Biochemical Pharmacology, Vol. 45, No. 10, pp. 2037–2043, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

IMMUNE STIMULATION BY AN ANTISENSE OLIGOMER COMPLEMENTARY TO THE *rev* GENE OF HIV-1*

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(*Received* 8 *October* 1992; *accepted* 22 *January* 1993)

Abstract—Mice developed massive splenomegaly and polyclonal hypergammaglobulinemia within 2 days after intravenous injection of a phosphorothioate oligomer that is antisense to a portion of the *rev* region of the HIV-1 genome. Histologic examination of spleens from injected animals showed marked expansion of a uniform-appearing population of small lymphocytes and many mitoses. Spleen mononuclear cells (SMNCs) from injected animals showed approximately a 10-fold-increased uptake of [^{3}H]thymidine and production of IgM and IgG. Flow cytometry analysis indicated that the responding cells were predominantly B-lymphocytes. The anti-*rev* oligomer also was mitogenic *in vitro* and stimulated immunoglobulin production by normal mouse SMNCs and human peripheral blood mononuclear cells. Similar immunologic effects were observed with an anti-*rev* 21-mer phosphorothioate, truncated at the 3′ end, but not with a 20-mer human p53 antisense phosphorothioate or a 28-mer anti-*rev* phosphodiester. These observations are consistent with the possibility that DNA sequences homologous to the *rev* gene participate in the regulation of mammalian lymphocyte activation, proliferation and maturation.

Human immunodeficiency virus (HIV‡) causes a chronic infection that usually eventuates in the acquired immunodeficiency syndrome (AIDS). Various strategies have been devised to prevent or treat HIV infection and thereby avoid the catastrophic consequences of AIDS. Among the most appealing is the use of antisense DNA because this approach offers the possibility to inhibit viral expression in chronically infected cells [1]. The feasibility of this technique was demonstrated in T cells infected with HIV. Exposure of these cells to a phosphorothioate oligodeoxynucleotide that is complementary (antisense) to the initiation sequence of HIV *rev* inhibited viral expression without killing the host cell [1].

The *rev* gene is an attractive target for this approach to antiviral therapy because it plays a key role in the HIV life cycle [2]. Rev protein is necessary for the expression of viral structural proteins, and inhibition of *rev* halts the generation of infectious viral progeny [1]. *Rev* encodes a protein, consisting of 116 amino acids, that is located mainly in the nucleolus of the cell [2]. The mechanism(s) by which Rev regulates viral RNA expression has not been fully elucidated. Experimental evidence has been presented that Rev regulates the splicing of messenger RNA (mRNA), mediates the export of viral mRNAs from the nucleus to the cytoplasm, and promotes the association of Rev-responsive element-containing mRNAs with polysomes [2–5]. Recent studies suggest that Rev protein selectively channels mRNA across the nuclear-envelope pore complex [5]. In the absence of Rev, viral mRNA species remain sequestered in the cell nucleus, and viral expression is inhibited [2]. Thus, Rev appears to be necessary for the correct localization and efficient translation of viral mRNA [6]. The regulatory activity exhibited by *rev* appears to be novel, but it has been postulated that Rev-like activities may represent a common form of cellular regulation [2, 7]. Intriguingly, human endogenous sequences share a common core of nucleic acid sequences with the *rev* gene of HIV-1 [8].

Although antisense oligonucleotides have been shown to inhibit *rev*-encoded protein synthesis and reduce unspliced (genomic) viral mRNA transcripts *in vitro* [1], the practical application of this promising new approach to anti-viral therapy will depend upon the outcome of pharmacologic and toxicologic studies [2]. Preliminary investigations in Swiss mice with a phosphorothioate oligodeoxynucleotide (27-mer), NSC 624958, which is antisense to a portion of the *rev* region of the HIV genome, found evidence of marked splenomegaly after chronic (14 days) s.c. administration.§ During analytical and pharmacokinetic studies with NSC 624958, our laboratory confirmed that parenteral administration of this

* The contents of this paper are solely the responsibility of the authors and do not necessarily represent the official views of the National Cancer Institute.

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‡ Abbreviations: HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome; ELISA, enzyme-linked-immunoadsorbent assay; Ig, immunoglobulin; TCR, T-cell receptor; PBMNCs, peripheral blood mononuclear cells; Cpd, compound; PBS, phosphate-buffered saline; PBS-T, phosphate-buffered saline-Tween 20; FBS, fetal bovine serum; PE, phycoerythrin; FITC, fluoroisothiocyanate; PWM, pokeweed mitogen; Con A, concanavalin A; PHA, phytohemagglutinin; and SMNCs, spleen mononuclear cells.

§ M. Hollingshead, personal communication, cited with permission.

antisense oligomer caused splenomegaly in two other mouse strains, CD_2F1 and C57BL/6. Since this drug eventually might be proposed for use in AIDS patients, who have profound immunologic abnormalities, it seemed important to characterize the effects of this antisense oligomer on the immune system.

MATERIALS AND METHODS

Oligomers. The following oligomers were used in the described studies: compound (Cpd) A, NSC 624958, an anti-*rev* 27-mer phosphorothioate; Cpd B, an anti-*rev* 21-mer phosphorothioate, truncated at the 3′ end; Cpd C, an anti-*rev* 28-mer phosphodiester; Cpd D, a 20-mer human p53 antisense phosphorothioate; and Cpd E, a 28-mer phosphorothioate homodeoxycytidine. All oligomers were obtained from Applied Biosystems, Foster City, CA. The sequences of the oligomers used are: Cpd A: 5′-TCG TCG CTG TCT CCG CTT CTT CTT GCC-3′; Cpd B: 5′-TCG TCG CTG TCT CCG CTT CTT-3′; Cpd C: 5′-TCG TCG CTG TCT CCG CTT CTT CTT GCC A-3′; Cpd D: 5′-CCC TGC TCC CCC CTG GCT CC-3′; and Cpd E: 5′-CCC CCC CCC CCC CCC CCC CCC CCC C-3′.

In vivo *treatment with oligomers.* Mice (CD_2F1 or C57BL/6) were injected in the tail vein with oligonucleotide (100 mg/kg) dissolved in 0.2 mL of vehicle (5% dextrose) or vehicle alone. The mice were bled retro-orbitally and the plasma was separated and frozen. Spleens were collected and weighed before mincing. A section was fixed and stained with hematoxylin and eosin for histopathology. The care of all animals was in accordance with the guidelines of the University of Vermont.

Cell separation. Human peripheral blood mononuclear cells (PBMNCs) were isolated by gradient centrifugation (Ficoll-Hypaque sp. gr. = 1.077) from the heparinized venous blood of normal volunteers after informed consent was obtained, using procedures approved by the Committee on Human Experimentation of the University of Vermont. PBMNCs were washed twice with RPMI 1640 medium, counted and then resuspended in RPMI 1640 with 10% heat-inactivated fetal bovine serum (FBS). Mononuclear cells from murine spleens (SMNCs) were prepared by mincing spleens over a stainless steel mesh and then isolating SMNCs by gradient centrifugation, washing twice and then resuspending in complete medium (RPMI 1640, 2 mM glutamine, 5% FBS, 15 mM Hepes, 2 mM pyruvate, and 0.05 mM mercaptoethanol).

In vitro *treatment with oligomers.* Human PBMNCs and murine SMNCs were incubated for 2 hr (37°) with log dilutions of oligomers (1 mg/mL to 100 ng/mL) or with medium alone. Cells were then washed and resuspended in culture medium at 10^6/mL for proliferation and immunoglobulin (Ig) studies.

Proliferation studies. Quadruplicate cultures of 10^5 murine SMNC from *in vivo* treated mice were incubated for 2 hr in a 96-well plate with 1 μCi of [^{3}H]thymidine. *In vitro* treated murine SMNCs were cultured for 18 hr, whereas human PBMNCs were cultured for 3 days before pulsing for 4 hr with [^{3}H]-thymidine. Pulsed cells were harvested on glass filters and counted in a beta scintillation counter. In addition, untreated, and in some cases treated, mononuclear cells (MNCs) were cultured with the mitogens concanavalin A (Con A 5 μg/mL) or pokeweed mitogen (PWM 1 μg/mL).

Immunoglobulin synthesis. To measure immunoglobulin synthesis, cells (10^6/mL) were incubated in 1-mL cultures for 24 hr (for *in vivo* treated murine SMNCs) or 9–12 days (for *in vitro* treated murine SMNCs or human PBMNCs). Untreated MNCs were cultured with PWM to provide a positive control for Ig synthesis. Tubes were then centrifuged and the supernatants were frozen until they were assayed for IgG and IgM levels using a standard enzyme-linked-immunoabsorbent assay (ELISA) as previously described [9, 10]. ELISA plates (96 well) were coated with goat anti-mouse IgG or IgM (1 μg/mL) diluted 1:1000 in bicarbonate buffer (pH 9.6) overnight at 4°, washed vigorously with phosphate-buffered saline-Tween 20 (0.05%) (PBS-T), and incubated overnight with culture supernatants diluted 1:10 or 1:20 with PBS-T. A standard of mouse IgG and IgM (1 mg/mL) was diluted with PBS-T to provide for a curve of 1–500 ng/mL. After vigorously washing with PBS-T, plates were incubated for 2–4 hr at room temperature with goat anti-mouse IgM or IgG conjugated to phosphatase diluted 1:1000 with PBS-T. After vigorously washing, phosphatase substrate (*p*-nitrophenyl phosphate 1 mg/mL) in diethanolamine buffer was added and the optical density (405 nm) was read. Based on the standard curve, nanograms of IgG or IgM per culture was determined.

Flow cytometry analysis. Murine SMNCs were suspended in 50 μL of phosphate-buffered saline-1% azide (PBS-azide) and reacted on ice with antibodies to mouse surface antigens: CD4, CD8, B220 (rat anti-mouse), CD3 (hamster anti-mouse), α/β and γ/δ T cell receptors, surface Ig, and CD5. Reacted cells were washed with cold PBS-azide and, if primary antibodies were not conjugated directly with phycoerythrin (PE) or fluoroisothiocyanate (FITC), then a secondary FITC-conjugated antibody (goat anti-mouse or hamster) was added. After a final wash with PBS-azide, cells stained for surface antigens were resuspended in 0.5 mL of 0.5% paraformaldehyde in PBS-azide for at least 10 min, and then the fixed cells were washed with PBS-azide. The cell pellet was gently resuspended with 0.5 mL of Bauer low salt stain (100 mL containing 3 g PEG-8000, 5 mg propidium iodide, 18,000 units of RNase (DNase free), 0.1% Triton-X 100, 4 mM sodium citrate buffer) diluted 1:10 in PBS-azide, mixed gently and then incubated at 37° for 20 min, mixing briefly during this period. To each tube was added 0.5 mL of Bauer high salt stain (per 100 mL: 3 g PEG-8000, 5 mg propidium iodide, 0.1% Triton-X 100, 400 mM sodium chloride), diluted 1:10 with PBS-azide, mixed gently and stored at 4° for at least 1 hr before flow cytometry analysis [11].

Statistical analyses. Statistical analyses were performed with the Mann–Whitney U test because the data were not normally distributed.

RESULTS

As early as 24 hr after injection of Cpds A and B,

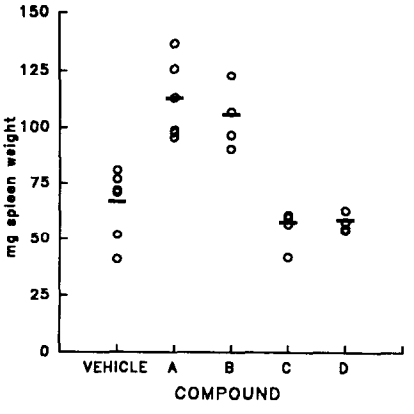

Fig. 1. Spleen weights for 4–6 animals collected 24 hr after intravenous injection of vehicle alone or the Cpds indicated. The horizontal bars represent the mean weight of each group.

the spleens of treated animals were approximately twice as heavy as animals treated with vehicle alone or with Cpds C and D (Fig. 1). Histologic examination of the spleens of animals injected with Cpd A showed large lymphoid nodules composed mainly of a uniform population of large cells with fine chromatin, irregular nuclei and many mitoses (Fig. 2). The enlarged spleens gradually shrunk to nearly normal size by 28 days post-injection.

In addition, by 24 hr, mice injected with Cpd A had about a 40% polyclonal increase in plasma globulin levels compared to those injected with vehicle alone. Twenty-four hours later, both IgM and IgG levels in the plasma were elevated: vehicle IgM, 50–135 μg/mL; Cpd A-injected IgM, 600–700 μg/mL; vehicle IgG, 750–1375 μg/mL; Cpd A IgG, 1875–2250 μg/mL.

To investigate further the splenomegaly and hypergammaglobulinemia observed following injection of the antisense oligomers, we measured the proliferation of SMNCs from mice 24 hr after intravenous injection of the oligomers. As shown in Fig. 3, there was striking uptake of tritiated thymidine by SMNCs from animals injected with Cpds A and B but not with Cpds C and D, compared to vehicle alone. Similarly, when these cells were cultured in 1 mL complete medium for 24 hr, there was a highly significant increase in production of both IgM and IgG by SMNCs from Cpd A-injected animals, and of IgM by Cpd B-injected animals, which was not observed in cultures of SMNCs from mice injected with Cpds C or D (Fig. 3). In other experiments, we found that SMNCs from animals injected with Cpd A were unresponsive to further stimulation by PWM, Con A, phytohemagglutinin (PHA) or Cpd A in concentrations which activated normal mouse SMNCs *in vitro* (data not shown). These observations suggested that anti-*rev* phosphorothioate oligomers, but not the nuclease-sensitive phosphodiester or a similar-sized human p53 antisense oligonucleotide, activated SMNCs and promoted immunoglobulin production within 24 hr of parenteral injection.

Consistent with the histologic observations, simultaneous analysis of cell surface components and DNA by flow cytometry demonstrated a marked increase in SMNC cell size and mitotic activity 24 hr after intravenous treatment of mice with Cpd A. If the lymphocyte gate (based on forward scatter and 90° light side scatter) was divided into two parts based upon size, 24 hr after treatment with Cpd A the majority of the cells had shifted into the large gate (77.6 to 80.2%) compared with vehicle-treated animals (12 to 21.5%). DNA staining with propidium iodide showed that there was approximately a 50% increase in the percentage of cells in $S + G_2 + M$ phases of the cell cycle 24 hr after treatment with Cpd A (28.3 to 33.3%) compared with vehicle treatment (18.8 to 20.7%). Combined DNA and surface antigen staining showed that as early as 4 hr after *in vivo* treatment, more B220 positive SMNCs (a marker of B-lymphocytes) were cycling from Cpd A-treated than vehicle-treated mice. By 12 hr, 36.7

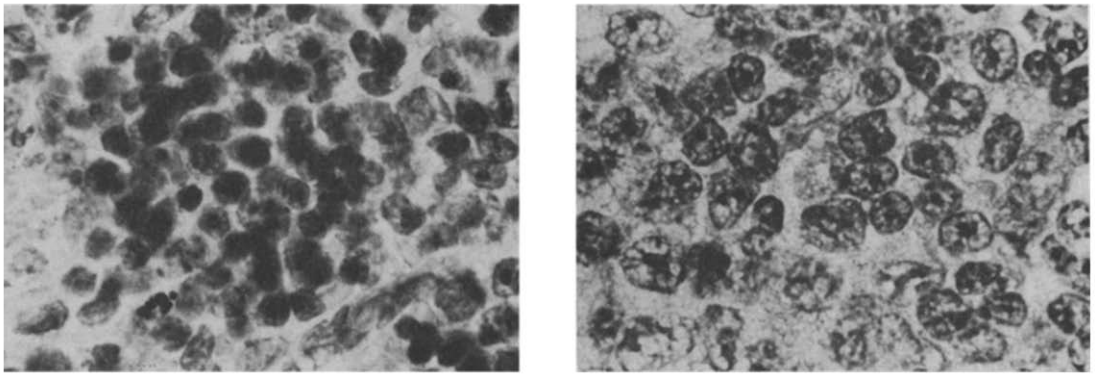

Fig. 2. Histologic analysis of mouse spleens collected 24 hr after intravenous injection of vehicle alone (left panel) or anti-*rev* 27-mer phosphorothioate (right panel). The tissue was fixed with formalin and stained with hematoxylin and eosin. Original magnification × 1000.

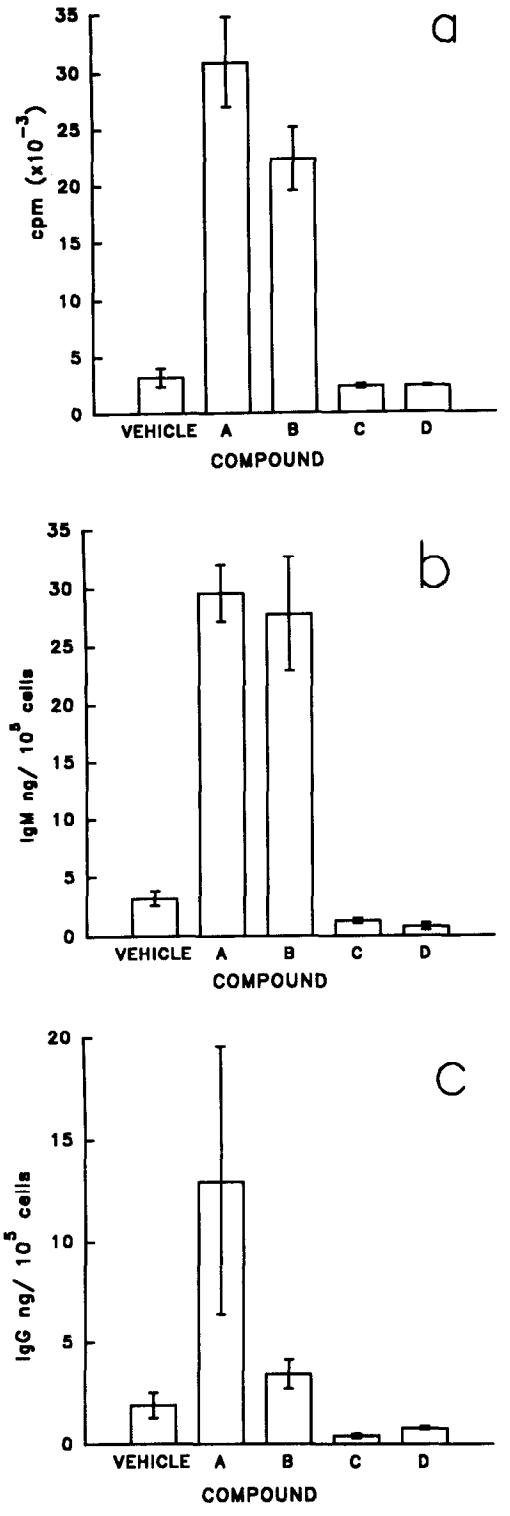

Fig. 3. Cellular proliferation and Ig production by mouse SMNCs measured 24 hr after intravenous injection of vehicle alone or oligomers. The brackets indicate the SEM. Each group contained 4–8 mice. Panel a: [^{3}H]thymidine incorporation, expressed as cpm $\times 10^{-3}$, by 10^5 cells from animals injected with Cpds A or B was statistically different from incorporation by control cells from mice injected with vehicle alone ($P < 0.005$). Panel b: IgM production by SMNCs, expressed as ng/10^5 cells, was statistically different from control cells ($P < 0.005$) in cells from mice injected with Cpds A or B. Panel c: IgG production was significantly higher than control levels ($P < 0.005$) by SMNCs from mice injected with Cpd A.

to 38.8% of the Cpd A-treated SMNCs were cycling and B220 positive, compared to 12.1 to 16.9% of the vehicle-treated SMNCs. This increase in cycling B220 positive cells continued through 24 hr post-treatment. There also was a marked increase in the percentage of CD3 positive cycling SMNCs 24 hr after Cpd A treatment (13.8 to 18.6%) as compared to vehicle treated (4 to 7.4%). However, no effects on the percentage of CD4 or CD8 positive cells were observed. Additional studies of SMNCs collected 24 hr after intravenous injection of mice with Cpd A indicated that these cells were predominantly B220 and surface Ig positive (>95%) and devoid (<5%) of CD5, CD3, CD4, CD8, α/β or γ/δ T-cell receptor (TCR). Thus, it appears that the anti-*rev* oligomer mainly activates B cells, but that a population of CD3 positive cells also enters mitosis.

In additional studies, the oligomers were incubated for 2 hr with isolated SMNCs from normal C57BL/6 mice. Then the cells were washed, resuspended in medium, counted and cultured. As shown in Table 1, 14 hr later Cpds A and B markedly stimulated tritiated thymidine incorporation by normal mouse SMNCs in a concentration-dependent fashion. The concentration of 100 μg/mL appeared to be optimal, but as little as 1 μg/mL of Cpd A caused a statistically significant activation of proliferation. At the highest concentration tested, 1000 μg/mL, Cpd A showed relative inhibition of thymidine incorporation compared to a concentration of 100 μg/mL ($P = 0.034$), whereas Cpd B did not. It is worth emphasis that Gao *et al.* [12] have observed that biochemical effects of phosphorothioates can be determined not only by the size of the molecule but also by the degree of substitution with thioate functions. It is possible that the "reversal" effect observed with high concentrations of Cpd A, but not Cpd B, is a consequence of the increased size and thioate substitution for A. Cpds C and D caused a negligible increase in thymidine incorporation. In experiments not shown, Cpd E, the deoxycytidine homo-oligomer, had no significant effect on thymidine incorporation. Cpds A and B were associated with a concentration-dependent stimulation of immunoglobulin production throughout a 2-week culture period. Illustrative measurements after 9 days of cell incubation are shown in Table 1. Levels of both IgM and IgG were increased. Some elevations of IgM and IgG were seen after exposure to Cpds C and D, particularly at 1000 μg/mL. These results suggested that anti-*rev* oligomers are potent stimulators of cell proliferation and immunoglobulin production by normal mouse SMNCs *in vitro*, but that at the highest concentrations there may be a non-specific effect.

It was of interest to test whether these effects of oligomers were species specific or also were applicable to human cells. As shown in Table 2, a 2-hr exposure to Cpds A and B stimulated [^{3}H]-thymidine incorporation by human PBMNCs, when measured 72 hr later, and production of both IgM

Table 1. Effect of a 2-hr exposure to oligodeoxynucleotides on mouse splenocyte proliferation and immunoglobulin production

Treatment	Thymidine incorporation (cpm)	N	Immunoglobulin production (ng/10^5 cells) IgM	IgG
Medium	1,200 ± 90	19	13 ± 3	1 ± 0.4
PWM	ND*	20	110 ± 10†	4 ± 1†
Cpd A				
1000 μg/mL	25,400 ± 5,500†	15	77 ± 7†	11 ± 2†
100 μg/mL	45,000 ± 8,000†	9	80 ± 19†	8 ± 2†
10 μg/mL	20,700 ± 2,500†	6	29 ± 2‡	4 ± 1‡
1 μg/mL	6,000 ± 900†	6	18 ± 4	2 ± 1
0.1 μg/mL	1,500 ± 230	6	9 ± 3	1 ± 1
Cpd B				
1000 μg/mL	29,000 ± 3,300†	11	84 ± 8†	8 ± 1†
100 μg/mL	29,700 ± 4,400†	7	81 ± 15†	7 ± 1†
10 μg/mL	20,600 ± 3,200†	4	61 ± 4†	4 ± 1‡
Cpd C				
1000 μg/mL	1,900 ± 170†	11	40 ± 11†	3 ± 1‡
100 μg/mL	1,100 ± 60	7	13 ± 4	2 ± 1
10 μg/mL	1,200 ± 120	4	22 ± 3	2 ± 0.4
Cpd D				
1000 μg/mL	1,900 ± 170‡	11	47 ± 8†	2 ± 0.2‡
100 μg/mL	2,100 ± 360‡	7	58 ± 8†	2 ± 0.4‡
10 μg/mL	1,800 ± 270‡	4	31 ± 4‡	2 ± 0.1

Values are means ± SEM.
* ND = not determined.
† $P < 0.005$.
‡ $P < 0.05$.

Table 2. Cellular proliferation and immunoglobulin production by human PBMNCs following a 2-hr exposure to oligodeoxynucleotides

Treatment	Thymidine incorporation (cpm)	N	Immunoglobulin production (ng/10^5 cells) IgM	IgG
Medium	880 ± 90	16	30 ± 14	10 ± 2
PWM	27,200 ± 6,600*	16	170 ± 70†	100 ± 40*
Con A	43,600 ± 7,100*	13	ND‡	ND
Cpd A				
100 μg/mL	9,200 ± 2,200*	12	760 ± 190*	200 ± 40*
10 μg/mL	2,400 ± 660†	9	370 ± 80†	68 ± 30†
1 μg/mL	1,300 ± 260	8	140 ± 80	23 ± 7
0.1 μg/mL	1,100 ± 180	8	20 ± 16	12 ± 3
Cpd B				
100 μg/mL	7,500 ± 2,000*	7	600 ± 150*	120 ± 30*
10 μg/mL	1,900 ± 350†	4	330 ± 70*	75 ± 40*
1 μg/mL	1,800 ± 470†	4	17 ± 13	11 ± 6
Cpd C				
100 μg/mL	1,600 ± 470	7	95 ± 50	24 ± 10
10 μg/mL	1,400 ± 270	4	19 ± 11	11 ± 4
1 μg/mL	1,600 ± 270†	4	9 ± 8	7 ± 1
Cpd D				
100 μg/mL	2,700 ± 600*	7	310 ± 90*	52 ± 10*
10 μg/mL	1,600 ± 380†	4	130 ± 60†	19 ± 4
1 μg/mL	1,300 ± 300	4	17 ± 17	10 ± 3

Values are means ± SEM.
* $P < 0.005$.
† $P < 0.05$.
‡ ND = not determined.

and IgG after 9 days of culture. These effects occurred at drug concentrations that were comparable to those that were effective with mouse cells. Daily measurements of thymidine incorporation indicated that peak uptake occurred at 72 hr (data not shown). As with murine SMNCs, the activation of PBMNCs and the stimulation of Ig production were concentration dependent with Cpd A. Exposure to Cpd C did not cause a significant increase of thymidine incorporation or Ig production. Incubation with Cpd D was associated with a modest increase of thymidine uptake and Ig production, particularly at the highest concentration tested.

DISCUSSION

Our studies suggest that this antisense oligomer is a mitogen for both mouse SMNCs and human PBMNCs and causes the development of Ig-producing cells. These effects on the immune system appear to be relatively specific to this anti-*rev* oligomer. Similar effects were not seen *in vivo* with a human p53 antisense oligomer of approximately the same size or with an anti-*rev* phosphodiester oligomer that is sensitive to nuclease degradation. The immune-stimulating properties of the anti-*rev* phosphorothioate oligodeoxynucleotide were confirmed *in vitro*, while the phosphodiester compound, the p53 antisense oligomer, and a deoxycytidine homo-oligomer were either inactive or capable of only modest stimulation at high concentrations. Different antisense oligonucleotides tested in pharmacokinetic and acute toxicity studies have not been associated with splenomegaly in rodents [13, 14], and the infusion of the p53 antisense oligomer does not cause splenomegaly in monkeys [15]. Oligodeoxynucleotides have been used specifically to inhibit T cell function [16–18]. Therefore, immune stimulation does not appear to be a general property of oligodeoxynucleotides.

Immune stimulation has been reported with another antisense oligomer. A deoxyoligonucleotide complementary to the mink cell focus-forming (MCF) envelope gene initiation region caused increased mouse spleen cell RNA synthesis and surface Ia and immunoglobulin expression [19]. Oligonucleotides complementary to two other regions of endogenous type C retroviral genomes, the gag-pol1 initiation site and the 3′ splice site, as well as randomly scrambled sequences of similar length, were not stimulatory [19]. These data were interpreted to suggest that endogenous retroviruses may suppress lymphocyte activation, and that antisense oligonucleotides specific for these inhibitory retroviruses may stimulate lymphocytes [19]. To the extent that the immune stimulation that we observed was specific to inhibition of *rev* gene activity, our work suggests that sequences resembling the *rev* region of the HIV genome may participate in the regulation of mammalian lymphocyte activation, proliferation and maturation. The recent description of human endogenous sequences with homology to the *rev* functional domain of HIV-1 supports this possibility [8].

Alternatively, the immune stimulation associated with this oligonucleotide may be independent of its antisense activity. B cells usually are activated from the resting state by antigen binding to surface Ig. In the mouse, activation also can be modulated by other physiologic mediators, including IL-4, γ-interferon, and lipopolysaccharide [20]. Growth and maturation factors subsequently are required to stimulate B cells to proliferate and mature [21]. We considered contamination with endotoxin as a possible explanation for the B lymphocyte excitation and maturation observed in our experiments [22], but measurements with a sensitive assay found no endotoxin activity (data not shown). Double-stranded RNAs promote production of lymphokines, including interferons and tumor necrosis factor [23, 24]. Immune augmentation of both T and B cells has been reported in studies of double-stranded RNAs. However, lymphocyte activation to the extent seen in these experiments with the antisense oligomer is not usually associated with exposure to these lymphokines [21].

Further characterization of the immunostimulatory properties of this antisense oligomer will be of interest, because its mechanism of action may be novel. The relatively small size and well defined structure of this compound should facilitate this analysis. Regardless of the mechanism the immunomodulatory effects of the anti-*rev* oligomer will need to be considered during any clinical trials of this agent in normal humans or in patients with HIV infection. The latter group, in particular, is characterized by abnormalities of B cell activation and immunoregulation [25]. Studies in patients with AIDS describe polyclonal B cell activation, decreased numbers of resting B cells, increased numbers of activated and fully differentiated B cells, and a decrease in response to mitogens [25]. It is interesting that we found many of these changes of the immune system in mice treated with the *rev* antisense oligomer. Whether these alterations in immune function in patients with AIDS and mice treated with the antisense oligomer are related remains to be determined. In any case, further testing of this promising therapy with an anti-viral agent should include consideration of its immunostimulatory effects.

Acknowledgement—This work was supported by grants from the National Cancer Institute (CA41843 and PO30 CA22435).

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