the hybrid *bcl-2–IgH* antisense RNA and regions against which complementary "sense" oligonucleotides have been designed. The sequences of the fusion point, in which random nucleotides are inserted (N region) during chromosome recombination of the DOHH2 and SU-DHL-4 cell lines, are shown. The *bcl-2* and IgH sequences, flanking the fusion region, are common to all t(14;18) cell lines. The 18-mer ODNs, either phosphodiester or phosphorothioate derivatives, were complementary to the following nucleotide numbers of the cDNA: for the N regions of DOHH2 cells (31), 3105 (ODN n1), 3110 (ODN n2), 3119 (ODN n3); for N regions of SU-DHL-4 cells (16), 2686 (ODN n4), 2692 (ODN n5), 2696 (ODN n6); for the *bcl-2* portion (16), 970 (ODN b1), 2479 (ODN b2), 2625 (ODN b3); for the IgH portion (36), 2956 (ODN h1), 3119 (ODN h2), 4218 (ODN h3).

containing 10% heat-inactivated fetal calf serum plus glutamine. The fetal calf serum used in ODN experiments was heated at 65°C for 30 min to inactivate nucleases.

ODNs. Different batches of phosphodiester or phosphorothioate ODNs were purchased from local (PRIMM, Milan, Italy; TIBB, Genoa, Italy) or foreign companies (MEDPROBE, Olso, Norway; GENSET, Paris, France), purified by Sephadex G-25 gel chromatography, and in some cases, analyzed by PAGE in 20% gels, reverse-phase HPLC, and capillary chromatography (33). Biological activity and nonspecific cytotoxicity were also evaluated. The 18-mer sense-oriented ODNs, complementary to the hybrid *bcl-2–IgH* antisense transcript, were designed to target either the individual cell-line-specific *bcl-2–IgH* N regions or the *bcl-2* and *IgH* regions shared by all t(14;18) cell lines. Control ODNs were designed in the antisense, inverted, scrambled, and mismatched orientation relative to the sense ODNs. The *in vitro* half-life of the ODNs administered to cells was about 12 h for the phosphorodiesters and 3 days for the phosphorothioate derivatives (5).

ODN Treatments and Cell Survival Analysis. For short-term cultures, 2–5 $\times$ 10^4 cells/200 μl were seeded in 96-microwell plates (Costar). For long-term cultures, 5 $\times$ 10^4 cells/500 μl were seeded in 24-well plates (Costar) and split twice weekly. Phosphodiester ODNs were supplied at the full dose at the time of cell plating and at half dose in the following days. Phosphorothioate ODNs were given as a single dose in the short-term cultures, and every 3 days in the long-term cultures. Irrespective of the nucleotide sequence, about 30% of the ODN batches were inactive or toxic and were, therefore, excluded from the study. Growth and viability of ODN-treated cultures were determined by microscopic cell counts with a hemocytometer chamber, and by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide-based colorimetric assay at the end of ODN experiments (34).

Quantitative Determinations of *bcl-2* mRNA and Protein. Total RNA from untreated and ODN-treated cells was extracted by RNAzol B (Biotecx Laboratories, Houston) and reverse-transcribed by using random hexamers and Moloney murine leukemia virus reverse transcriptase (Promega). Quantitative reverse transcription-coupled PCR determination of *bcl-2* mRNA was performed as described (5) on the reverse transcription product by using the following primer sets (Genset, Paris, France): 5′-CAATTCCGCATTTAATTCATGGTATTCAG-GAT-3′ (bases 2866–2899 of the *bcl-2* cDNA sequence) (16) and 5′-GGTGACCAGGGTCCCTTGGCCCAG-3′ (bases 2973–2998 of the *IgH* sequence) (35) for the *bcl-2–IgH* amplification; 5′-ACCCCACTGAAAAAGAT ~GA-3′ (bases 1544–1563) and 5′-ATCTTCAAACCTCCTCCATGATG-3′ (bases 2253–2262 and 3508–3517) of the β_2-microglobulin (36) for the amplification of β_2-microglobulin used as internal standard. To determine BCL-2 protein cellular level, 2 $\times$ 10^6 ODN-treated cells were fixed for 10 min in 2% paraformaldehyde plus 1% Triton X-100, washed in Tris-buffered saline, and incubated with anti-BCL-2 mAb or normal mouse serum (for negative control) after preincubation for 10 min with 2% heat-inactivated human AB serum. Indirect immunofluorescence staining for BCL-2 was analyzed by flow cytometry with an EPICS-C instrument (Coulter), as published elsewere in detail (37).

Apoptosis Determinations. Apoptotic cells present in ODN-treated cultures were detected both by propidium iodide staining and *in situ* fluorescent dUTP end labeling of fragmented DNA (TUNEL; terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling). For propidium iodine staining, cells were resuspended in 1.5 ml of hypotonic solution containing 0.1% sodium cytrate, propidium iodine (25 μg/ml), and 0.1% Triton X-100 and kept at 4°C in the dark prior to being analyzed by flow cytometry. TUNEL was performed as described (38) on cells fixed for 10 min in 2% paraformaldehyde, washed twice in 0.1 M Tris buffer, fixed again for 1 min in acetone, washed, and incubated for 1 h at 37°C with 1.5 μM fluorescein isothiocyanate-coupled dUTP (Boehringher Mannheim) plus terminal deoxynucleotidyltransferase (TdT, 0.5 international unit/μl; Boehringher Mannheim) in 1$\times$ TdT buffer. After two more washes, the cells were analyzed by flow cytometry.

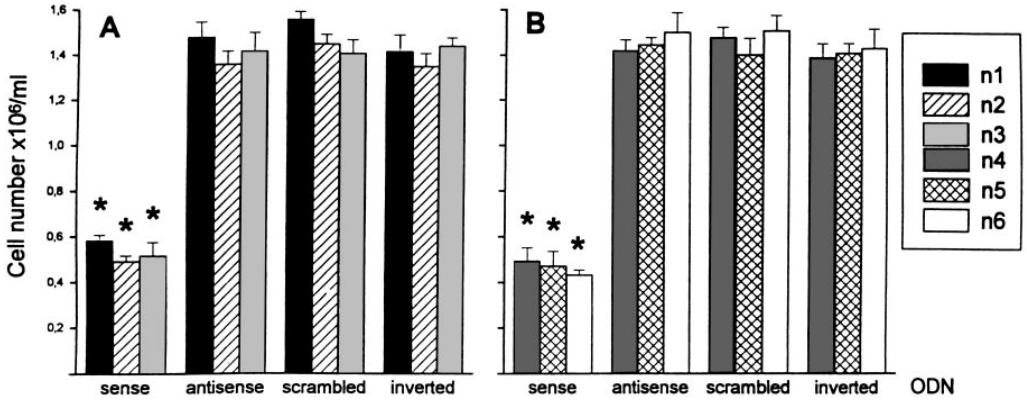

FIG. 2. Sequence-specific activity of ODNs targeting the N regions of the DOHH2 or the SU-DHL-4 cells. Three phosphodiester sense ODNs complementary to discrete segments of the N regions of DOHH2 cells (*A*) or SU-DHL-4 cells (*B*), as shown in Fig. 1, were employed. Antisense, inverted, and scrambled ODNs were used as controls. Untreated cells were considered as further control. All ODNs were administered at 10 μM on day 0 and at 5 μM on days 1 and 2. Cell counting and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assays were carried out on day 3. Data represent the means $\pm$ SEM of five experiments. Statistical significance was measured by Student's *t* test; *, $P < 0.01$.

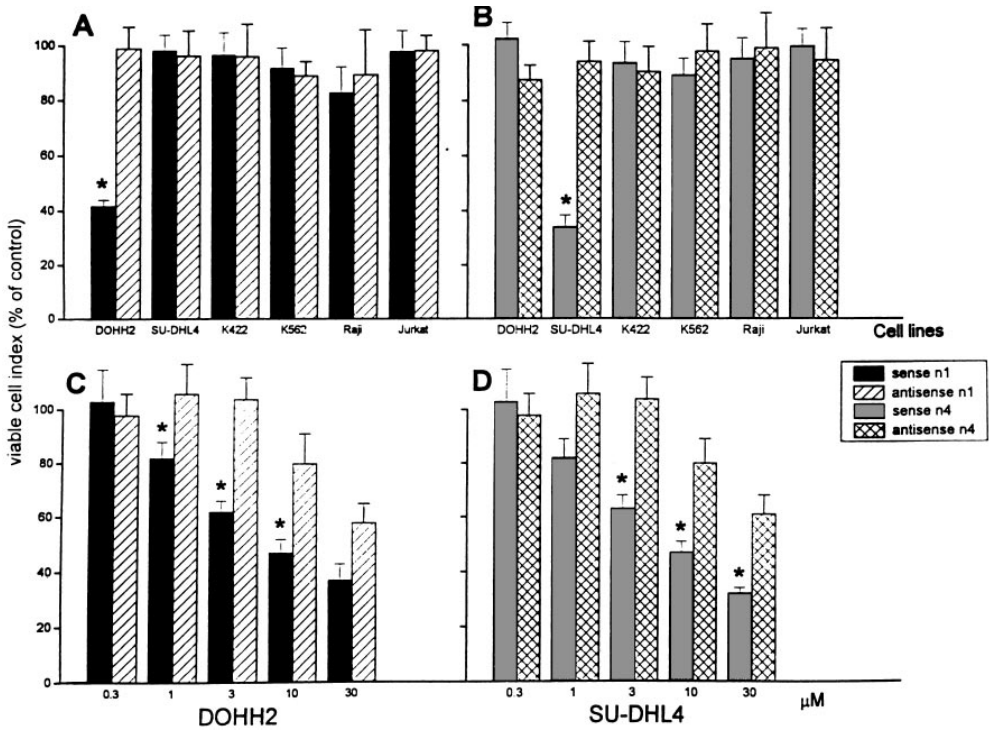

FIG. 3. Cell line specificity and dose–response activity of ODNs targeting the N regions of DOHH2 (*A* and *C*) and of DHL-4 (*B* and *D*) cells. Phosphorothioate sense ODN n1 (*A*) and ODN n4 (*B*) were given as a single dose of 10 μM on day 0. Control ODN were the phosphorothioate antisense ODN n1 or ODN n4, respectively. Dose–response activity of ODN n1 (*C*) or of ODN n4 (*D*) is shown. Cell count and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assays were done on day 5. Data, expressed as the viable cell index (48), are the mean ± SEM of three experiments. *, $P < 0.01$.

RESULTS

By up-regulating *bcl-2* expression, the hybrid *bcl-2–IgH* gene endows cells with a survival advantage that is probably responsible for neoplastic transformation. Although the molecular mechanisms of this *bcl-2* up-regulation remain to be fully elucidated, some role might be played by the chimeric *bcl-2–IgH* antisense RNA, recently identified in the t(14;18) cells. The targeting of the *bcl-2–IgH* antisense RNA with complementary ODNs (Fig. 1) results in decreased *bcl-2* gene expression and induction of apoptotic cell death. In this study, we have extended these observations by treating t(14;18) tumor cell lines with a number of ODNs complementary to different regions of the *bcl-2–IgH* antisense RNA.

Biological Activity and Specificity of ODNs Targeting Individual N Regions. The N nucleotide insertion sequences present in the junction region of the *bcl-2–IgH* antisense RNA are potential targets for the antisense approach. We asked whether ODNs complementary to the N regions of the antisense RNA of DOHH2 (n1, n2, and n3) and of SU-DHL4 (n4, n5, and n6) cells had any sequence-specific biological activity. The data (Fig. 2) show that exposure of these cell lines for 72 h with their respective sense ODNs results in almost 60–70% reduction in viable cell number, compared with cells that were untreated or treated with control ODNs.

Moreover, although the sense ODN n1 was active on DOHH2 cells but not on SU-DHL4 (Fig. 3), and *vice versa* for ODN n4, neither of these ODNs was active on the follicular lymphoma cell line K422, whose N region sequence in the *bcl-2–IgH* antisense transcript differs from that of DOHH2 and SU-DHL4 cells. Likewise, the cell lines K562, Raji, and Jurkat, which carry a normal *bcl-2* gene and are thus negative for the presence of *bcl-2–IgH* sense transcripts, were unresponsive to ODNs n1 and n4. Though not shown, the activity of ODNs n2 and n3 was superimposable on that of ODN n1, and the activity of ODNs n5 and n6 was identical to that of ODN n4. Collectively, these results indicate that sense oligonucleotides complementary to N region sequences of the endogenous *bcl-2–IgH* antisense RNA markedly affect cell survival in a sequence- and dose-dependent fashion.

Antitumor Activity of Sense ODNs Targeting Regions of the *bcl-2–IgH* Antisense Transcript Shared by the t(14;18) Cell Lines. The *bcl-2–IgH* antisense RNA carries invariant nucleotide sequences shared by all the t(14;18)-positive cell lines, located upstream and downstream from the N region, in particular in the 5′ region of the major breakpoint cluster region of *bcl-2* and in the

Table 1. t(14;18) specific activity of ODNs targeting the *bcl-2* regions or the *IgH* regions

	No. of cells × 10^{-4} per ml						
Cell line	None	b1	b2	b3	h1	h2	h3
DOHH2	85 ± 6	40 ± 3*	46 ± 7*	43 ± 6*	45 ± 4*	45 ± 6*	43 ± 4*
DHL-4	71 ± 2	29 ± 3*	28 ± 5*	32 ± 4*	35 ± 5*	39 ± 6*	34 ± 5*
K422	72 ± 9	36 ± 4*	34 ± 4*	32 ± 7*	37 ± 3*	33 ± 4*	35 ± 4*
K562	62 ± 8	60 ± 6	60 ± 5	80 ± 4	62 ± 6	57 ± 5	59 ± 3
LCL	75 ± 4	70 ± 6	69 ± 6	72 ± 6	70 ± 3	63 ± 6	67 ± 5
RAJI	73 ± 87	74 ± 4	66 ± 7	67 ± 3	68 ± 7	70 ± 7	69 ± 7

Phosphorothioate ODNs were given as a single dose at 10 μM on day 0 and cells were counted on day 5. Data are the mean ± SEM from three experiments.
*$P < 0.01$.

3′ region of the J locus. We have therefore synthesized sense ODNs targeting the *bcl-2* region (b1, b2, and b3) and the *IgH* region (h1, h2, and h3) of the *bcl-2–IgH* antisense transcript (Fig. 1) and examined their biological effects. All t(14;18)-positive cell lines appeared particularly susceptible to these ODNs (Table 1), as indicated by the 60–70% reduction of viability after 5 days of treatment. These effects were dose-dependent (Table 2). These findings therefore indicate (*i*) that ODN targeting sequences of the *bcl-2–IgH* antisense transcript common to all t(14;18)-positive cell lines are biologically active and (*ii*) that this activity is not influenced by the individual nucleotide sequence present in the region fusing *bcl-2* to *IgH*.

Down-Regulation of *bcl2–IgH* mRNA and BCL-2 Protein in ODN-Treated Cells. Cellular levels of *bcl-2–IgH* mRNA and BCL-2 protein in cells treated with ODNs were evaluated by quantitative reverse transcription-coupled PCR using β_2-microglobulin or β-actin (data not shown) as internal standards, and by immunofluorescence, respectively. In Fig. 4 the relative expression of the *bcl-2–IgH* transcript in K422 cells treated with ODN b1 in the antisense (lanes 1–4) and sense (lanes 5–8) orientation is shown. It can be seen that, compared with cells untreated or treated with control ODNs, those treated with the sense ODN b1 presented a marked reduction of *bcl2–IgH* mRNA levels. Importantly, this reduction was paralleled by a sharp decrease in BCL-2 protein levels, as revealed by flow cytometric analysis (Fig. 5). In contrast, the amount of BCL-2 protein in cells treated with control ODNs was similar to that of untreated cells (Fig. 5).

Massive Apoptosis After Long-Term Exposure to Sense ODNs. The fate of lymphoma cell lines after long-term incubation with ODNs was investigated. Both t(14;18)-positive and -negative cell lines were treated daily with control or sense ODNs and examined at various times for cell viability. During the first 10–12 days, there was a 40–70% drop in viable cells in cultures treated with sense ODNs, relative to cultures treated with control ODNs (Figs. 6 and 7). Strikingly, however, by 14 days sudden and complete cell death occurred in sense-ODN-treated cultures, and this event, which took place quickly (2–3 h; Fig. 8) and was consistently reproducible in several independent experiments, was sequence-specific, in agreement with the results shown in Figs. 2 and 3 and Tables 1 and 2. No significant growth alterations were noted either in samples treated with control ODNs or in cells not expected to be targeted by the sense ODN.

DISCUSSION

The expression of the antiapoptotic gene *bcl-2* can be down-regulated in a sequence-specific manner by antisense ODNs targeting the AUG initiation codon of the *bcl-2* mRNA (40). Because this event is accompanied by the onset of apoptotic cell death (41), it has been proposed that *bcl-2*-specific antisense ODNs may play a relevant role in the treatment of follicular lymphomas, characterized by overexpression of *bcl-2* as result of the t(14;18) chromosomal translocation (42, 43). Nevertheless, since the sequence of this ODNs is not tumor-specific, their therapeutic advantage may be limited by the fact that they can have profound effects on a wide variety of tissues that normally express *bcl-2*. To overcome this limitation, we have explored the possibility of down-regulating the chimeric *bcl2–IgH* transcript of follicular lymphomas without affecting the normal *bcl-2* transcript, by using ODN sequences targeting the tumor-specific junction region originating from the fusion between the *bcl-2* and *IgH* genes that includes the N region insertions (16).

Table 2. t(14; 18) dose–response activity of ODNs targeting the *bcl-2* regions of the *IgH* regions

	No. of DOHH2 cells ($\times 10^{-4}$) per ml			
	b1		h1	
μM	S	AS	S	AS
—	76 ± 4	78 ± 7	83 ± 9	80 ± 7
0.3	78 ± 4	75 ± 7	84 ± 6	78 ± 10
1	52 ± 7*	79 ± 9	55 ± 8*	79 ± 6
3	44 ± 4*	78 ± 7	49 ± 7*	81 ± 5
10	31 ± 6*	67 ± 3	34 ± 8*	68 ± 11
30	27 ± 6	39 ± 9	27 ± 9	43 ± 8

Phosphorothioate ODNs were given as a single dose on day 0. Data are the mean ± SEM from three experiments.
*$P < 0.01$.

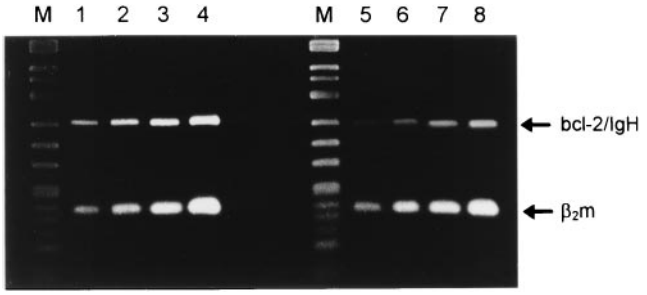

FIG. 4. *bcl-2* mRNA determination in K422 cells after a 5-day exposure to 10 μM phosphorothioate ODN. Increasing volumes (1, 2, 4, and 8 μl) of amplification products of cDNA from antisense h1 ODN (lanes 1–4)- or from sense h1 ODN (lanes 5–8)-treated cells were used.

During the course of these studies, we have unexpectedly found that all t(14;18)-positive follicular lymphomas do express, in addition to the normal oriented *bcl-2–IgH* transcript, a *bcl-2–IgH* transcript in the antisense orientation. Because endogenous antisense transcripts, already described for other genes (44), seem to influence the stability of the relevant mRNA regulating gene expression, we reasoned that the modulation of the antisense *bcl-2–IgH* mRNA by specific ODNs could affect the expression of the sense *bcl-2–IgH* mRNA, and this might open up a new perspective in antisense strategy. Indeed, the results provided in the present study point out that the *bcl-2–IgH* antisense transcript is a biologically relevant target for the ODN strategy. We have in fact shown that phosphodiester and phosphorothioate ODNs targeting different parts of the *bcl-2–IgH* mRNA induce programmed death efficiently in cells carrying the t(14;18) translocation but not in those without this cytogenetic abnormality. In addition, we show that the sense ODNs are equally effective in inducing apoptotic response, irrespective of the nucleotide sequence used to target the *bcl-2–IgH* antisense RNA. This finding is actually in contrast with data indicating that the effectiveness of antisense ODNs is largely dependent on the targeted region of the mRNA. This could be explained by the different features of the *bcl-2–IgH* antisense RNA compared with mRNAs in regard to nuclear compartimentalization, lack of an open reading frame or splicing sequences, or half-life.

Recently, the antisense activity of ODNs has been questioned because of the evidence that these molecules can elicit sequence-independent effects, particularly, the phosphorothioate deriva-

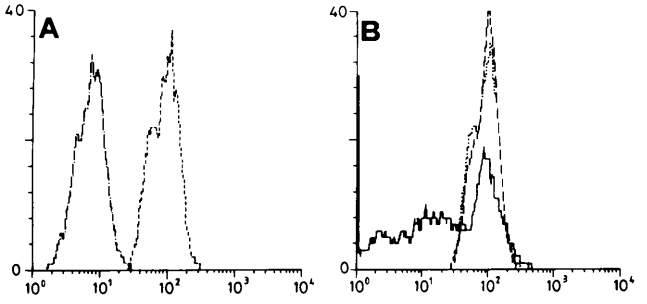

FIG. 5. BCL-2 protein levels in K422 cells exposed for 3 days to 10 μM phosphodiester ODN h1. Flow cytometry histograms of untreated cells labeled with an irrelevant mouse immunoglobulin (-·-·-) or with a BCL-2 mAb (- - -) (*A*) or of cells treated with sense (—), antisense (· · ·), or inverted (- - -) h1 ODNs (*B*) are shown.

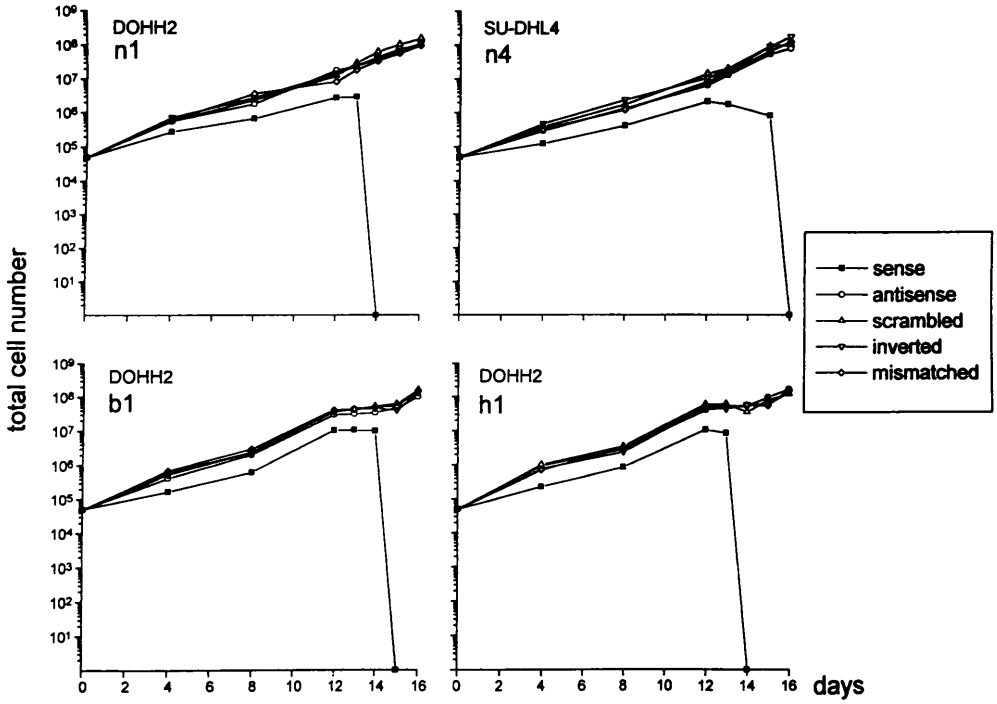

FIG. 6. Sequence-specific cell death induced by sense ODNs targeting different regions of the hybrid *bcl-2–IgH* antisense RNA. Phosphodiester sense ODNs complementary to sequences of the N region of the DOHH2 (ODN n1) (*A*) and of the SU-DHL-4 cells (ODN n4) (*B*) or the *bcl-2* (ODN b1) (*C*) and the IgH regions (ODN h1) (*D*) of the *bcl-2–IgH* antisense transcript were given at 10 μM on day 0 and at 5 μM the following days as in Fig. 2. The respective antisense, inverted, scramble, and mismatched ODNs were also used. Cells were counted every 4 days. Data are the means from three experiments.

tives also used for *in vivo* studies (45). To rule out the possibility that our results were due to nonspecific effects of the sense ODNs, their activity was always compared with that of several control ODNs (unmodified and phosphorothioate-modified) used at equimolar doses on cell lines carrying or not carrying the t(14;18) translocation. The antisense mechanism is further supported by the absolute cell specificity shown by the ODNs used in these studies. ODNs targeting the unique nucleotide sequence present in the N region of the DOHH2 antisense transcript were characterized by an absolute DOHH2-restricted apoptotic activ-

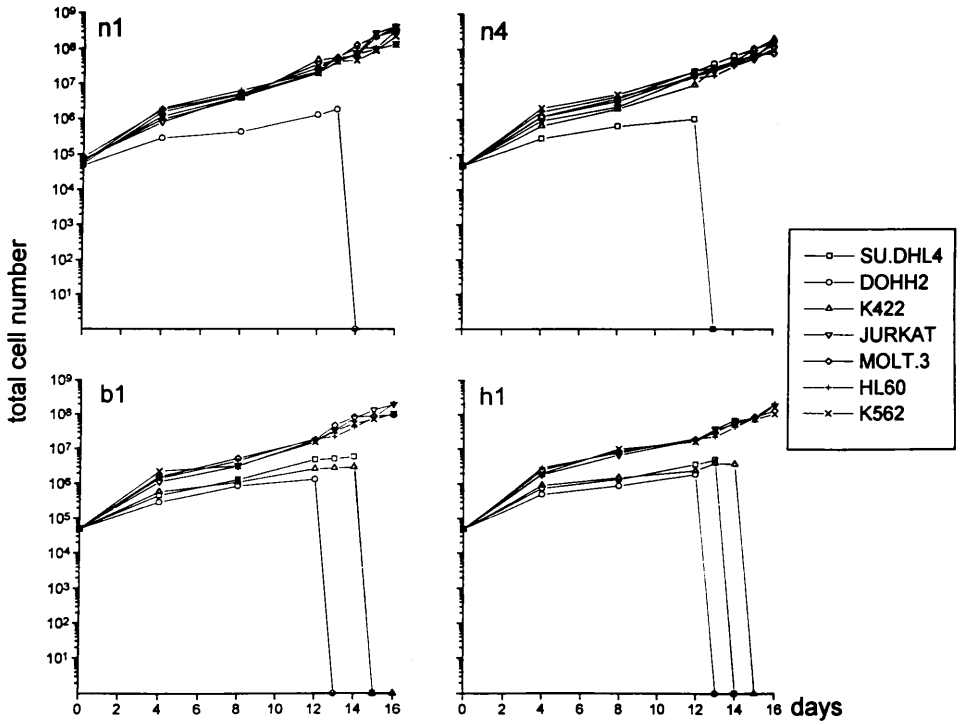

FIG. 7. Cell-line-specific death by ODNs targeting different regions of the hybrid *bcl-2–IgH* antisense RNA. Sense phosphodiester ODNs spanning the N region of the DOHH2 cell line (ODN n1) (*A*), the N region of the SU-DHL-4 cell line ODN n4 (*B*), the *bcl-2* portion (ODN b1) (*C*), or the IgH portion (ODN h1) (*D*) of the hybrid antisense RNA, were given as indicated in Fig. 4. Cells were counted every 4 days. Data are means from three experiments.

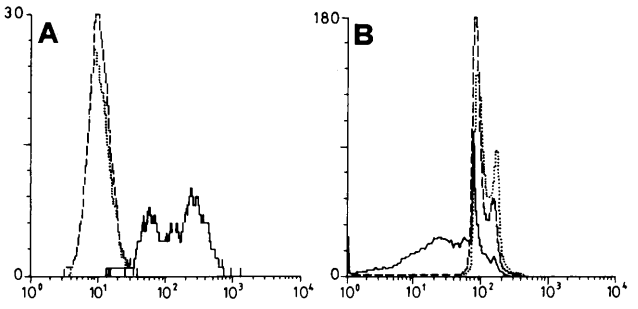

FIG. 8. TUNEL assay and DNA distribution in DHL-4 cells after exposure to ODNs. DHL-4 cells, either untreated (158 158 158) or treated with sense (190) or antisense (- - -) ODN h1 (1 μM/day) were labeled either by the TUNEL assay and analyzed by flow cytometry (*A*) or were stained with propidium iodine as indicated in MATERIALS AND METHODS and analyzed by flow cytometry (*B*).

ity. In a specular way, the activity of ODNs targeting the N region of the SU-DHL-4 cell line was restricted to the SU-DHL-4 cell line. The ODN treatment of other human cell lines or normal bone marrow cells (data not shown) was not effective. Besides the junction-targeted oligonucleotides, other active sense-oriented ODNs were those complementary upstream from the *bcl-2* major breakpoint region and downstream from the J segment of the *IgH* locus. The activity of these sense ODNs with all t(14;18) cell lines but not with those negative for this translocation provides the basis for the development of tumor-specific ODNs for the treatment of t(14;18) follicular lymphomas.

The finding that none of the antisense ODNs targeting the *bcl-2–IgH* mRNA affected the growth of the follicular lymphoma cells remains to be considered. Apart from the possibile competition exerted by the antisense transcript with the ODN-targeted sequences in the mRNA, it must be considered that most of the effective anti-*bcl-2* ODNs so far described are clustered in a region straddling the transcription initiation site that is generally believed to be very sensitive to ODN targeting (1). The different localization of our targeted regions (the 3′ untranslated region) and the generally higher level of mRNAs compared with the relative antisense transcripts (26) may account for these negative results. The low expression level of antisense transcripts did not allow us to provide direct evidence for the ODN-mediated down-regulation of the *bcl-2–IgH* antisense RNA. However, our results strongly argue in favor of a true antisense mechanism of action of the sense ODNs used.

The massive cell death observed at the end of the long-term t(14;18) cell treatment is noteworthy. The growth rate inhibition (survival disadvantage) observed during the first 12-day ODN exposure was followed by a fulminant unexpected death of the t(14;18) cell population. Although difficult to explain, this finding might be reminiscent of the massive disappearance, by 2–4 weeks of age, of the B and T cells in the lymphoid organs of the *bcl-2* null homozygous mice (46, 47). Irrespective of the targeted region in the *bcl-2–IgH* antisense transcript, the complete cell death produced by our ODNs was strictly restricted to t(14;18) cell lines. In conclusion, this finding that the antisense transcripts can be efficiently targeted by ODNs opens new possibilities for the pharmacological control of gene expression by these compounds.

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