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Synergism between CpG-Containing Oligodeoxynucleotides and IL-2 Causes Dramatic Enhancement of Vaccine-Elicited CD8+ T Cell Responses

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Novel anticancer vaccination regimens that can elicit large numbers of Ag-specific T cells are needed. When we administered therapeutic vaccines containing the MHC class I-presented self-peptide tyrosinase-related protein (TRP)-2180–188 and CpG-containing oligodeoxynucleotides (CpG ODN) to mice, growth of the TRP-2-expressing B16F1 melanoma was not inhibited compared with growth in mice that received control vaccinations. When we added systemic IL-2 to the TRP-2180–188 plus CpG ODN vaccines, growth of B16F1 was inhibited in a CD8-dependent, epitope-specific manner. Vaccines containing TRP-2180–188 without CpG ODN did not cause epitope-specific tumor growth inhibition when administered with IL-2. The antitumor efficacy of the different regimens correlated with their ability to elicit TRP-2180–188-specific CD8+ T cells. When we administered TRP-2180–188 plus CpG ODN-containing vaccines with systemic IL-2, 18.2% of CD8+ T cells were specific for TRP-2180–188. Identical TRP-2180–188 plus CpG ODN vaccines given without IL-2 elicited a TRP-2180–188-specific CD8+ T cell response of only 1.1% of CD8+ T cells. Vaccines containing TRP-2180–188 without CpG ODN elicited TRP-2180–188-specific responses of 2.8% of CD8+ T cells when administered with IL-2. There was up to a 221-fold increase in the absolute number of TRP-2180–188-specific CD8+ T cells when IL-2 was added to TRP-2180–188 plus CpG ODN-containing vaccines. Peptide plus CpG ODN vaccines administered with IL-2 generated epitope-specific CD8+ T cells by a mechanism that depended on endogenous IL-6. This is the first report of synergism between CpG ODN and IL-2. This synergism caused a striking increase in vaccine-elicited CD8+ T cells and led to epitope-specific antitumor immunity.

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models (1, 16, 23, 24), we hypothesized that peptide plus CpG ODN vaccines would be more effective at eliciting Ag-specific CD8+ T cell responses and at mediating therapeutic antitumor responses if they were combined with exogenous IL-2. As a rigorous test of the vaccination regimens used in our experiments, we used B16F1, a rapidly growing murine melanoma that expresses very low levels of MHC class I molecules and is poorly immunogenic (25). B16F1 expresses tyrosinase-related protein-2 (TRP-2). Because TRP-2 is also expressed by normal melanocytes, it is subject to self-tolerance (26). Amino acids 180–188 of TRP-2 (TRP-2180–188) form an immunogenic MHC class I-presented epitope (2, 3, 26). We found that synergism between IL-2 and CpG ODN led to massive epitope-specific CD8+ T cell responses, and vaccines containing TRP-2180–188 plus CpG ODN in IFA could only mediate epitope-specific antitumor immunity when combined with IL-2. The antitumor effect of TRP-2180–188 plus CpG ODN-containing vaccines combined with IL-2 was dependent on CD8+ T cells. Our results demonstrated that generation of CD8+ T cell responses by peptide plus CpG ODN-containing vaccines administered with exogenous IL-2 depended on endogenous IL-6.
Materials and Methods
Mice and tumor cells
C57BL/6 and BALB/c mice were obtained from the National Cancer Institute (Frederick, MD) animal production area. IL-6-deficient mice (27) on a C57BL/6 background were obtained from The Jackson Laboratory. Type I IFNR-deficient mice on a BALB/c background were provided by Dr. H. Young (National Cancer Institute, Frederick, MD). These mice were derived from type I IFNR-deficient mice on a 129 SvEv background (28) by Dr. J. Durbin (Ohio State University, Columbus, OH). The genotype of

FIGURE 2.  A, Vaccines containing TRP-2180-188 and CpG ODN elicit large TRP-2180-188-specific CD8 T cell responses when administered with IL-2. On days 0, 3, and 6 priming vaccines consisting of TRP-2180-188, HBC128-140, and CpG ODN in IFA were administered. IL-2 was administered on days 7–9. On day 14, a boost vaccine was administered and IL-2 was given on days 15–18. On day 19, the mice were sacrificed, splenocytes were stimulated ex vivo for 6 h with either TRP-2180-188 or the negative control peptide OVA257-264 and intracellular staining for IFN-γ was performed (ICCS assay). Robust TRP-2180-188-specific CD8 T cell responses, but minimal OVA257-264-specific responses, were detected in a mouse that received TRP-2180-188-containing vaccines. Plots are gated on CD3 lymphocytes. The percentage of CD3 CD8 T cells that produced IFN-γ is shown on each plot. B, When mice were treated as described in A, a mean of 18.3% of CD3 CD8 T cells were induced to produce IFN-γ in response to TRP-2180-188 stimulation, but only 0.1% of CD3 CD8 T cells produced IFN-γ in response to OVA257-264 stimulation (n = 11 mice/group). Synergism between IL-2 and CpG ODN increases the magnitude of TRP-2180-188-specific CD8 T cell responses. C, Examples are shown of TRP-2180-188-specific CD8 T cell responses in mice that received either the vaccination regimen described in A consisting of TRP-2180-188, HBC128-140, and CpG ODN in IFA combined with systemic IL-2 or this regimen with CpG ODN but without IL-2 or with IL-2 but with CpG ODN omitted. The ICCS assay was performed as in A. The percentage of CD3 CD8 T cells that produced IFN-γ is shown on each plot. D, Mice received either the vaccination regimen described in A consisting of TRP-2180-188, HBC128-140, and CpG ODN in IFA administered with systemic IL-2, or regimens with IL-2 or CpG ODN omitted. TRP-2180-188-specific CD8 T cell responses were measured by ICCS assay as in A. Regimens containing both CpG ODN and IL-2 elicited larger TRP-2180-188-specific CD8 T cell responses as a percentage of total CD3 CD8 T cells than regimens containing CpG ODN without IL-2, regimens containing IL-2 without CpG ODN, or regimens containing only peptides in IFA with neither CpG ODN nor IL-2 (n = 8–12/group). E, In the same mice described in D, vaccination regimens containing both CpG ODN and IL-2 generated a larger absolute number of TRP-2180-188-specific CD3 CD8 T cells than regimens containing CpG ODN without IL-2, regimens containing IL-2 without CpG ODN, or regimens containing only peptides in IFA with neither CpG ODN nor IL-2 (n = 8–12/group).
these mice was confirmed by PCR (data not shown). All animal studies were approved by the National Cancer Institute Center for Cancer Research Animal Care and Use Committee. The B16F1 melanoma (H-2b) and EL4 lymphoma cells (H-2d) were purchased from American Type Culture Collection. The TC-1 fibrosarcoma tumor cell line (H-2d) (29) was provided by T. C. Wu (Johns Hopkins University, Baltimore, MD).

**Vaccine ingredients**

Amino acids 180–188 of TRP-2 (TRP-2<sub>180–188</sub>) (2, 3, 26), amino acids 257–264 of the OVA protein (OVA<sub>257–264</sub>) (10), amino acid 366–374 of the influenza nucleoprotein (NP<sub>366–374</sub>) (1), amino acids 82–90 of the RSV (respiratory syncytial virus) M2 protein (RSV M2<sub>82–90</sub>) (30), amino acids 25–33 of the human gp100 protein (gp100<sub>25–33</sub>) (1), and amino acids 197–205 of the HIV gag protein (gag<sub>197–205</sub>) (31) and amino acids 49–57 of the human papillomavirus E7 protein (E7<sub>49–57</sub>) (31) form immunogenic epitopes that are presented by MHC class I molecules. Peptides with cell-penetrating peptide moieties (CPP) can penetrate dendritic cell membranes and elicit T cell responses (32). We used a peptide made up of a cell-penetrating moiety and TRP-2<sub>180–188</sub> (CPP-TRP-2) in our work (32). Amino acids 128–140 of the hepatitis B core protein (HBC<sub>128–140</sub>) form an epitope presented by H-2 I-Ab that can elicit CD<sup>8</sup>T cell responses that are capable of enhancing vaccine-elicted CD<sup>8</sup>T cell responses (33). Peptides were synthesized and purified to >95% purity by Biopeptide or New England Peptide. Human recombinant IL-2 was obtained from the National Cancer Institute Biological Resources Branch. A sterile, endotoxin-free solution of CpG ODN 1826 (CpG ODN) (14) in Tris-EDTA buffer was purchased from Copley Pharmaceutical Group. IFA was purchased from Sigma-Aldrich.

**Vaccine preparation**

Each priming vaccine in experiments that used TRP-2<sub>180–188</sub> or OVA<sub>257–264</sub> consisted of the following ingredients emulsified in IFA: 50 µg of CpG ODN, 50 µg of TRP-2<sub>180–188</sub> or OVA<sub>257–264</sub> and, in some experiments, 60 µg of HBC<sub>128–140</sub>. Boost vaccines were prepared in an identical manner as priming vaccines except 100 µg of TRP-2<sub>180–188</sub> or OVA<sub>257–264</sub> was included. In experiments in which the HBC<sub>128–140</sub> Peptide was used, 120 µg of HBC<sub>128–140</sub> was included in boost vaccinations. All E7<sub>49–57</sub> vaccine doses consisted of 50 µg of CpG ODN and 50 µg of E7<sub>49–57</sub> in IFA. All RSV M2<sub>25–33</sub> vaccine doses consisted of 50 µg of CpG ODN and 50 µg of RSV M2<sub>25–33</sub> in a PBS/IFA emulsion. All CPP-TRP-2 vaccine doses consisted of 50 µg of CpG ODN and 100 µg of CPP-TRP-2 in a PBS/IFA emulsion. The volume of all vaccine injections was 100 µl. When CpG ODN was eliminated in experiments, it was replaced with Tris-EDTA buffer. When IL-2 was given, 40,000 IU was administered i.p. twice daily. When IL-2 was not administered, control buffer, containing human serum albumin and mannitol in PBS, was injected in place of IL-2 as a control. In all experiments, priming vaccinations were given as a series of three injections, 3 days between injections. The first and third priming vaccines were given s.c. at the base of the tail; the second priming vaccination was administered s.c. on the left side. Boost vaccinations were given at the base of the tail s.c.

**Antibodies**

The following Abs from BD Pharmingen were used: anti-CD3e (clone 145-2C11), anti-IFN-γ (clone XMG1.2), anti-I-A/I-E (clone 2G9), anti-Ly6G (clone RB6-8C5), anti-CD28 (clone 37.51), and anti-CD16/CD32 (clone 2.4G2). Anti-CD8a (clone CT-CD8a) from Caltag Laboratories was used.

**Peptide stimulation followed by intracellular cytokine staining (ICCS) experiments**

The percentage of CD<sup>8</sup>T cells specific for TRP-2<sub>180–188</sub> was determined by stimulating splenocytes with peptides followed by ICCS. Mice were sacrificed, splenocytes were RBC depleted, washed, and suspended at 3.5 × 10<sup>6</sup> live cells/ml. For each mouse, two 15-ml tubes were prepared. Each tube contained 3.5 × 10<sup>5</sup> splenocytes, 2 µg/ml of a stimulatory anti-CD28 Ab, and 1 µl of Golgi Plug (BD Pharmingen). For each mouse, 20 µl of TRP-2<sub>180–188</sub>, was added to one tube and 20 µg/ml of the negative control peptide OVA<sub>257–264</sub> was added to the other tube. All tubes were incubated at 37°C for 6 h. The cells were then washed and surface stained for CD3 and CD8. The cells were then permeabilized with Cytofix/Cytoperm and stained for intracellular IFN-γ according to the instructions of the Cytofix/Cytoperm kit (BD Pharmingen). Flow cytometry acquisition was performed with a BD Biosciences FACSort. Analysis was performed with CellQuest software (BD Biosciences). For each mouse, a tube containing cells stimulated with TRP-2<sub>180–188</sub> and another tube containing cells stimulated with OVA<sub>257–264</sub> were analyzed. The percentage of CD<sup>8</sup>T cells specific for TRP-2<sub>180–188</sub> was calculated as the percentage of CD<sup>3</sup>CD<sup>8</sup>T IFN-γ<sup>+</sup> events with TRP-2<sub>180–188</sub> stimulation minus the percentage of CD<sup>3</sup>CD<sup>8</sup>T IFN-γ<sup>+</sup> events with OVA<sub>257–264</sub> stimulation. ICCS assays to measure RSV M2<sub>25–33</sub> specific responses were performed in an identical manner except for the substitution of RSV M2<sub>25–33</sub> for TRP-2<sub>180–188</sub> and gag<sub>97–108</sub> for OVA<sub>257–264</sub>. ICCS assays to measure E7<sub>49–57</sub>-specific responses were also performed in an identical manner except for the substitution of E7<sub>49–57</sub> for TRP-2<sub>180–188</sub> and NP<sub>366–374</sub> for OVA<sub>257–264</sub>. Anti-CD28 was used in all ICCS experiments except those reported in Fig. 9D. ICCS assays of peripheral blood cells were performed in the same manner as for splenocytes except that 1 × 10<sup>6</sup> peripheral blood cells were used in each tube instead of 3.5 × 10<sup>6</sup> splenocytes.

**Quantification of the absolute number of splenic CD<sup>8</sup>T cells specific for TRP-2<sub>180–188</sub> in ICCS experiments**

RBC-depleted splenocytes from vaccinated mice were prepared. Trypan blue was used for dead cell discrimination and live cells were counted. The cells were then stained with anti-CD3 and anti-CD8 and suspended in PBS. 7-Aminoactinomycin D (7-AAD; BD Pharmingen) was added for dead cell discrimination and the cell number was determined by flow cytometry.
exclusion. A region enclosing all live cells was analyzed for dual expression of CD3 and CD8. The percentage of live cells expressing both CD3 and CD8 was multiplied by the total number of live splenocytes to determine the absolute number of splenic CD3<sup>+</sup>/CD8<sup>+</sup> cells. The absolute number of splenic CD3<sup>+</sup>/CD8<sup>+</sup> RP-2180-188-specific T cells was determined by multiplying the absolute number of CD3<sup>+</sup>/CD8<sup>+</sup> splenocytes by the percentage of CD8<sup>+</sup> T cells specific for RP-2180-188 determined during the ICCS assay.

**Measurement of CD8<sup>+</sup> T cell avidity**

To measure the avidity of vaccine-elicited CD8<sup>+</sup> T cells, EL4 cells were incubated with either 10, 0.01, 0.001, or 0 μM TRP-2<sub>2180-188</sub> peptide for 3 h. The EL4 cells were then washed and incubated with splenocytes from vaccinated mice for 5 h. Following the incubation, CD8<sup>+</sup> T cells producing IFN-γ were enumerated by ICCS assay as described above. For these assays, an equal number of splenocytes from four mice in each vaccination category were combined and the percentage of CD8<sup>+</sup> T cells that were TRP-2<sub>2180-188</sub>-specific was defined as the percentage of CD8<sup>+</sup> T cells producing IFN-γ in response to unpulsed EL-4 cells minus the percentage of CD8<sup>+</sup> T cells producing IFN-γ after stimulation with EL4 cells pulsed with a given concentration of TRP-2<sub>2180-188</sub> peptide.

**Quantitation of CD8<sup>+</sup> T cell responses with MHC class I multimers**

To measure TRP-2<sub>2180-188</sub>-specific or OVA<sub>257–264</sub>-specific CD8<sup>+</sup> T cell responses, single-cell suspensions of splenocytes were stained with either TRP-2<sub>2180-188</sub>-K<sup>b</sup> or OVA<sub>257–264</sub>-K<sup>b</sup> tetramers (both from Coulter) along with anti-CD8 (clone KT15; Coulter), anti-I-A/I-E, anti-Ly6G, and 7-AAD. To measure RSV M282–90-specific CD8<sup>+</sup> T cell responses, we stained splenocytes with RSV M282–90-K<sup>d</sup> pentamers (Proimmune). The

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**FIGURE 4.** An MHC class II-presented helper epitope is not required for synergism between CpG ODN and IL-2 to generate large TRP-2<sub>190–118</sub>-specific CD8<sup>+</sup> T cell responses that cause epitope-specific antitumor immunity. A, Mice were vaccinated with TRP-2<sub>190–118</sub> in IFA with or without CpG ODN on days 0, 3, 6, and 14. The mice received IL-2 or control injections on days 7–9 and 15–18. On day 19, TRP-2<sub>190–118</sub>-specific CD8<sup>+</sup> T cell responses were measured by ICCS assay (n = 8–10 mice/group). Vaccines containing both CpG ODN and IL-2 elicited larger TRP-2<sub>190–118</sub>-specific CD8<sup>+</sup> T cell responses as a percentage of total CD3<sup>+</sup> CD8<sup>+</sup> splenocytes than regimens containing CpG ODN without IL-2, regimens containing IL-2 without CpG ODN, or regimens containing TRP-2<sub>190–118</sub> in IFA with neither CpG ODN nor IL-2 (n = 8–10/group). B, In the same mice described in A, vaccination regimens containing both CpG ODN and IL-2 generated a larger absolute number of TRP-2<sub>190–118</sub> CD3<sup>+</sup> CD8<sup>+</sup> splenocytes than regimens containing CpG ODN without IL-2, regimens containing IL-2 without CpG ODN, or regimens containing TRP-2<sub>190–118</sub> in IFA with neither CpG ODN nor IL-2 (n = 8–10/group). C, Mice were injected with 30,000 B16F1 cells on day 0. Mice were vaccinated with peptide plus CpG ODN in IFA vaccines and IL-2 was administered as described in A starting on day 0. One group received vaccines containing TRP-2<sub>190–118</sub> and another group received vaccines containing the negative control peptide OVA<sub>257–264</sub>. Aside from the different peptides, both groups were treated identically. There was a statistically significant difference (p < 0.006) at the indicated (*) time points (n = 12 mice/group). D, The antitumor effect observed with TRP-2<sub>190–118</sub> plus CpG ODN in IFA vaccines administered with IL-2 is dependent on CD8<sup>+</sup> cells. Mice were Ab depleted of CD8<sup>+</sup> cells or treated with isotype-matched control Abs. The mice were then challenged with B16F1 cells on day 0. All mice received TRP-2<sub>190–118</sub> plus CpG ODN-containing vaccines and IL-2 as described in A. Tumor growth was inhibited in control mice compared with CD8-depleted mice. A statistically significant difference (p < 0.001) occurred at the indicated (*) time points (control n = 12, CD8-depleted n = 10).
A

10 μM Peptide Concentration

% CD8^+ T cells specific

Vaccine Category

B

0.01 μM Peptide Concentration

% CD8^+ T cells specific

Vaccine Category

C

0.001 μM Peptide Concentration

% CD8^+ T cells specific

Vaccine Category

D

TRP-2_180–188 Concentration

% CD8^+ T cells specific

TRP-2_180–188

*CD8^+ T cell responses were measured by stimulating splenocytes from vaccinated mice ex vivo using EL4 cells pulsed with either 10 μM (A), 0.01 μM (B), or 0.001 μM (C) TRP-2_180–188 peptide and then performing intracellular staining for IFN-γ. D. The normalized percentage of CD8^+ T cells that were TRP-2_180–188 specific was defined as the percentage of the maximum CD8^+ T cell response for each vaccine category that was elicited by APCs pulsed with either 10, 0.01, or 0.001 μM TRP-2_180–188 Peptide. The maximum response for each vaccine category was defined as the response elicited by APCs pulsed with 10 μM TRP-2_180–188 peptide. Equal numbers of splenocytes from each of four mice in each vaccine category were combined for this experiment. All mice were vaccinated with TRP-2_180–188 plus CpG ODN vaccines and all mice received IL-2 as described in Fig. 4A. This was one of two experiments with nearly identical results.

FIGURE 5. TRP-2_180–188-specific CD8^+ T cell responses were measured by stimulating splenocytes from vaccinated mice ex vivo using EL4 cells pulsed with either 10 μM (A), 0.01 μM (B), or 0.001 μM (C) TRP-2_180–188 peptide and then performing intracellular staining for IFN-γ. D. The normalized percentage of CD8^+ T cells that were TRP-2_180–188 specific was defined as the percentage of the maximum CD8^+ T cell response for each vaccine category that was elicited by APCs pulsed with either 10, 0.01, or 0.001 μM TRP-2_180–188 Peptide. The maximum response for each vaccine category was defined as the response elicited by APCs pulsed with 10 μM TRP-2_180–188 peptide. Equal numbers of splenocytes from each of four mice in each vaccine category were combined for this experiment. All mice were vaccinated with TRP-2_180–188 plus CpG ODN vaccines and all mice received IL-2 as described in Fig. 4A. This was one of two experiments with nearly identical results.

Tumor therapy experiments

B16F1 cells were rapidly proliferating with a viability of >95% at the time of injection. The cells were kept on ice and mixed well before each injection. Mice were injected with either 100,000 (see Figs. 1 and 3) or 30,000 (see Figs. 4 and 7) tumor cells s.c. on the right side. For each tumor therapy experiment, mice were injected with tumor cells and then randomly distributed to either the treatment group or the control group. Mice then received vaccination regimens that included either TRP-2_180–188 (treatment group) or OVA257–264 (control group). In tumor therapy experiments using the TC-1 tumor cell line, mice were injected with 50,000 tumor cells s.c. on the right side 3 days before the initiation of vaccination regimens. The mice then received vaccination regimens as described in Results. Tumor size was measured with calipers every 3 days starting 10–13 days after tumor injection. The longest length and the length perpendicular to the longest length were multiplied to obtain the tumor size (area) in mm². When the tumor size reached 200 mm² or ulceration developed, the mice were sacrificed. Some experiments assessed therapy of B16F1 in mice that had been depleted of CD8^+ cells. Five hundred micrograms of the anti-CD8 Ab 2.43 (obtained from the National Cell Culture Center) were injected on days −3, −2, −1, 6, and 14 with day 0 being the day of tumor injection and initiation of vaccination. This regimen eliminated >99% of CD8^+ splenocytes. In experiments that used 2.43 for CD8 depletion, a control group was included that received injections of ChromPure rat IgG (Jackson ImmunoResearch Laboratories) in place of 2.43.

Statistical analysis

Groups were compared using the two-tailed Mann-Whitney U test. In cases where four groups are compared (see Figs. 2, D and E, and 4, A and B), p < 0.01 should be considered statistically significant in accordance with the Bonferroni correction. In all other cases, p < 0.05 should be considered statistically significant. In all graphs, the mean and the SEM are shown. Unless otherwise stated, two to three experiments of each type were conducted and the results of all experiments were combined for graphical presentation and statistical analysis.
Results

Vaccines containing TRP-2<sub>180–188</sub> and CpG ODN can inhibit growth of B16F1 only when exogenous IL-2 is provided

In preliminary experiments, we confirmed that vaccines containing TRP-2<sub>180–188</sub>, HBC<sub>128–140</sub>, and CpG ODN in IFA were consistently capable of eliciting CD8<sup>+</sup> T cell responses against TRP-2<sub>180–188</sub>. Because we were interested in studying CD8<sup>+</sup> T cell responses, we designed our tumor therapy experiments to detect epitope-specific differences in tumor growth. We injected two groups of mice with the TRP-2-expressing melanoma B16F1. We administered vaccines consisting of TRP-2<sub>180–188</sub>, CpG ODN, and HBC<sub>128–140</sub> emulsified in IFA to one of the groups, and we administered vaccines containing the same ingredients, except that a negative control peptide, OVA<sub>257–264</sub>, replaced TRP-2<sub>180–188</sub>, to the second group. Aside from the different peptides, both groups were treated identically. Tumor growth was not inhibited by vaccination with TRP-2<sub>180–188</sub> compared with vaccination with OVA<sub>257–264</sub> (Fig. 1A). In contrast, when we added IL-2 to the same peptide plus CpG ODN vaccination regimen, epitope-specific inhibition of B16F1 growth occurred (Fig. 1B). Although CpG ODN is known to inhibit growth of B16F1 as a single agent (14), all of our experiments were performed by vaccinating one group of mice with vaccines containing TRP-2<sub>180–188</sub> plus CpG ODN and simultaneously vaccinating a second group with vaccines containing OVA<sub>257–264</sub> plus CpG ODN. Because both groups were treated identically aside from the difference in peptide, the difference in tumor growth between mice that received vaccines containing TRP-2<sub>180–188</sub> and those that received vaccines containing OVA<sub>257–264</sub> could be concluded to be epitope specific and not due to the activation of non-epitope-specific effector cells such as NK cells. Both CpG ODN and IL-2 were required for epitope-specific tumor growth inhibition, because epitope-specific tumor growth inhibition did not occur when mice were vaccinated with TRP-2<sub>180–188</sub> in IFA without CpG ODN and given IL-2 (Fig. 1C). Because the tumor growth inhibition observed with TRP-2<sub>180–188</sub> plus CpG ODN vaccination combined with IL-2 was epitope specific, we hypothesized that IL-2 caused an increase in TRP-2<sub>180–188</sub>-specific CD8<sup>+</sup> T cells.

Synergism between CpG ODN and IL-2 leads to a striking enhancement of TRP-2<sub>180–188</sub>-specific CD8<sup>+</sup> T cell responses

Administration of vaccines consisting of TRP-2<sub>180–188</sub>, HBC<sub>128–140</sub>, and CpG ODN in IFA combined with systemic IL-2 resulted in large CD8<sup>+</sup> T cell responses that were specific for TRP-2<sub>180–188</sub> (Fig. 2A). In mice vaccinated with TRP-2<sub>180–188</sub> plus CpG ODN-containing vaccines and given IL-2, a mean of...
18.3% of CD3⁺CD8⁺ splenocytes produced IFN-γ after a 6-h ex vivo peptide stimulation with TRP-2₁₈₀₋₁₈₈, but only 0.1% of CD3⁺CD8⁺ splenocytes produced IFN-γ in response to the negative control peptide OVA₂₅₇₋₂₆₄; therefore, the mean TRP-2₁₈₀₋₁₈₈-specific CD8⁺ T cell response was 18.2% of CD3⁺CD8⁺ splenocytes (Fig. 2B). Naïve mice tested with the same assay had 0.01% of CD3⁺CD8⁺ splenocytes specific for TRP-2₁₈₀₋₁₈₈ (data not shown). To assess the roles of IL-2 and CpG ODN in the vaccination regimen, we eliminated either IL-2 or CpG ODN from the regimen of TRP-2₁₈₀₋₁₈₈ plus CpG ODN-containing vaccines administered with systemic IL-2.

Mice were vaccinated and received IL-2 or control injections as described in A. ICCS assay was performed on peripheral blood cells 5 days after the final vaccination in the same manner that it was performed on splenocytes in all other experiments (n = 8 mice/group). E. Mice received TRP-2₁₈₀₋₁₈₈ plus CpG ODN in IFA priming vaccinations on days 0, 3, and 6 and a boost vaccination on day 14. All mice were treated with IL-2 on days 7–9 and 15–18. One group of mice received vaccines that contained the HBC₁₂₈₋₁₄₀ “helper epitope” and another group was treated identically except that the HBC₁₂₈₋₁₄₀ epitope was omitted. When the two groups were assessed by ICCS assay 30 days after the boost vaccination, there was not a significant difference in the percentage of CD₈⁺ T cells specific for TRP-2₁₈₀₋₁₈₈ or in the absolute number of TRP-2₁₈₀₋₁₈₈-specific CD₈⁺ T cells (F) (n = 8 mice/group).
that received vaccines containing TRP-2_{180–188} plus CpG ODN combined with IL-2 had a mean absolute number of 5.6 × 10^6 TRP-2_{180–188}-specific CD3^+ T cell responses measured by ICCS assay. Vaccines containing both CpG ODN and IL-2 elicited larger TRP-2_{180–188}-specific CD8^+ T cell responses as a percentage of total CD3^+CD8^+ splenocytes than regimens containing CpG ODN without IL-2, regimens containing IL-2 without CpG ODN, or regimens containing CPP-TRP-2 in IFA with neither CpG ODN nor IL-2 (n = 8/group). B. In the same mice described in A, vaccination regimens containing both CpG ODN and IL-2 generated a larger absolute number of TRP-2_{180–188}-specific CD3^+CD8^+ splenocytes than regimens containing CpG ODN without IL-2, regimens containing IL-2 without CpG ODN, or regimens containing CPP-TRP-2 in IFA with neither CpG ODN nor IL-2 (n = 8/group).

B16F1 tumor-bearing mice generate TRP-2_{180–188}-specific CD8^+ T cell responses only when vaccinated against TRP-2_{180–188} despite having tumors that express the TRP-2 protein. When CpG ODN was omitted from the vaccine regimen, but IL-2 was administered, a response was elicited in which 2.8% of CD3^+CD8^+ splenocytes were specific for TRP-2_{180–188} and the absolute number of CD3^+CD8^+TRP-2_{180–188}-specific splenocytes was 0.5 × 10^6 (Fig. 2, C–E).

Vaccination of tumor-bearing mice with TRP-2_{180–188} plus CpG ODN-containing vaccines administered with systemic IL-2 elicited strong TRP-2_{180–188}-specific CD8^+ T cell responses. Tumor-bearing mice that received identical regimens except for the replacement of TRP-2_{180–188} by OVA257–264 did not generate a TRP-2_{180–188}-specific response despite the presence of large B16F1 tumors that expressed TRP-2 (Fig. 2, C–E). B16F1 was confirmed to express mRNA for TRP-2 by RT-PCR (our unpublished data).

In experiments described so far, HBC_{128–140} a peptide that can elicit CD4^+ T cell responses that are capable of enhancing vaccine-elicited CD8^+ T cell responses (33), was included in all peptide plus CpG ODN vaccines. To assess the importance of HBC_{128–140} as a vaccine component, we vaccinated mice using TRP-2_{180–188} plus CpG ODN in IFA vaccines without including HBC_{128–140} and administered IL-2. The mean TRP-2_{180–188}-specific CD8^+ T cell response elicited by this regimen made up 32.1% of total CD8^+ T cells (Fig. 4A). Synergism between CpG ODN and IL-2 was evident when IL-2 was omitted from the vaccination regimen TRP-2_{180–188} plus CpG ODN (Fig. 4A). Synergism between CpG ODN and IL-2 was evident because when IL-2 was omitted from the vaccination regimen TRP-2_{180–188} plus CpG ODN in IFA vaccines (Fig. 4B). We found that the IL-2 administration schedule used in all of the experiments reported in this work, which consisted of moderate dose i.p. IL-2 administered after vaccinations, was superior to low-dose s.c. IL-2 administered at the same time as vaccinations at increasing TRP-2_{180–188}-specific CD8^+ T cell responses (data not shown).

Tumor growth inhibition mediated by TRP-2_{180–188} plus CpG ODN-containing vaccines combined with IL-2 is dependent on CD8^+ T cells

The finding that the tumor growth inhibition mediated by peptide plus CpG ODN-containing vaccines administered with IL-2 was dependent on the presence of the MHC class I-presented peptide TRP-2_{180–188} indicated that CD8^+ T cells were causing the anti-tumor response. To conclusively demonstrate this, we depleted CD8^+ cells from one group of mice and left another group undepleted. Next, we treated both groups with vaccines consisting of TRP-2_{180–188} plus CpG ODN in IFA and administered IL-2 to both groups. We found that tumor growth inhibition was dependent on CD8^+ T cells (Fig. 4D).

CD8^+ T cells elicited by a CpG ODN plus IL-2-containing vaccination regimen or vaccination regimens in which either CpG ODN or IL-2 were omitted have similar avidities

A vaccination regimen containing the combination of IL-2 plus CpG ODN elicited larger TRP-2_{180–188}-specific CD8^+ T cell responses than regimens in which either IL-2 or CpG ODN were omitted when T cell responses were measured using APCs pulsed with a wide range of TRP-2_{180–188} peptide concentrations (Fig. 5, A–C). The avidity of vaccine-elicited CD8^+ T cells has been defined as the percentage of the maximum peptide-specific CD8^+ T cell responses...
cell response elicited by APCs pulsed with graded concentrations of peptide (34). When measured in this manner, the avidity of CD8 T cells elicited by vaccination regimens containing both IL-2 and CpG ODN was not different from the avidity of CD8 T cells elicited by vaccination regimens containing only CpG ODN or only IL-2 (Fig. 5D).

CpG ODN plus E749–57-containing vaccines plus systemic IL-2 eradicate TC-1 tumor cells

To demonstrate the antitumor efficacy of CpG ODN-containing vaccines combined with IL-2 in a different tumor model, we used the TC-1 fibroblast tumor cell line. TC-1 expresses the human papillomavirus E7 protein (29). Amino acid 49–57 of the E7 protein (E749–57) form an immunogenic H-2 D b-presented epitope (11). Mice were injected with TC-1 tumor cells and then 3 days later they were treated with a vaccination regimen that included E749–57 plus CpG ODN in IFA vaccines and systemic IL-2. This regimen produced E749–57-specific CD8 T cell responses that were detected ex vivo by ICCS assay (Fig. 6A). These experiments also demonstrated a dramatic inhibition of tumor growth (Fig. 6B) and increased survival (Fig. 6C) in E749–57-vaccinated mice compared with mice that were treated identically except that a negative control peptide, NP366–374, replaced E749–57 in their vaccines. Ten of 13 mice that received the regimen consisting of E749–57 plus CpG ODN in IFA vaccines and systemic IL-2 continue to survive tumor-free >2 mo after injection of TC-1 cells.

The increase in vaccine-elicited TRP-2180–188-specific CD8 T cell responses caused by IL-2 persists for at least 21 days after the final vaccination

Tumor-bearing mice received TRP-2180–188 plus CpG ODN in IFA vaccines and were treated with IL-2 or control injections. TRP-2180–188-specific CD8 T cell responses were increased in mice that received IL-2 compared with mice that received control injections when TRP-2180–188-specific CD8 T cell responses were measured 21 days after the final vaccination (Fig. 7, A–C). All of the experiments described so far have measured splenic TRP-2180–188-specific CD8 T cell responses. Addition of IL-2 to TRP-2180–188 plus CpG ODN in IFA vaccines also caused a dramatic increase in peripheral blood TRP-2180–188-specific CD8 T cells (Fig. 7D).

A “helper epitope” did not enhance TRP-2180–188-specific memory CD8 T cell responses generated by CpG plus IL-2-containing vaccination regimens

Experiments were conducted to directly assess the importance of the HBC128–140 “helper epitope” in long-term TRP-2180–188-specific memory CD8 T cell responses. These experiments did not demonstrate a significant difference between mice that received vaccines containing HBC128–140 or not containing HBC128–140 in the percentage of CD8 T cells specific for TRP-2180–188 (Fig. 7E) or the absolute number of TRP-2180–188-specific CD8 T cells (Fig. 7F) 30 days after the final vaccination.

Synergism between CpG ODN and IL-2 is evident when mice are vaccinated with long CPP

To demonstrate that synergism between CpG ODN and IL-2 occurs with vaccination using immunogens other than minimal MHC class I-binding peptides, we replaced the minimal MHC-binding peptides used in the rest of our experiments with a 21-aa peptide that is made up of a cell-penetrating moiety and the TRP-2180–188 peptide. This peptide is referred to as CPP-TRP-2 (32). We chose to use this peptide because it can penetrate the cell membrane of...
dendritic cells and then be presented on the dendritic cell surface
where it can be recognized by CD8+ T cells (32).

Consistent with our previous findings, strong synergism be-
tween CpG ODN and IL-2 at enhancing vaccine-elicited CD8+
T cell responses was evident when vaccines contained the CPP-
TRP-2 peptide (Fig. 8). These results suggest that synergism be-
tween CpG ODN and IL-2 can increase CD8+ T responses elicited
by intracellularly processed Ags.

Adding systemic IL-2 to TRP-2180–188 plus CpG ODN
containing vaccines increases the number of CD8+ T cells
with TCR capable of binding TRP-2180–188-Kb tetramers

Our findings demonstrated that addition of IL-2 to TRP-2180–188
plus CpG ODN-containing vaccines dramatically increased CD8+
T cell responses as measured by intracellular cytokine staining for
IFN-γ. Because this is a functional assay, the increase in TRP-
2180–188-specific CD8+ T cells could have been due to an increase
in the number of cells bearing TCRs capable of recognizing TRP-
2180–188 or the addition of IL-2 could have increased the fraction
of TRP-2180–188-specific CD8+ T cells that could produce IFN-γ
without actually increasing the number of T cells bearing TCR that
could recognize TRP-2180–188. We hypothesized that addition of
IL-2 increased the number of CD8+ T cells with TCR that recog-
nized TRP-2180–188. To test this hypothesis, we vaccinated two
groups of mice with TRP-2180–188 plus CpG ODN in IFA and
administered IL-2 to one of the groups and control injections to
the other group. We measured vaccine-elicited, TRP-2180–188-specific
CD8+ T cell responses directly ex vivo with TRP-2180–188-Kb
tetramers (Fig. 9A). Addition of IL-2 to TRP-2180–188 plus CpG
ODN vaccines increased the number of CD8+ splenocytes capable
of binding TRP-2180–188-Kb tetramers as a percentage of total
CD8+ splenocytes (Fig. 9B) or as an absolute number of CD8+
splenocytes (Fig. 9C). These data demonstrate that addition of IL-2
to TRP-2180–188 plus CpG ODN-containing vaccines increased the number of CD8+ T cells with TCR that recognized TRP-2180–188. Addition of IL-2 to TRP-2180–188 plus CpG ODN in IFA vaccines
causd a 32.8-fold increase in the mean percentage of CD8+ splenocytes
that were TRP-2180–188-specific as measured by direct
ex vivo analysis with TRP-2180–188-Kb tetramers (Fig. 9B). The
same mice in which TRP-2180–188-specific CD8+ T cell responses

**FIGURE 10.** A, Representative examples are shown of ex vivo OVA257–
264-Kb tetramer binding to CD8+ T cells from mice that received OVA257–264 plus
CpG ODN in IFA priming vaccines on days 0, 3, and 6 and a boost vaccination
on day 14. The mice received either IL-2 or control injections on days 7–9 and
15–18. OVA257–264-specific CD8+ T cell responses were measured with OVA257–
264-Kb tetramers on day 19. An example of OVA257–264-Kb tetramer binding to
cells from control-vaccinated mice that were vaccinated with gp10025–33 plus
CpG ODN in IFA and treated with IL-2 is also shown. The numbers on the plots
refer to the fraction of CD8+ splenocytes that bound OVA257–264-Kb tetramers. In
mice treated as described in A, IL-2 in-
creased the fraction (B) and the absolute number (C) of OVA257–264-specific
CD8+ splenocytes (with IL-2 n = 9, no IL-2 n = 10). D, Enhancement of RSV
M282–90 plus CpG ODN in IFA vaccination by IL-2 occurs in BALB/c mice.
Mice were vaccinated with RSV M282–90 plus CpG ODN in IFA on days 0, 3, 6,
and 14. The mice received either IL-2 or control injections on days 7–9 and
15–18. On day 19, RSV M282–90-specific CD8+ T cell responses were measured
by RSV M282–90-Kd pentamers and by ICCS assay. IL-2 increased RSV M282–
90-specific CD8+ T cell responses as measured by direct ex vivo quantitation
with RSV M282–90-Kd pentamers (with IL-2 and no IL-2 n = 10). E, Ex vivo
quantitation of RSV M282–90-specific CD8+ T cells by ICCS assay was per-
formed on the same mice described in D. The ICCS assay detected a similar in-
crease in vaccine-elicited RSV M282–90 specific responses as was detected by
the RSV M282–90-Kd pentamers when IL-2 was added to RSV M282–90 plus CpG
ODN in IFA vaccines.
were measured using tetramers were also assessed by ICCS assay for TRP-2\textsubscript{180–188}-specific IFN-γ production. As measured by ICCS assay, a 12.6-fold increase in the mean percentage of CD8\textsuperscript{+} splenocytes that were TRP-2\textsubscript{180–188} specific occurred when IL-2 was added to TRP-2\textsubscript{180–188} plus CpG ODN in IFA vaccines (Fig. 9D). Because a greater increase in TRP-2\textsubscript{180–188}-specific CD8\textsuperscript{+} T cell responses with addition of IL-2 to TRP-2\textsubscript{180–188} plus CpG ODN-containing vaccines was detected when responses were measured by direct binding to tetramers than by the ICCS assay for IFN-γ, we concluded that most of the increase in TRP-2\textsubscript{180–188}-specific CD8\textsuperscript{+} T cell responses detected by the ICCS assay was due to an increase in the number of CD8\textsuperscript{+} T cells with TCR that recognized TRP-2\textsubscript{180–188} and not to functional enhancement leading to increased IFN-γ production.

TRP-2\textsubscript{180–188} is a self-Ag. To determine whether addition of IL-2 to peptide plus CpG ODN-containing vaccines could also enhance responses to foreign Ags, we vaccinated two groups of mice with OVA\textsubscript{257–264} plus CpG ODN in IFA and administered IL-2 to one of the groups and control injections to the other group. We measured vaccine-elicited, OVA\textsubscript{257–264}-specific CD8\textsuperscript{+} T cell responses directly ex vivo with OVA\textsubscript{257–264}-K\textsuperscript{b} tetramers (Fig. 10A). Addition of IL-2 to the OVA\textsubscript{257–264} plus CpG ODN-containing vaccines increased the number of CD8\textsuperscript{+} splenocytes capable of binding OVA\textsubscript{257–264}-K\textsuperscript{b} tetramers as a percentage of total CD8\textsuperscript{+} splenocytes (Fig. 10B) or as an absolute number of CD8\textsuperscript{+} splenocytes (Fig. 10C).

Administration of IL-2 to BALB/c mice vaccinated with peptide plus CpG ODN-containing vaccines dramatically enhances epitope-specific CD8\textsuperscript{+} T cell responses

A series of experiments was conducted in which BALB/c mice were vaccinated with a peptide from the respiratory syncytial virus M2 protein (RSV M\textsubscript{282–290}) emulsified in IFA with CpG ODN. These experiments extend our previous results because the BALB/c strain is quite different in cytokine production and other immune characteristics from the C57BL/6 mice used in the rest of our experiments (35). Addition of IL-2 to RSV M\textsubscript{282–290} plus CpG ODN-containing vaccines caused a 6.8-fold increase in the mean percentage of CD8\textsuperscript{+} splenocytes that were RSV M\textsubscript{282–290} specific as measured by direct ex vivo analysis with RSV M\textsubscript{282–290}-K\textsuperscript{b} pentamers (Fig. 10D) and a 5.1-fold increase in the mean percentage of CD8\textsuperscript{+} splenocytes that were RSV M\textsubscript{282–290}-specific of as measured by ICCS assay (Fig. 10E). These data demonstrated that the increase in RSV-M2\textsubscript{282–290}-specific CD8\textsuperscript{+} T cells detected by ICCS assay when IL-2 was added to RSV M\textsubscript{282–290} plus CpG ODN-containing vaccines was due to an increase in the number of CD8\textsuperscript{+} T cells with TCR capable of recognizing the peptide included in the vaccine, because equivalent increases in RSV-M2\textsubscript{282–290}-specific CD8\textsuperscript{+} T cells were detected by pentamer binding and by ICCS assay for IFN-γ.

**Generation of epitope-specific CD8\textsuperscript{+} T cell responses by peptide plus CpG ODN-containing vaccines combined with exogenous IL-2 depends on endogenous IL-6**

Because CpG ODN have been shown to make conventional CD4\textsuperscript{+} T cells resistant to the suppressive effects of Tregs by an IL-6-dependent mechanism (15), we hypothesized that IL-6 could be important in generating the large CD8\textsuperscript{+} T cell responses elicited by peptide plus CpG ODN-containing vaccines combined with exogenous IL-2. When we administered OVA\textsubscript{257–264} plus CpG ODN in IFA vaccines and IL-2 to wild-type mice and IL-6-deficient mice, OVA\textsubscript{257–264}-specific responses were attenuated in the IL-6-deficient mice (Fig. 11A). Type I IFNs have been shown to be important in generation of CD8\textsuperscript{+} T cell responses by CpG ODN-containing vaccines (36). When we administered RSV M\textsubscript{282–290} plus CpG ODN in IFA vaccines and IL-2 to wild-type mice and to type I IFN-deficient mice, the vaccine-elicited RSV M\textsubscript{282–290}-specific responses were not different (Fig. 11B).

**Discussion**

We have demonstrated for the first time that CpG ODN and IL-2 synergize to dramatically increase the magnitude of peptide-vaccine-elicited CD8\textsuperscript{+} T cell responses (Figs. 2, 4, 7–10). The absolute number of epitope-specific CD8\textsuperscript{+} T cells increased up to 221-fold when exogenous IL-2 was added to a peptide plus CpG ODN vaccination regimen (Figs. 2E, 4B, 7C, 9C, and 10C). Synergism between CpG ODN and IL-2 increased epitope-specific CD8\textsuperscript{+} T cell responses as measured by a functional assay for IFN-γ (Figs. 2, 4, 7, 9D, and 10E), or as measured by direct ex vivo binding of the epitope-specific CD8\textsuperscript{+} T cells to peptide-MHC multimers (Figs. 9, A–C, and 10, A–D). The increase in vaccine-elicited CD8\textsuperscript{+} T cells caused by addition of IL-2 to TRP-2\textsubscript{180–188} plus CpG ODN-containing vaccines persisted for at least 3 wk after the last vaccination (Fig. 7, A–C). Peptide plus CpG ODN vaccines administered with IL-2 generated epitope-specific CD8\textsuperscript{+} T cell responses by a mechanism that involved endogenous IL-6 (Fig. 11A).
Addition of IL-2 to TRP-2180–188 plus CpG ODN vaccines increased the number of TRP-2180–188–specific CD8+ T cells by a greater degree when measured by TRP-2180–188–Kb tetramers than when measured by the ICCS assay for IFN-γ production (Fig. 9). Moreover, addition of IL-2 to RSV M2 82–90 plus CpG ODN vaccines increased the number of RSV M2 82–90–specific CD8+ T cells by a similar degree when measured by direct ex vivo binding to RSV M2 82–90–Kb pentamers or when measured by ICCS assay (Fig. 10). Taken together, these data demonstrate that the main effect of IL-2 is an increase in the number of epitope-specific CD8+ T cells rather than an enhancement of functional status manifested by an enhanced ability to produce IFN-γ.

We observed epitope-specific inhibition of tumor growth by TRP-2180–188 plus CpG ODN in IFA vaccines only when the vaccines were combined with IL-2 (Fig. 1B). Vaccination with TRP-2180–188 plus CpG ODN in IFA without IL-2 was ineffective at mediating epitope-specific inhibition of B16F1 growth (Fig. 1A). In addition, TRP-2180–188 in IFA vaccines without CpG ODN did not cause epitope-specific tumor growth inhibition when combined with systemic IL-2 (Fig. 1C). Tumor growth inhibition was demonstrated to be dependent on CD8+ T cells (Fig. 4D), and TRP-2180–188 plus CpG ODN vaccines combined with IL-2 generated strikingly increased numbers of TRP-2180–188–specific CD8+ T cells compared with regimens with either CpG ODN or IL-2 omitted (Figs. 2, C–E, 4, A and B, and 7). These data make the increased number of TRP-2180–188–specific CD8+ T cells with addition of IL-2 to TRP-2180–188 plus CpG ODN-containing vaccines the most likely reason that epitope-specific tumor growth inhibition was only observed when TRP-2180–188 plus CpG ODN vaccines were combined with IL-2.

Our tumor therapy experiments used wild-type mice and the highly aggressive, poorly immunogenic, TRP-2-expressing B16F1 melanoma. When B16F1 tumor-bearing mice were vaccinated with the negative control peptide OVA257–264 and CpG ODN in IFA and received systemic IL-2, TRP-2180–188–specific CD8+ T cell responses were not elicited. Generation of TRP-2180–188–specific CD8+ T cell responses depended on the presence of TRP-2180–188 in vaccines (Fig. 3). This result shows that TRP-2180–188–specific CD8+ T cell responses were not generated by direct priming by B16F1 or cross-priming by tumor-infiltrating dendritic cells and confirms that B16F1 is a very poorly immunogenic tumor that is a difficult test for any CD8+ T cell-mediated therapy.

To our knowledge, the CD8+ T cell responses elicited by TRP-2180–188 plus CpG ODN-containing vaccines administered with IL-2 are the largest ever measured against this epitope by an ex vivo assay. The CD8+ T cell responses elicited by vaccines containing TRP-2180–188 plus CpG ODN administered with IL-2 were larger in magnitude than the responses elicited against this epitope by a whole tumor cell vaccine combined with CTLA-4 blockade and Treg depletion (37). The magnitude of responses obtained by vaccination with TRP-2180–188 plus CpG ODN-containing vaccines administered with IL-2 were larger than those elicited by recombinant viral vaccines encoding the TRP-2 protein (38).

Other investigators have shown that the same CpG ODN used in our work and CD40 agonists synergize to enhance CD8+ T cell responses elicited by OVA257–264 peptide vaccination. These investigators found that vaccine responses to the OVA257–264 peptide combined with CpG ODN and CD40 agonists were dependent on endogenous type I IFN (36). In contrast, we demonstrated that CD8+ T cell responses elicited by peptide plus CpG ODN-containing vaccines combined with IL-2 were not dependent on type I IFN (Fig. 8B).

Although one previous study has shown that the antitumor activity of a vaccine regimen containing CpG ODN was dependent on endogenous IL-6 (39), our work is the first to demonstrate that the magnitude of epitope-specific CD8+ T cell responses elicited by CpG ODN-containing vaccines is dependent on endogenous IL-6 (Fig. 11A). Other investigators have shown that the TLR ligands LPS and CpG ODN can make conventional CD4+ T cells resistant to the suppressive effects of Tregs by an IL-6-dependent mechanism. These same investigators went on to show an attenuation of CD4+ T cell responses elicited by a protein plus LPS plus IFA vaccine in IL-6-deficient mice. This attenuation of vaccine responses in IL-6-deficient mice was reversed by depletion of Tregs (15). Because CD8+ T cell responses elicited by peptide plus CpG ODN in IFA vaccines combined with exogenous IL-2 were attenuated in IL-6-deficient mice, IL-6 might also make CD8+ T cells resistant to suppression by Tregs.

One of the most important aspects of our work is the potential clinical relevance. It was recently shown in a clinical trial that the type-B CpG ODN, CpG 7909, dramatically increased CD8+ T cell responses when added to a Melan-A peptide in IFA vaccine (12). The CD8+ T cell responses detected in this clinical trial were some of the largest vaccine-elicited CD8+ T cell responses ever reported in humans. Systemic side effects in patients that received Melan-A peptide plus CpG 7909 in IFA vaccines were minimal. Like CpG 7909, the CpG ODN used in our work is also a type-B CpG ODN (13, 14). Our work suggests that addition of short courses of low to moderate doses of IL-2 to peptide plus CpG ODN 7909 vaccines might cause dramatic increases in the magnitude of vaccine-elicited CD8+ T cell responses and might enhance the antitumor efficacy of these vaccines. The doses of IL-2 administered in our work were substantially less than those used in some other murine models (1, 24). In addition, synergism between CpG ODN and IL-2 is still evident at lower doses of both reagents (J. D. Kochenderfer, C. D. Chien, J. L. Simpson, and R. E. Gress, manuscript in preparation). Because IL-2 is already an approved drug, an extensive experience with dosages, administration schedules, and potential side-effects has been accumulated (5, 22, 40). A clinical trial of peptide plus CpG 7909 vaccination combined with IL-2 is feasible. In addition to peptide vaccines, other types of vaccines have been combined with CpG ODN (39, 41). IL-2 might be added to these vaccination regimens to determine whether the combination of CpG ODN and IL-2 could enhance vaccine-elicited, Ag-specific T cell responses. Our results demonstrating dramatic enhancement of CD8+ T cell responses against the viral epitope RSV M2 82–90 show that peptide plus CpG ODN vaccines combined with exogenous IL-2 can elicit large CD8+ T cell responses against viral Ags (Fig. 10). Combining CpG ODN and IL-2 might be a useful strategy for generating antiviral T cell responses.

In conclusion, IL-2 synergizes with CpG ODN to dramatically increase vaccine-elicited CD8+ T cell responses. TRP-2180–188 plus CpG ODN in IFA vaccination was not an effective therapy for B16F1 melanoma unless IL-2 was also administered. Vaccine responses elicited by peptide plus CpG ODN vaccination combined with exogenous IL-2 were dependent on endogenous IL-6. Finally, addition of exogenous IL-2 to clinical antitumor and antiviral vaccination strategies that include CpG ODN is a promising area for future research.

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Disclosures

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