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Phosphorothioate-Modified TLR9 Ligands Protect Cancer Cells against TRAIL-Induced Apoptosis

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Hypomethylated CpG oligodeoxynucleotides (CpG ODNs) target TLR9 expressed by immune cells and are currently being evaluated as adjuvants in clinical trials. However, TLR signaling can promote some tumor growth and immune evasion, such as in multiple myeloma (MM). Therefore, deciphering the effects of CpG ODNs on cancer cells will help in preventing these adverse effects and in designing future clinical trials. TLR activation induces multiple signaling pathways, notably NF-κB that has been involved in the resistance to TRAIL. Thus, we wondered if CpG ODNs could modulate TRAIL-induced apoptosis in different models of tumors. Here, we show that TLR9+ (NCI-H929, NAN6, KMM1) and TLR9− MM cells (MM1S) were protected by CpG ODNs against recombinant TRAIL-induced apoptosis. By using two fully human, agonist mAbs directed against TRAIL receptors DR4 and DR5 (mapatumumab and lexatumumab, respectively), we show that the protection was restricted to DR5-induced apoptosis. Similar results were observed for two colon cancer (C45 and Colo205) and two breast cancer cell lines (HCC1569 and Cal51). The protection of CpG ODNs was mediated by its nuclease-resistant phosphorothioate backbone independent of TLR9.

We next demonstrated by surface plasmon resonance that phosphorothioate-modified CpG ODNs directly bound to either TRAIL through TRAIL receptor interaction.

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various mechanisms including translocation of NF-κB, and activation of PKB/Akt or Erk MAPK seem to be involved (17, 20, 21). These pathways can be induced by TLRs, including TLR9 (22–24), therefore jeopardizing the efficacy of the therapeutic use of TLR9 ligands. The aim of this work was to study the effects of CpG ODNs on TRAIL-induced cell death of tumors by using MM, colon, and breast cancer cell lines.

Materials and Methods

Human myeloma cell lines (HMCLs), colon cancer cell lines, and breast cancer cell lines

The HMCL named MDN has been established in our laboratory and is cultured in the presence of 3 mg/ml RIL-6 (Novartis Pharmaceuticals). The KMM1 HMCL was provided by Dr. T. Otsuki (Okayama, Japan) and the Karpas 620 cell line by Dr. A. Karpas (Cambridge, U.K.). The MM.1S HMCL was provided by Dr. S. T. Rosen (Chicago, IL). NCI-H929 was purchased from Deutsche Sammlung von Mikroorganismen and Zellkulturen. Karpas 620 was established in our laboratory from ascites of a stage IV colon cancer patient. The colon cancer cell line Colo205 and the breast cancer cell line HCC1569 were obtained from the American Type Culture Collection. Cal51 was provided by Dr. J.-L. Merlin (Nancy, France). All cell lines were maintained in RPMI 1640 medium supplemented with 5% FCS, 2 mM glutamine, and antibiotics.

RNA isolation and RT-PCR

Total RNA was extracted, treated by DNase, and reverse transcribed as previously described (11). cDNA concentration was quantified by a spectrophotometer (NanoDrop) before amplification by PCR (35 cycles) using the following specific primers for TLR9 (Sigma-Genosys): 5'-TTATGGACTTCC TGCTGGAGGGTC-3' and 5'-CTCGCTTTTTTGCGAAAGCCA-3'.

Cell viability assay

Cell lines (10^5 cells/200 μl) were cultured with or without mapatumumab, lexatumumab, recombinant human TRAIL, or agonist Fas Ab for 48 h in the presence or absence of CpG ODN. Cell viability was determined with PE-conjugated Apo2.7 labeling and analyzed on a FACScalibur flow cytometer (BD Biosciences).

Western Blotting

Cells (10^5 cells/ml) were serum-starved for 18 h, treated or not, harvested, and resuspended in lysis buffer (10 mM Tris-HCl (pH 7.6), 150 mM NaCl, 5 mM EDTA, 1 mM PMSF, 2 mM Na3VO4, 1 mM NaF, 2 mM glutamine, and 2% FCS, 2 mM glutamine, and antibiotics.

Abs, cytokines, and reagents

Human TRAIL-R1 or -R2 agonist mapatumumab and lexatumumab mAbs, respectively, were provided by Human Genome Sciences. Recombinant human TRAIL and recombinant TRAIL receptors DR4 and DR5 were purchased from R&D Systems. mAb CD95 (FAS clone CH11) was purchased from Chemicon International. U0126 and Bay11-7082 were purchased from Alexis. CpG ODNs were provided by Invitrogen. Bases shown in uppercase letters are phosphodiester and those in lowercase letters are phosphorothioate (nuclease resistant): the ODN 2006 sequence was 5'-CTGCGTTTTGTCGAAGACCA-3', the ODN 2007 sequence was 5'-TCGTCGTTTTGTCGTTTTGTCGTTGGGGG-3', and the 3' CPG ODN sequence was 5'-TCGTCGTTTTGTCGTTTTGTCGTTGGGGG-3'.

Surface plasmon resonance (SPR)

Experiments were conducted at 25°C on a BIAcore 3000 instrument. For binding analysis, either 200 resonance units of DR4 or DR5, 2000 resonance units of recombinant TRAIL, or 8000 resonance units of lexatumumab were covalently coupled to a research grade CM5 chip (GE Healthcare) by amine coupling as recommended by the manufacturer. All measurements were baseline-corrected by subtracting the values obtained from the analyte being injected over a control flow cell. Binding of mapatumumab (3 μg/ml), lexatumumab (3 μg/ml), or recombinant TRAIL (2 μg/ml) either alone or preincubated for 60 min with CpG ODN (0.78–12.5 μg/ml) was assayed for 2 min at a flow rate of 40 μl/min on DR4- or DR5-immobilized chips. Dissociation was monitored for 3 min and then the surface was regenerated by injection of 10 mM glycine (pH 2.0). Measurement of the direct binding of CpG ODN to recombinant TRAIL-immobilized or lexatumumab-immobilized chips was performed as follows. Five concentrations of CpG ODN ranging from 3.25 to 52 nM for CpG ODN injected over a LexA-immobilized chip and from 103 nM to 3.3 μM for CpG ODN injected over a recombinant TRAIL-immobilized chip were titrated from low to high concentrations in a single-cycle mode for 3 min at 40 μl/min. Dissociation was monitored for 4 min. Data were analyzed using the BioEval 4.1 software (Biacore).

NK cell expansion and cytotoxic assays

Assessment of NK cell expansion was performed as previously described (25). Briefly, PBMCs from normal volunteers were purified by Ficol-Hypaque centrifugation and cocultured with irradiated EBV-transformed B lymphoblastoid cells in 8% human serum and in the presence of IL-2 for 4–6 wk. The proportion of CD3−/CD16+ NK cells increased to >50% during the culture. Expanded CD3−/CD16+ NK cells were then purified using anti-CD16 microbeads (Miltenyi Biotech) and the cytotoxic assay was performed. 51Cr-labeled target cells were incubated for 15 min with CpG ODN, and cytotoxic assays were performed in 96-well V-bottom plates with 3000 51Cr-labeled target cells in 200 μl (4 h). The percentage of specific cytotoxicity was determined as follows: (experimental release – spontaneous release)/(total release – spontaneous release) x 100.

Results

CpG ODN inhibits the apoptosis of HMCLs induced by TRAIL through DR5

We first determined the expression of TLR9 on a panel of six representative HMCLs. As shown in Fig. 1, we detected TLR9 at various levels in the HMCLs KARPAS 620, MDN, NCI-H929, NAN6, and KMM1, but not in MM1S. Incubation of KARPAS 620, MDN, and KMM1 with CpG ODN did not modify the apoptosis induced by 50 ng/ml human recombinant TRAIL. On the contrary, CpG ODN decreased the apoptosis of NCI-H929, NAN6, and, more surprisingly, TLR9-negative MM1S cells (mean of ratios 53 ± 3, 84 ± 6, and 62 ± 12%, respectively) (see Fig. 2A and Table I). The effect of CpG ODN on TRAIL-induced apoptosis was dose dependent as shown in Fig. 2B, and the inhibition of apoptosis was obtained over a wide range of TRAIL concentrations as shown in Fig. 2C for NCI-H929. It is known that TRAIL induces apoptosis through the activation of two receptors, TRAIL-R1 (DR4) and/or TRAIL-R2 (DR5). To better characterize the mechanism of apoptosis inhibition observed with CpG ODN, we replaced human recombinant TRAIL with mapatumumab and lexatumumab, two agonist Abs directed against DR4 or DR5, respectively (26, 27). As summarized in Table I for all six HMCLs and illustrated in Fig. 3A, HMCLs were either solely sensitive through DR4 (KARPAS 620), DR5 (NCI-H929, NAN6, and MM1S), or both (MDN, KMM1). Strikingly, CpG ODN completely blocked the apoptosis induced by lexatumumab in MDN, NCI-H929, KMM1, NAN6, and MM1S, but not that induced by mapatumumab. The effect of CpG ODN on DR5-induced apoptosis was dose dependent, as shown in Fig. 3B. No inhibition of mapatumumab-induced apoptosis was observed in MDN even at
suboptimal concentrations of Ab (Fig. 3C). As shown in Fig. 3D, the cleavage of caspase-8 in NCI-H929 induced by lexatumumab was blocked by the addition of CpG ODN to the culture. This result suggested that CpG ODN could act at the initiation of the cell death signaling pathway.

As Fas is a member of the TNF ligand superfamily that also induces apoptosis of tumor cells, we wondered whether CpG ODN could also inhibit its function. NCI-H929 was Fas-resistant, but KMM1 and MDN were Fas-sensitive (Fig. 3E and data not shown). Stimulation of KMM1 or MDN by an anti-Fas mAb agonist induced a dose-dependent apoptosis that was not inhibited by CpG ODN. Taken together, our results suggested that CpG ODN specifically inhibited TRAIL-induced apoptosis through DR5.

CpG ODN inhibits the apoptosis of colon and breast cancer cell lines induced by TRAIL through DR5
As CpG ODNs are intended for use in both solid and hematologic cancer patients, we also performed the experiment on two colon cancer cell lines, C45 and Colo205 (Fig. 4), and two breast cancer cell lines, HCC1569 and Cal51 (data not shown). Both colon cancer cell lines express TLR9 (Fig. 4A), and CpG ODN decreased the apoptosis of the Colo205 line induced by recombinant human TRAIL (mean of reduction, 60 ± 14%). The colon and breast cancer cell lines were only sensitive to DR5 stimulation. As illustrated in Fig. 4B for Colo205, addition of CpG ODN decreased the lexatumumab-induced apoptosis, either partially in Colo205, C45, and HCC1569 (mean of reduction, 47 ± 15%, n = 3; 60%, n = 2; and 74%, n = 2, respectively) or completely in Cal51 (mean of reduction, 97%, n = 2).

The blocking of lexatumumab-induced apoptosis depends on the phosphorothioate modification of CpG ODN
Synthetic CpG ODNs that are currently used in vitro and in clinical investigations bear a nuclease-resistant phosphorothioate backbone that increases their half-life. In contrast, the phosphodiester

Table I. CpG inhibition of TRAIL-induced apoptosis does not correlate with TLR9 expression

<table>
<thead>
<tr>
<th>TLR9 Expression</th>
<th>rhTRAIL Sensibility (% Apo2.7)</th>
<th>CpG Inhibition of rhTRAIL Apoptosis (%)</th>
<th>Mapatumumab Sensibility (% Apo2.7)</th>
<th>Lexatumumab Sensibility (% Apo2.7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K620</td>
<td>+ +</td>
<td>74.33 ± 12.5</td>
<td>8.52</td>
<td>81.00 ± 3.61</td>
</tr>
<tr>
<td>MDN</td>
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<td>48.33 ± 8.08</td>
<td>16.55</td>
<td>78.00 ± 4.36</td>
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<tr>
<td>NCI-H929</td>
<td>+</td>
<td>50.67 ± 9.29</td>
<td>53.28</td>
<td>11.67 ± 2.08</td>
</tr>
<tr>
<td>KMM1</td>
<td>+/-</td>
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<td>15.24</td>
<td>55.67 ± 9.71</td>
</tr>
<tr>
<td>NAN6</td>
<td>+/-</td>
<td>34.00 ± 6.94</td>
<td>84.31</td>
<td>3.00 ± 1.73</td>
</tr>
<tr>
<td>MM1S</td>
<td>-</td>
<td>35.33 ± 5.03</td>
<td>62.26</td>
<td>13.00 ± 2.65</td>
</tr>
</tbody>
</table>

*TLR9 expression was determined by PCR. Survival of six HMCLs in the presence of rhTRAIL (50 ng/ml), mapatumumab, or lexatumumab (6 μg/mL) was determined by Apo2.7 staining at day 2. The mean values ± SD of three independent experiments are shown. rhTRAIL, recombinant human TRAIL.*
bond of native DNA is highly sensitive to degradation by endonucleases. Both forms of CpG ODNs stimulate TLR9 and induce immunostimulatory activities. To determine whether the native form of CpG ODN was as potent as the synthetic form in the inhibition of apoptosis, we measured the TRAIL-induced apoptosis in the presence of either phosphorothioate-modified CpG ODN (CpG 2006) or a synthetic phosphodiester CpG ODN (CpG 2006-G5). The later sequence had a higher capacity to be internalized and to stimulate TLR9 than did native CpG ODN due to the addition of a 3′ poly-G string. As shown in Fig. 5, the phosphodiester CpG ODN did not inhibit the lexatumumab-induced apoptosis, suggesting that the protection was TLR9-independent. Furthermore, addition of a non-CpG ODN sequence designed to compete with the binding of CpG ODN 2006 to its receptor and that lacks TLR9 stimulatory capacity totally abrogated the apoptosis. As this CpG ODN also has a phosphorothioate backbone, our data suggest that the protection of tumors cells against TRAIL depends on the phosphorothioate modification of CpG ODN rather than on TLR9 stimulation.

FIGURE 3. CpG ODN inhibits the apoptosis induced by an agonist Ab directed against DR5. A, Percentage of cell death of KARPAS 620, MDN, and NCI-H929 induced by either mapatumumab or lexatumumab (6 μg/ml) in the absence or presence of CpG ODN (5 μg/ml) determined at day 2 by Apo2.7 staining. Three independent experiments are shown for each cell line. B, Percentage of cell death of NCI-H929 induced by lexatumumab (6 μg/ml) in the presence of increasing doses of CpG ODN (0.039, 0.156, 0.625, 2.5, and 10 μg/ml) and determined at day 2 by Apo2.7 staining. The mean values ± SD of three independent experiments are shown. C, Percentage of cell death of MDN induced by increasing doses of either mapatumumab or lexatumumab in the presence of CpG ODN (5 μg/ml) and determined at day 2 by Apo2.7 staining. The mean values ± SD of three independent experiments are shown. D, Expression of caspase 8 (full-length and cleaved form) determined by immunoblot analysis in NCI-H929 treated by 6 μg/ml lexatumumab, 5 μg/ml CpG ODN, or both. Actin was used as a protein loading control. E, Percentage of cell death of KMM1 induced by increasing doses of anti-Fas Ab (clone CH11) in the presence of CpG ODN (5 μg/ml) and determined at day 2 by Apo2.7 staining. The mean values ± SD of three independent experiments are shown.

FIGURE 4. CpG ODN inhibits the apoptosis of colon cell lines induced by human recombinant TRAIL and by an agonist Ab directed against DR5. A, Expression of TLR9 mRNA in two colon cancer cell lines analyzed by RT-PCR as described in Materials and Methods. B, Percentage of cell death of Colo205 induced by human recombinant TRAIL (50 ng/ml), mapatumumab (6 μg/ml), or lexatumumab (6 μg/ml) in the absence or presence of CpG ODN (5 μg/ml) determined at day 2 by Apo2.7 staining. Three independent experiments are shown.
Apo2.7 staining. The mean values with phosphorothioate modification. Survival was determined at day 2 by Phodiester CpG ODN 2006-G5, or a nonstimulatory TLR9 ODN sequence.

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involved in the immune surveillance of cancer, we wondered if NK, NKT, and CD8 T cells to eliminate infected cells, tumor cells, TRAIL is expressed and used by immune effector cells such as Phosphorothioate-modified CpG ODN inhibits the killing of various DR5-sensitive tumor cells by NK cells.

Phosphorothioate-modified CpG ODN inhibits the killing of lexatumumab to DR5

It has been previously shown that the internalization of DR4 and DR5 occurs within minutes of TRAIL binding (28). In agreement, we observed a decrease of DR5 cell surface staining in the presence of lexatumumab (data not shown). Surprisingly, the addition of phosphorothioate-modified CpG ODN blocked the disappearance of DR5 from the cell surface. This result suggested that phosphorothioate-modified CpG ODN could perturb the interaction of TRAIL or anti-DR4/DR5 mAbs with their respective receptors in a TLR9-independent manner.

To test this hypothesis, we immobilized recombinant DR4 or DR5 on an SPR chip surface and observed the binding of either mapatumumab or lexatumumab, respectively, in the presence or absence of CpG ODN. DR4 and DR5 formed a saturated complex with mapatumumab and lexatumumab mAbs, respectively (Figs. 6A and B). In agreement with our hypothesis, incubation of phosphorothioate-modified CpG ODN with DR5 (Fig. 6A), but not with DR4 (Fig. 6B), inhibits the interaction with the corresponding Ab in a dose-dependent manner. Furthermore, the binding of recombinant TRAIL to DR5 was also inhibited by phosphorothioate-modified CpG ODN in a dose-dependent manner (Fig. 6C). Conversely, phosphodiester CpG ODN did not inhibit these interactions (supplemental Fig. S1).

Taken together, these results suggested that phosphorothioate-modified CpG ODN could directly bind to DR5, recombinant human TRAIL, or lexatumumab. Surprisingly, no direct binding of CpG ODNs to DR5 was observed (data not shown). In contrast, we observed binding of CpG ODNs to lexatumumab when it was immobilized on an SRP chip surface, but not to mapatumumab (Fig. 6D). Similarly, binding of CpG ODNs to recombinant human TRAIL immobilized on an SRP chip surface was also observed (Fig. 6E).

Phosphorothioate-modified CpG ODN inhibits the killing of DR5-sensitive tumor cells by NK cells

TRAIL is expressed and used by immune effector cells such as NK, NKT, and CD8 T cells to eliminate infected cells, tumor cells, or autoreactive cells (18). As NK cells have been reported to be involved in the immune surveillance of cancer, we wondered if CpG ODN could alter their cytotoxicity toward tumor cells. To this end, we incubated NCI-H929 with or without a phosphorothioate-modified CpG ODN and then measured the cytotoxicity induced by NK cells. As shown in Fig. 7A, the lysis of NCI-H929 by NK cells was significantly reduced in the presence of CpG ODN (mean of lysis reduction at an E:T ratio of 1:15, 45 ± 23%, n = 3). A similar result was obtained for another DR5-sensitive cell line (Culo205) but not with the doubly DR4- and DR5-sensitive MDN cell line (data not shown). Finally, neutralization of TRAIL on NK cells by a blocking Ab partially decreased the cytotoxicity of NK cells to the same level as that obtained upon CpG ODN incubation (mean of lysis reduction at E:T ratio of 1:15, 49 ± 1%, n = 3; Fig. 7B). These data suggested that the component of NK cell cytotoxicity involving TRAIL was inhibited by CpG ODN.

Discussion

TLRs are expressed by cells of the immune system, as well as by tumor cells of various origins. Although biological consequences of TLR activation have been extensively studied in immune cells, the effect on cancer cells is largely unknown. In the present study, we aimed to determine the effect of CpG ODN on the TRAIL-induced cell death of cancer cells. We show that a synthetic phosphorothioate-modified CpG ODN inhibits the killing of various DR5-sensitive tumor cells through a TLR9-independent binding to TRAIL or to lexatumumab. This inhibition reduces the cytotoxic activity of NK cells through TRAIL and could therefore dampen the clinical efficacy of CpG ODN-based adjuvants.

Although synthetic CpG ODN sequences have been widely used in vitro to decipher the TLR9 signaling pathway and to stimulate the immune system, their exact mechanism of action has only been recently uncovered (2, 29). Indeed, TLR9 recognizes natural phosphodiester DNA through the DNA sugar backbone 2'-deoxyribose rather than through CpG motifs, as thought so far. In contrast, phosphorothioate-modified synthetic ODNs, despite strong binding, are dependent on CpG motifs to activate TLR9. Our results extend the structure-dependent function of phosphorothioate-modified ODNs in the cell death pathway.

So far, known effects of CpG ODN on cell death resistance have involved TLR9 stimulation and Erk1/2 or NF-κB signaling pathway activation. Additionally, it has been shown that NF-κB activation contributed to LPS/TLR4-induced apoptosis resistance of lung cancer cells against TRAIL (30). We also observed NF-κB activation through phosphorylation of IκB in the presence of CpG ODN; however, inhibition of either NF-κB or Erk1/2 activation by specific inhibitors did not reverse the effects of CpG ODNs (supplemental Fig. S2). Therefore, the survival effect was not mediated by these previously described pathways. On the contrary, despite activation of TLR9 signaling by classical synthetic CpG ODNs, the mechanism of survival was TLR9-independent, as it was also observed in a TLR9−/− cell line and with a sequence designed to act as an antagonist of TLR9. Furthermore, we showed that the phosphorothioate modification of the CpG ODN, necessary for the nuclease resistance of the sequence, conferred the inhibitory capacity. In agreement with this finding, it has been previously shown that the phosphorothioate backbone of ODN sequences strongly increased nonspecific binding to various proteins. In particular, phosphorothioate sequences inhibit basic fibroblast growth factor binding to its receptor (31, 32). This stickiness of phosphorothioate sequences is also observed in the binding to TLR9, as a phosphorothioate-modified sequence, regardless of the bases, has a higher TLR9 affinity than does the same phosphodiester sequence (29). Taken together, these data imply that natural DNA sequences from bacteria would trigger TLR9 signaling in tumors and immune cells, leading to activation. On the other hand, synthetic phosphorothioate CpG ODNs would both mediate the previous effects and induce TLR9-independent phenomena such as receptor-ligand.

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binding inhibition. This phenomenon could be extended to the inhibition of virus entry, as it has been recently shown that human cytomegalovirus, hepatitis C virus, and HIV entry are blocked by phosphorothioate-modified ODNs, regardless of CpG motifs (33–36). One could therefore take advantage of this to design antiviral vaccines that target virus entry.

**FIGURE 6.** Phosphorothioate-modified CpG ODN inhibits the binding of recombinant human TRAIL and of lexatumumab to DR5. A. SPR analysis of lexatumumab binding to DR5 in the presence or absence of increasing doses of CpG ODNs. CpG ODNs were perfused over a DR5-immobilized chip for 2 min at a flow rate of 40 µl/min and the resonance changes were recorded. B. SPR analysis of mapatumumab binding to DR4 in the presence or absence of CpG ODNs (25 µg/ml). C. SPR analysis of recombinant TRAIL binding to DR5 in the presence or absence of increasing doses of CpG ODNs. D. SPR analysis of binding activity of increasing concentrations of CpG ODNs on either mapatumumab- or lexatumumab-immobilized chips. E. SPR analysis of binding activity of increasing concentrations of CpG ODNs on a recombinant TRAIL-immobilized chip.

**FIGURE 7.** Phosphorothioate-modified CpG ODN inhibits the killing of the HMCL NCI-H929 by NK cells. A. Cytotoxicity of NK cells against NCI-H929 in the absence or presence of 5 µg/ml CpG ODN. Data shown are means ± SD of one experiment performed in triplicate and are representative of three independent experiments. B. Cytotoxicity of NK cells against NCI-H929 was determined in the presence of 5 µg/ml CpG ODN, 5 µg/ml azide-free TRAIL-neutralizing Ab, or an isotype control Ab. Data shown are means ± SD of one experiment performed in triplicate and are representative of three independent experiments.
Given our present finding, it is likely that the capacity of phosphorothioate CpG ODNs to inhibit TRAIL-induced apoptosis could have an influence on the outcome of previously published studies. Therefore, interpretation of experiments should be done with caution and would better be completed with phosphodiester sequences stabilized by 3′ poly-G extensions that confer a nanoparticle-like structure (37).

Regarding the inhibition of TRAIL−induced apoptosis, we observed a decrease in the cytotoxicity mediated by NK cells. This result suggests that cells of the immune system could be partially dampened in their function of tumor surveillance, particularly in the case of CpG peritumoral injection. Fortunately, note that the lysis machinery of immune effector cells is not restricted to TRAIL and therefore could be protected from a major shutdown.

In conclusion, our results confirm the need for an encapsulation of CpG ODNs that would avoid unspecific binding with unexpected consequences. Finally, these data increase our understanding of the function of CpG ODNs in tumors.

Disclosures

The authors have no financial conflicts of interest.

References