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The Role of Cell Surface Receptors in the Activation of Human B Cells by Phosphorothioate Oligonucleotides

Hua Liang,* Charles F. Reich, † David S. Pisetsky, † and Peter E. Lipsky2*

Phosphorothioate oligodeoxynucleotides (sODNs) containing the CpG motif or TCG repeats induce T cell-independent polyclonal activation of human B cells. To elucidate the mechanism of this response, the role of cell surface receptors was investigated. Sepharose beads coated with stimulatory but not nonstimulatory sODNs induced B cell proliferation comparably with soluble sODNs. The B cell stimulatory activity of Sepharose-bound sODN did not result from free sODN released from the beads since media incubated with coated beads were inactive. Using FITC-labeled sODNs as probes, binding to human B cells could be detected by flow cytometry. Binding was rapid, saturable, initially temperature independent, but with a rapid off-rate. Competition studies indicated that both stimulatory sODNs and minimally stimulatory sODNs bound to the same receptor. By contrast, phosphodiester oligonucleotides with the same nucleotide sequence as sODNs and bacterial DNA inhibited the binding of sODNs to B cells minimally. Charge appeared to contribute to the binding of sODNs to B cells since binding of sODNs was competitively inhibited by negatively charged molecules, including fucoidan, poly I, and polyvinyl sulfate. These data indicate that human B cells bind sODNs by a receptor-mediated mechanism that is necessary but not sufficient for polyclonal activation. The Journal of Immunology, 2000, 165: 1438–1445.

Dexyribonucleic acid from various bacteria and some phosphorothioate oligodeoxynucleotides (sODNs)3 have widespread immunological effects, including induction of polyclonal activation of murine B cells (1–10). The stimulatory activity of bacterial DNA and sODNs has been attributed to short sequence motifs called immunostimulatory sequences or CpG motifs, in which an unmethylated CpG dinucleotide is flanked by two 5’ purines and two 3’ pyrimidines (3, 11, 12). Studies using a murine pre-B cell line, WEHI 231, and murine splenocytes suggested that cellular uptake of CpG-containing sODNs is required for the functional activities of sODNs (3, 13, 14).

Previous studies demonstrated that certain sODNs can directly induce T cell-independent polyclonal activation of human B cells (15). However, the mechanism for human B cell activation by sODNs may differ from that of murine B cells and occur without endocytosis of the sODNs. Thus, a highly active sODN (HIVas, an antisense to the rev region of HIV) immobilized onto beads stimulated B cells as effectively as soluble HIVas. This finding suggested that human B cell activation by HIVas results from engagement of surface receptors and that cellular entry of HIVas is not necessary for activation (15).

To investigate the mechanism of human B cell activation induced by sODNs in greater detail, the current study examined the capacity of human B cells to bind sODNs. The results indicate that the binding of sODNs by human B cells is receptor mediated and that sODNs appear to bind to the same receptor regardless of stimulatory potential. Moreover, sODNs coupled to Sepharose beads stimulate B cells as effectively as soluble sODNs, whereas non-stimulatory sODNs do not activate B cells even when bound to Sepharose beads. These results indicate that sODNs bind to a B cell surface receptor and the binding is necessary but not sufficient for polyclonal activation of human B cells.

Materials and Methods

DNA and oligodeoxynucleotides

Oligodeoxynucleotides were synthesized using standard phosphoramidite chemistry methods (16). sODNs and sODNs with a six-carbon spacer and amino linker were purchased from Midland Certified Reagent (Midland, TX). The sequences and activity of sODNs used in this study are shown in Table I. Calf thymus DNA was purchased from Life Technologies (Grand Island, NY). Escherichia coli and Micrococcus lysodeikticus DNA were purchased from Sigma (St. Louis, MO) and further purified by repeated phenol/chloroform extraction, followed by ethanol precipitation.

Cell preparation and purification

PBMCs were isolated from heparinized blood of healthy adult volunteers by centrifugation over sodium diatrizoate/Ficoll gradients (Sigma). PBMC were depleted of NK cells and monocytes by incubation with 5 μg/ml 1–leucine methylster (Sigma) in serum-free RPMI 1640 (BioWhittaker, Walkersville, MD) (17–19). After washing, the remaining cells were stained with biotinylated anti-CD19 mAb (Coulter, Hialeah, FL) and passed through a Ceprate streptavidin column, according to the manufacturer’s procedures, to separate B cells from other cells (CellPro, Bothell, WA). The resultant positively selected B cells were analyzed by flow cytometry and found to be >97% CD20+ using a PE-conjugated anti-CD20 mAb (Caltag, South San Francisco, CA). In some experiments, PBMC were treated with isotypic NH4Cl to lyse RBC and stained with a mixture of dextran-linked mAbs (anti-CD3, anti-CD14, anti-CD2, anti-CD16, and anti-CD56), followed by a magnetic colloid. The cells were purified over a StemSep column to separate B from other cells according to the manufacturer’s instructions (StemCell Technologies, Vancouver, British Columbia, Canada). The resultant negatively selected B cells were analyzed by...
The categories were defined according to their ability to induce human B cell proliferation. Most active, >3010 cpm; moderately active >1510 to <3000 cpm; minimally active, <1500 cpm. There was no significant difference in the magnitude of activation by any pair of sODNs within each category although there were significant differences between activation induced by at least one sODN from different categories (p < 0.05) and between activation induced by any sODN and response of B cells alone.

flow cytometry and found to be >90% CD19<sup>+</sup> after staining with PE-conjugated anti-CD19 mAb (PharMingen, San Diego, CA).

Cell lines
The ML1 B cell line was generated by EBV transformation of PBMC (20). B cell hybridomas (2X) were generated by fusing activated human peripheral blood B cells with the Spaz-4 fusion partner (21).

Culture conditions
B cells (5 x 10<sup>5</sup>/well) were cultured with sODNs or sODN-coated beads in U-bottom 96-well microtiter plates (Costar, Cambridge, MA). All cultures were conducted in RPMI 1640 medium supplemented with penicillin G (200 U/ml), gentamicin (10 μg/ml), L-glutamine (0.3 g/ml), and 10% FBS (10% RPMI). Modified sODNs were synthesized with a lysine residue linked to the 5' end, as described (14). CNBr-activated Sepharose 4B beads were coated with modified sODNs, according to the manufacturer's instructions (Pharmacia Biotech). Briefly, modified sODNs were dissolved in coupling buffer (0.1 M NaHCO<sub>3</sub>, pH 8.3, containing 0.5 M NaCl) and incubated overnight at 4°C with constant rotation with Sepharose beads that had been swollen and washed in 1 mM HCl (pH 2–3). After washing with coupling buffer and blocking by incubation with 0.1 M glycine at room temperature for 2 h, the beads were washed with three cycles of alternating pH buffers (coupling buffer (pH 8.3) and acetate buffer (pH 4)). The beads were then resuspended at a 1/10 dilution (10 μl solution = 1 μg soluble sODNs) and stored in RPMI 1640 medium supplemented with penicillin G (200 U/ml), gentamicin (10 μg/ml), and nystatin (1:100). Control beads were coupled with glycine only.

Results
Proliferation induced by Sepharose-bound sODNs
A previous study showed that Sepharose beads coated with HIVas, a highly stimulatory sODN, were as effective as soluble HIV as at

![Figure 1](http://www.jimmunol.org)
inducing B cell proliferation and IgM production (15). The current experiments examined whether other sODNs stimulated highly purified human B cells when coupled to beads. As can be seen in Fig. 1, Sepharose-bound TCG4, 20 mer, TMCM, and HSVas-stimulated B cell DNA synthesis comparably with their soluble counterparts, whereas glycine-coated beads did not induce B cell proliferation. Neither soluble C20 nor Sepharose-bound C20 activated human B cells. These results suggest that sODNs stimulate human B cells by interacting with cell surface molecules.

To investigate the possibility that soluble sODNs were released from the Sepharose beads and activated human B cells, sODN beads were incubated in medium at 37°C overnight, after which the incubated beads were centrifuged to separate them from their supernatants. The incubated beads and their supernatants were then tested for the ability to induce B cell proliferation. As can be seen in Fig. 2A, HIVas beads preincubated with medium overnight induced comparable DNA synthesis as unincubated HIVas beads, indicating that HIVas beads did not lose their activity after overnight incubation. In addition, supernatant from these incubated HIVas beads did not stimulate B cells. Similar results were obtained with TCG4, as shown in Fig. 2B. These data suggest that sODNs stimulate human B cells by interacting with cell surface molecules.

To test the binding of HIVas, highly purified human peripheral blood B cells were incubated with or without various concentrations of FITC-labeled HIVas at either 37°C or 4°C for different time periods and analyzed by flow cytometry. As can be seen in Figs. 3 and 4, the binding of HIVas is comparable at 37°C and 4°C within 30 min. However, after 30 min, the percentage of B cells binding HIVas-FITC and the density of bound HIVas at 37°C became significantly greater than that noted at 4°C. Fluorescence-microscopic examination indicated that HIVas-FITC accumulated in intracellular vacuoles when cells were incubated for more than 30 min at 37°C, but not at 4°C. These results indicate that binding

![Graph](image1.png)

**FIGURE 2.** B cell proliferation induced by Sepharose-bound sODNs and their supernatants. Sepharose-bound sODNs (100–200 µl/well, 10 µl sODN bead = 1 µg soluble sODN) were washed with 10% RPMI and incubated overnight. 10% RPMI incubated overnight were used as a control. The incubated sODN beads were separated from their supernatants by centrifugation at 2000 rpm for 2 min. The supernatant was transferred to another tube and centrifuged again to remove any remaining beads. The sODN beads were washed and resuspended in 10% RPMI B cells (50 × 10^5/well) were cultured with various amounts of incubated sODN beads or their supernatants (0.5–10 µl/well for HIVas bead and its supernatant, and 1–25 µl/well for TCG4 bead and its supernatant) for 3 days, and proliferation was measured as described. B cells cultured with various amounts of unincubated sODN beads (0.5–10 µl/well for HIVas bead, and 1–25 µl/well for TCG4 bead) or glycine bead (0.5–25 µl/well) or control supernatant (0.5–25 µl/well) were used as controls. The data are the mean of triplicate cultures with an SEM of less than 10%. The results of one of three experiments, each using B cells from an individual donor, with similar findings are shown.

![Graph](image2.png)

**FIGURE 3.** Binding of HIVas-FITC by B cells at different temperatures. B cells (2 × 10^5/sample) were incubated with medium (PBS supplemented with 2% normal human serum) alone (dotted line) or 10 µg/ml of HIVas-FITC (black line) at 37°C or 4°C for various lengths of time. The binding of HIVas-FITC by B cells was analyzed by flow cytometry. The results of one of two experiments, each using B cells from an individual donor, with similar findings are shown.
of HIVas is initially temperature independent with a temperature-dependent component observed thereafter.

To determine whether HIVas bound to all cells comparably, binding to B cells and T cells was assessed. Previous data had demonstrated that HIVas did not directly stimulate T cells (15). As shown in Fig. 5, HIVas did not bind to T cells.

**FIGURE 4.** Binding of HIVas-FITC by B cells at different temperatures. Transformation of Fig. 3. The x-axis represents incubation time. The y-axis represents percentage of B cells binding HIVas-FITC (upper panel) or binding MFI (lower panel).

**FIGURE 5.** Binding of HIVas-FITC by B cells, not T cells. PBMC (2 x 10^6/well) were incubated with medium alone (dotted line) or HIVas-FITC (20 μg/ml) (black line) at 37°C for 30 min. After washing to remove unbound HIVas-FITC, cells were stained with PE-conjugated mAbs against CD19, or CD3, or their isotype-matched controls. Binding of HIVas-FITC by CD3^+ or CD19^+ cells was analyzed by flow cytometry, as described. The results shown here are representative of three separate experiments, each conducted with cells from a different donor.

**FIGURE 6.** Binding of HIVas-FITC by peripheral B cells and B cell lines. Peripheral blood B cells and B cell lines 2X and ML1 cells (2 x 10^6/sample) were incubated with medium alone or various concentrations of HIVas-FITC (1–50 μg/ml) at 37°C for 30 min, and the binding of HIVas-FITC was analyzed as described. The y-axis represents the percentage of B cells binding HIVas-FITC (A) or binding density (B–D); the x-axis represents the concentration of FITC-labeled HIVas. For B cell lines, cells were gated to remove dead cells. The results shown in A and B are representative of five separate experiments, each conducted with B cells from a different donor; the results in C are representative of two separate experiments; and the results in D are representative of six separate experiments.
To examine whether the binding of HIVas by human B cells is saturable, B cells were incubated with various concentrations of FITC-labeled HIVas at 37°C for 30 min and analyzed by flow cytometry. As can be seen in Fig. 6, both the percentage of B cells (peripheral blood B cells and the B cell lines 2X and ML-1) binding HIVas-FITC and the density of bound HIVas tended to reach a plateau when the concentration of HIVas-FITC was higher than 10 μg/ml. This result indicates that binding of HIVas is saturable.

Finally, the off-rate of bound HIVas was found to be rapid. About 50% of B cell-bound HIVas-FITC remained after a 2-min incubation in the presence of unlabeled HIVas, and about 40% of bound HIVas remained in the absence of unlabeled HIVas (data not shown). The apparent off-rate exhibits a \( t_{1/2} \) of 2 min in the presence of unlabeled HIVas-FITC, and 1.6 min in the absence of unlabeled HIVas.

**sODNs bind to the same receptor(s)**

To determine the specificity of sODN binding, competitive inhibition studies were conducted. In these experiments, B cells were preincubated with various unlabeled sODNs, followed by HIVas-FITC. The representative sODNs can be divided into three groups according to their ability to compete. HSVas and NKNSO inhibited the binding of HIVas-FITC by B cells comparably with HIVas. TMCM, G\(_{20}\), and CG\(_{7.5}\) inhibited binding modestly, whereas TCG\(_{4}\) and C\(_{20}\) did not inhibit the binding (Fig. 7). Because TCG\(_{4}\), TMCM, HSVas, and HIVas are highly stimulatory, NKNSO is moderately stimulatory, and C\(_{20}\), G\(_{20}\), and CG\(_{7.5}\) are minimally stimulatory, the capacity to compete with HIVas binding alone did not predict functional activity.

To determine whether TCG\(_{4}\), C\(_{20}\), and other sODNs that failed to inhibit B cell binding of HIVas bound to the same receptor(s), the binding of TCG\(_{4}\)-FITC by B cells was examined. As can be seen in Fig. 8, the binding of TCG\(_{4}\)-FITC by B cells tends to reach a plateau when the concentration of TCG\(_{4}\)-FITC was higher than 10 μg/ml. These results indicate that binding of TCG\(_{4}\)-FITC is similar to that of HIVas in that it is saturable. The ability of sODNs to inhibit binding of TCG\(_{4}\)-FITC by B cells was examined next. As can be seen in Fig. 9, all sODNs tested, including TCG\(_{4}\), HIVas, C\(_{20}\), TMCMC\(_{4}\)D\(_{4}\), and TMCM, inhibited the binding of TCG\(_{4}\)-FITC by B cells. These results suggest that TCG\(_{4}\), HIVas, C\(_{20}\), TMCMC\(_{4}\)D\(_{4}\), and TMCM bind to the same receptor(s), but apparently with different avidities.

To assess whether sODNs inhibit the stimulatory activity of HIVas, B cells were incubated with various concentrations of sODNs in the presence or absence of unlabeled sODNs was examined. As can be seen in Fig. 10, maximal proliferation of B cells induced by HIVas was inhibited by increasing concentrations of G\(_{20}\). Similar results were obtained with other sODNs, except C\(_{20}\) and TCG\(_{4}\) (not shown), which did not inhibit maximal B cell proliferation induced by HIVas. These results indicate that sODNs, except TCG\(_{4}\) and C\(_{20}\), inhibited the stimulatory activity of HIVas, suggesting that these sODNs bind to the same stimulatory receptor on human B cells.

**FIGURE 7.** sODNs, except TCG\(_{4}\) and C\(_{20}\), compete the binding of HIVas-FITC by B cells. B cells (2 × 10^5/well) were incubated with various concentrations of HIVas, HSVas, TMCM, NKNSO, TCG\(_{4}\), G\(_{20}\), CG\(_{7.5}\) (1–100 μg/ml), and C\(_{20}\) (1–200 μg/ml) at 37°C for 30 min, followed by HIVas-FITC (10 μg/ml) for another 30 min at 37°C. A total of 4 × 10^5 B cells was collected from each culture and analyzed as described. The percentage of fluorescence-positive B cells or the mean fluorescence intensity of B cells incubated in medium alone was subtracted from the percentage binding to HIVas-FITC (A) or the mean density of HIVas-FITC bound, MFI (B). The data presented are representative of three separate experiments, each conducted with B cells from a different donor.

**FIGURE 8.** Binding of TCG\(_{4}\)-FITC by B cells. B cells (2 × 10^5/sample) were incubated with medium alone or various concentrations of TCG\(_{4}\)-FITC (1–50 μg/ml) at 37°C for 30 min. The binding of TCG\(_{4}\)-FITC was analyzed by flow cytometry, as described. The results shown here are representative of two separate experiments, each conducted with B cells from a different donor.
ODNs and DNA do not bind human B cells comparably

To determine whether DNA and the diester form of ODNs bind B cells as well as sODNs, B cells were preincubated with various unlabeled sODNs or their diester counterparts or DNAs, followed by HIVas-FITC, and analyzed for binding of HIVas-FITC. As can be seen in Fig. 11, phosphodiester ODNs (o-HIVas, o-TMCM, o-NKNSO) did not inhibit binding of HIVas-FITC as effectively as their sODN counterparts (HIVas, TMCM, NKNSO). Similar results were obtained with other ODNs (o-TCG4, o-G20, o-C20) and their sODN counterparts. Bacterial DNA from M. lysodeikticus (Fig. 11), E. coli (data not shown), and calf thymus DNA (data not shown) had little effect on the binding of HIVas. Notably, bacterial DNA and calf thymus DNA did not inhibit HIVas-induced B cell stimulation (Fig. 12). These results suggest that human B cells do not bind microbial and calf thymus DNA and ODNs as effectively as they bind sODNs.

Negatively charged molecules inhibit the binding of HIVas

DNA and sODNs are negatively charged molecules. To examine whether negative charge may play a role in the binding of HIVas-FITC by human B cells, binding of HIVas-FITC by B cells in the presence or absence of increasing concentrations of a variety of negatively charged molecules was analyzed. A number of the negatively charged molecules tested (fucoidan, poly I, and polyvinyl sulfate) inhibited binding of HIVas by B cells (data not shown).

FIGURE 9. sODNs compete with TCG4-FITC for B cell binding. B cells (2 × 10⁵/sample) were incubated with various concentrations of HIVas, TCG4, C20, TMCM, or TMCMCΔT (1–200 µg/ml) at 37°C for 30 min, followed by TCG4-FITC (5 µg/ml) for another 30 min at 37°C. The binding of TCG4-FITC was analyzed as described. The percentage or MFI of B cells cultured with medium alone was subtracted from the percentage of B cells binding to TCG4-FITC (A) or the mean density of TCG4-FITC bound, MFI (B). The data presented are representative of two separate experiments, each conducted with B cells from a different donor.

FIGURE 10. G20 but not C20 inhibits the stimulatory activity of HIVas. B cells (50 × 10⁵/well) were cultured with various concentrations of G20 or C20 (1–100 µg/ml) alone or with HIVas (1 or 2 µg/ml) for 3 days, and proliferation was analyzed as described. The data are the mean of triplicate cultures with an SEM of less than 10%. The results of one of five experiments, each using B cells from an individual donor, with similar findings are shown.

FIGURE 11. ODNs and DNA fail to compete the binding of HIVas-FITC by B cells. B cells (2 × 10⁵/well) were incubated with various concentrations of sODNs (HIVas, TMCM, NKNSO), or their diester form counterparts (1–100 µg/ml) or M. lysodeikticus DNA (1–200 µg/ml) at 37°C for 30 min, followed by HIVas-FITC (10 µg/ml) for another 30 min at 37°C. The binding of HIVas-FITC was analyzed by flow cytometry, as described. The percentage of fluorescence-positive B cells or the mean fluorescence intensity of B cells incubated in medium alone was subtracted from the percentage binding to HIVas-FITC (upper panel) or the mean density of HIVas-FITC bound (lower panel). The data presented are representative of three separate experiments, each conducted with B cells from a different donor.
These results suggest that negative charge may play a role in binding of HIVas by B cells.

Discussion

The current data demonstrate that binding of sODNs by human B cells is rapid, saturable, specific, and initially temperature independent, indicating that binding of sODNs by B cells is receptor mediated. Of note, all sODNs tested appear to bind the same receptor regardless of their stimulatory potential. However, binding is specific for sODNs, not for DNA and ODNs. Finally, active sODNs bound to Sepharose beads induced comparable activation of B cells as the free sODNs, and the stimulatory activity of the Sepharose-bound sODN did not result from the free sODN released from the beads. These results indicate that sODNs bind human B cells by engaging cell surface receptors, and binding appears to be necessary, but not sufficient, for B cell activation.

Our studies on human B cells indicated that Sepharose beads coated with active sODNs stimulate B cells as effectively as soluble sODNs. Because the active sODNs form a stable covalent bond with the beads, free or released sODN does not appear to account for the stimulatory activity of sODN beads. Of note, in other experiments, supernatants from large amounts of some, but not all, sODN-coupled Sepharose beads stimulated B cells, but supernatant from at least a 5-fold larger amount of sODN beads was required to achieve equal activation. Therefore, it is unlikely that soluble sODNs released from sODN beads account for the stimulatory activity of sODN beads. Taken together, our results indicate that sODNs stimulate human B cells by binding to cell surface receptors, and that cell entry of sODN is not necessary.

As our findings indicate, the mechanism of polyclonal activation of human B cells by sODNs may differ from that of murine B cells. Previous studies showed that CpG containing sODNs immobilized on the tissue culture plates were nonstimulatory, suggesting that cellular uptake was required for stimulation of murine B cells (3). In contrast, our data indicate that cell entry is not required for the stimulatory activity of sODNs. Therefore, the mechanism of sODNs-induced activation of B cells might be different in mice and humans.

The identity of the B cell receptor(s) involved in human B cell activation induced by sODNs has not yet been defined. A variety of evidence suggests the presence of surface proteins on human lymphocytes that bind to DNA and ODNs. For instance, a 75–80-kDa nucleic acid-binding protein was detected by chromatography and on immunobots of cell membrane preparations of human peripheral blood cells, including B cells (22, 23). Of importance, it has been reported that sODNs can bind serum IgM, IgG, and IgA, suggesting that Ig on human B cell surface might be the sODN receptor (24). In some circumstances, nucleic acid-binding receptors may play a role in stimulation by sODNs. Thus, runs of guanine (G), which can bind to the type I scavenger receptor by forming base-quartet-stabilized four-stranded helices, can promote the activity of stimulatory ODN for macrophages and lead to enhanced NK cell lytic activity and IFN-γ production (25, 26). Other cell surface molecules may also function as ODN receptors. MAC-1 (CD11b/CD18) is an oligodeoxynucleotide-binding protein on human polymorphonuclear leukocytes (PMN), and binding of SdC28 (a 28-bp phosphorothioate derivative of poly C) inhibited the migration of PMN and selectively enhanced reactive oxygen species (ROS) production by TNF-α-stimulated PMN (27). Of importance, dsDNA or ODNs can bind HLA class II molecules specifically, and dsDNA or ODNs bound HLA-IIβ Raji cells, but not HLA-IIβ−RJ2.2.5 cells. Moreover, the mixed lymphocyte reaction and Ag-specific T cell proliferation were inhibited by preincubation of stimulator cells or Ag-specific T cell lines or anti-CD3 mAb-stimulated human PBMC with dsDNA (28). These results suggest that dsDNA can bind HLA class II molecules and can inhibit Ag presentation. Together, this evidence suggests that a variety of surface receptors for DNA and ODNs may be present on human immune cells. Engagement of one or more of these receptors may be involved in the capacity of sODNs to stimulate human B cells.

The current study also demonstrated that sODNs, except TCG4, and C20 inhibited the binding and stimulatory activity of HIVas, suggesting that sODNs, except TCG4 and C20, bind to the same set of receptor(s). However, TCG4-coated beads activated B cells as effectively as soluble TCG4, suggesting that TCG4 activated human B cells by the same mechanism as HIVas. In addition, binding of FITC-labeled TCG4 and C20 by B cells is saturable, and unlabeled HIVas, TCG4, and C20 inhibited the binding of TCG4-FITC. These results indicate that HIVas, TCG4, and C20 bind to the same set of B cell surface receptor(s). Because TCG4 (12 bp) and C20 (20 bp) are shorter in length than HIVas (27 bp), they may have lower avidity and/or a faster off-rate for the sODN receptor(s) than HIVas. Therefore, TCG4 and C20 did not inhibit binding and the stimulatory activity of HIVas, whereas HIVas inhibited binding of TCG4. It is also possible that HIVas contains a specific sequence or can form specific structures, which may account for its relatively higher avidity for the sODN receptor(s) compared with TCG4 and C20. It is noteworthy that combinations of stimulatory
sODNs, especially HIVas and TCG₄, had no additive or synergistic effect on human B cell responses (data not shown), whereas inactive sODNs inhibited B cell activation induced by stimulatory ODNs. These results suggest that stimulatory sODNs activate B cells by engaging the same receptor, but in a unique manner. Whether this interaction reflects higher avidity or a unique conformation has not been determined. In summary, all of these data are consistent with the conclusion that each of the sODNs tested binds to the same set of receptor(s).

Although all sODNs tested bind to the same receptor, they do not all activate human B cells comparably (Table I). Moreover, some moderately active sODN (i.e., NKNSO) bound B cells comparably with HIVas, whereas a more stimulatory sODN (TCG₄) appeared to have lower avidity than some minimally active sODNs (e.g., G₃₋₀) (Fig. 7). Thus, there does not appear to be a direct correlation between the stimulatory activity and the binding avidity of sODNs.

Our studies indicate that DNA and phosphodiester ODNs do not bind B cells as well as phosphorothioate ODNs (Fig. 11). This finding is consistent with previous observations indicating sODNs bind murine bone marrow cells and spleen cells much better than phosphorothioate ODN (29, 30). This difference may relate to the distribution of charge in the backbone of sODNs compared with ODNs (31). All of these data suggest that sODNs may bind B cells differently from ODNs and DNA. sODNs activate human B cells by engaging surface receptors, whereas ODNs only induce minimal activation of human B cells (15). Therefore, the data suggest that B cells have receptors specific for sODN, but not ODN-binding receptors, and that engaging these surface receptors by sODNs, but not by ODNs, activates human B cells.

The results presented herein suggested that negative charge may play a role in binding of sODNs. This is consistent with other observations. It was reported that binding of 20-bp sODNs by human intestinal epithelial Caco-2 cells was receptor mediated and that NaCl washing removed up to 68% of cell-associated sODNs without affecting monolayer viability and appearance (32). These results suggested that the association between sODNs and Caco-2 cell surface receptors might be based on charge interaction. In addition, it has been shown that interaction between ODNs and thrombin is based on multiple site charge-charge interactions. Neutralizing the negative charge of ODNs by replacing the negatively charged phosphodiester groups with neutral charged groups decreased their thrombin-inhibitory activities (33). Therefore, charge-charge interactions may play a role in binding of sODNs and ODNs to their receptors. Experiments are in process to define these receptors and the mechanism of signaling.

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