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Stimulation by Soluble CD70 Promotes Strong Primary and Secondary CD8+ Cytotoxic T Cell Responses In Vivo

Tania F. Rowley and Aymen Al-Shamkhani

Identification of the signals required for optimal differentiation of naive CD8+ T cells into effector and memory cells is critical for the design of effective vaccines. In this study we demonstrate that CD27 stimulation by soluble CD70 considerably enhances the magnitude and quality of the CD8+ T cell response. Stimulation with soluble CD70 in the presence of Ag significantly enhanced the proliferation of CD8+ T cells and their ability to produce IL-2 and IFN-γ in vitro. Administration of Ag and soluble CD70 resulted in a massive (>300-fold) expansion of Ag-specific CD8+ T cells in vivo, which was due to the enhanced proliferation and survival of activated T cells. In mice that received Ag and soluble CD70, CD8+ T cells developed into effectors with direct ex vivo cytotoxicity. Furthermore, unlike peptide immunization, which resulted in a diminished response after rechallenge, CD27 stimulation during the primary challenge evoked a strong secondary response upon rechallenge with the antigenic peptide. Thus, in addition to increasing the frequency of primed Ag-specific T cells, CD27 signaling during the primary response instills a program of differentiation that allows CD8+ T cells to overcome a state of unresponsiveness. Taken together these results demonstrate that soluble CD70 has potent in vivo adjuvant effects for CD8+ T cell responses. The Journal of Immunology, 2004, 172: 6039–6046.

The ability of T cells to elicit an effective immune response is governed by signals generated through recognition of Ag/MHC by the TCR and the interaction of costimulatory molecules with their ligands on APCs (1–6). By delivering signals that synergize with those initiated by the TCR, costimulatory molecules positively regulate various aspects of T cell activation, including T cell proliferation, cytokine production, and cell survival (1–7). In addition, costimulation may influence the T cell response to a subsequent antigenic challenge. T cells that receive signals via the TCR in the absence of costimulation can enter a state of hyporesponsiveness, termed anergy, and as a result they fail to elicit a proliferative response upon Ag re-encounter (1–3, 8). Furthermore, the stimulatory effects of natural adjuvants, such as LPS, are more, the stimulatory effects of natural adjuvants, such as LPS, are...
with an antigenic peptide. Our results show that stimulation via CD27 has profound effects on both the magnitude and the quality of the CD8⁺ T cell response generated and suggest a novel strategy to boost CD8⁺ T cell responses in vivo.

Materials and Methods

Abs, reagents, and cells

OVA peptide 257–264 (OVA257–264) with the sequence SIINFEKL was obtained from Peptide Protein Research ( Fareham, U.K.). LPS from Salmonella minnesota was obtained from Sigma-Aldrich (Poole, U.K.). The hybridomas 1610A1 (anti-B7-1) and GL-1 (anti-B7-2) were obtained from American Type Culture Collection (Manassas, VA). The following hybridomas were generated in-house: SB2H2 (anti-human Fc), Mc39-16 (anti-A31 lymphoma Id), and AT65 (anti-BCL1 lymphoma Id). Normal human IgG (hlgG) was prepared in-house. Allophycocyanin-labeled anti-CD8α and an anti-Ki67/iso type control kit were obtained from BD Pharmingen (San Diego, CA). PE-labeled H-2Kb OVA257–264 tetramers were obtained from Proimmune (Oxford, U.K.). The EL4 T cell thymoma was maintained in vitro in RPMI 1640 and 10% FBS. E.G7, a derivative of EL4, which had been transfected with chicken OVA cDNA, was maintained in RPMI 1640 and 10% FBS with 0.4 mg/ml G418. Both tumor lines were obtained from American Type Culture Collection.

Generation of sCD70

A cDNA fragment corresponding to the extracellular domain of murine CD70 (aa residues 41–195) was amplified by PCR using a cDNA template prepared from Con A-activated mouse splenocytes. The PCR fragment was cloned downstream of a cDNA encoding a modified human IgG1 Fc region designed to express type II transmembrane proteins as soluble proteins. The human Fc-CD70 fragment was excised and then ligated into the pEE14 expression vector. The sCD70 was expressed in Chinese hamster ovary cell line/spleen cells (1 × 10⁶). The structure and purity were confirmed by SDS-PAGE and size exclusion chromatography.

Mice and in vivo experiments

OT-I TCR transgenic mice (26) crossed onto a recombinase-activating gene 1 knockout (RAG1−/−) background, were obtained from Dr. B. Stockinger (National Institute of Medical Research, London, U.K.), and homozygous OT-I TCR transgenic C57BL/6 mice were obtained from Dr. M. Merkenschlager (Imperial College, London, U.K.). Wild-type C57BL/6 mice were purchased from Harlan (Blackthorn, U.K.). For adoptive transfer of OT-I T cells to naive C57BL/6 recipients, a single-cell suspension of lymph node (inguinal, brachial, and mesenteric) and spleen cells was prepared from OT-I C57BL/6 mice, and the proportion of transgenic T cells was determined by K⁺ OVA257–264 tetramer and anti-CD8 staining. OT-I transgenic T cells (1 × 10⁶) were then transferred by i.v. injection into sex-matched C57BL/6 recipients. The transferred transgenic T cells had a naive phenotype (CD62Lhigh, CD44low, CD25−). Two or 3 days later, T cells were primed in vivo by i.v. administration of OVA257–264 (20 nmol) in combination with the following: LPS (10 μg), sCD70 (200 μg), or, as a control, hlgG (200 μg), as indicated in the figure legends. Soluble CD70 or hlgG was administered for an additional 2 days (two doses of 200 μg). Secondary stimulation was conducted by i.v. injection of OVA257–264 (20 nmol). Reagents were tested for LPS contamination and contained <0.05 ng of LPS/20 nmol of OVA257–264 or 200 μg of sCD70. All mice were used at ~8–12 wk of age. Animal experiments were conducted according to the U.K. Home Office license guidelines and were approved by the University of Southampton’s ethical committee.

Flow cytometry

For tracking Ag-specific T cells in vivo, blood samples (50 μl) or lymph node/spleen cells (1 × 10⁶) were stained with titrated amounts of PE-labeled H-2Kb OVA257–264 tetramers and allophycocyanin-labeled anti-CD8α for 30 min at room temperature. The cells were then washed, and the RBCs were lysed before analysis on a FACSCalibur (BD Biosciences, San Diego, CA).
Mountain View, CA) flow cytometry. To stain for intracellular expression of Ki67 proliferation Ag, PBL were first stained with tetramer and anti-CD8α as described above. Cells were fixed and permeabilized in 4% formaldehyde/0.1% saponin for 15 min at room temperature, washed, and then stained with anti-Ki67 FITC (BD Biosciences, San Diego, CA) or an isotype control Ab for 15 min at room temperature in the presence of 0.1% saponin before three-color analysis on a FACSCalibur. Seventy thousand live lymphocyte events were typically collected per sample.

CFSE dilution analysis
OT-I T cells from OT-I RAG1−/− mice were prepared by RBC lysis of splenocytes. Cells (2–4 x 10^7/ml) were incubated for 10 min at 37°C with 5 μM CFSE (Molecular Probes, Leiden, The Netherlands) in serum-free RPMI 1640 and then washed twice with RPMI 1640 and 10% FBS. OT-I T cells (2 x 10^5) were cultured for 72 h with various stimuli as indicated in the figure legends. Cells were then stained with anti-CD8α, and ~6000 viable CD8+ T cells analyzed per sample by flow cytometry. The scD70 was used at 10 μg/ml. To monitor T cell division in vivo, OT-I T cells were labeled with CFSE (1 μM) for 10 min in PBS containing 0.1% BSA. Cells were washed once with RPMI 1640 and 10% FBS and then twice with PBS. One million cells were administered by i.v. injection to C57BL/6 mice. Twenty-four hours later, mice received OVA257-264 (20 nmol) with sCD70 (200 μg) or hlgG as a control by i.v. injection. Mice were administered two further injections of scD70 or hlgG (200 μg). Some mice were left unstimulated. At various times after immunization, splenocytes were stained with PE-labeled H-2Kb OVA257-264 tetramers and allophycocyanin-labeled anti-CD8α. The level of CFSE in CD8+ tetramer− cells was then analyzed by flow cytometry.

[^3]H]thymidine incorporation and cytokine assays
OT-I T cells (2 x 10^3) from OT-I RAG1−/− mice were cultured in 0.2 ml in 96-well, U-bottom plates for a total period of 72 h in the presence of OVA257-264 peptide at the indicated concentrations or gamma-irradiated E.G7 cells (360 Gy) and either control hlgG or scD70 at a final concentration of 10 μg/ml. In some experiments anti-B7-1 and anti-B7-2 mAbs or isotype-matched control mAbs (anti-A31 and anti-BCL1Id) were also added (10 μg/ml). [Methyl-[^3]H]thymidine (0.5 μCi/well) was added to the cultures 16 h before harvesting. For cytokine analysis, cell supernatant was harvested after 48 h, and the levels of IL-2 and IFN-γ were determined by ELISA. Viable T cells were enumerated, and this value was used to calculate the amount of cytokine produced per 10^6 T cells.

Cytotoxicity assay
Ex vivo cytotoxicity of pooled splenocytes was measured on day 10 after Ag priming by a standard 51 Cr release assay. EL4 or E.G7 target cells (~1 x 10^7) were labeled with 0.1 mCi of 51 Cr (Amersham Pharmacia Biotech, Little Chalfont, U.K.) for 1 h at 37°C. EL4 cells were simultaneously pulsed with 5 μM OVA257-264 peptide. Cells were washed and 5 x 10^5 target cells incubated with the indicated ratio of effector cells for 4 h at 37°C. The percentage of 51 Cr released was calculated by: %Cr release = [(sample cpm − background cpm) / (total cpm − background cpm)] / 100, where background cpm represents spontaneous 51 Cr release from targets in medium alone. The value for total cpm was obtained by lysis of labeled cells by the addition of Nonidet P-40 (1%). Nonpeptide-pulsed EL4 targets were used as a control for nonspecific killing.

Results
Costimulatory effects of scD70 in vitro
We produced a soluble recombinant form of CD70 by fusing the RAG1−/− Ag priming by a standard 51 Cr release assay. EL4 or E.G7 target cells (~1 x 10^7) were labeled with 0.1 mCi of 51 Cr (Amersham Pharmacia Biotech, Little Chalfont, U.K.) for 1 h at 37°C. EL4 cells were simultaneously pulsed with 5 μM OVA257-264 peptide. Cells were washed and 5 x 10^5 target cells incubated with the indicated ratio of effector cells for 4 h at 37°C. The percentage of 51 Cr released was calculated by: %Cr release = [(sample cpm − background cpm) / (total cpm − background cpm)] / 100, where background cpm represents spontaneous 51 Cr release from targets in medium alone. The value for total cpm was obtained by lysis of labeled cells by the addition of Nonidet P-40 (1%). Nonpeptide-pulsed EL4 targets were used as a control for nonspecific killing.

We then examined the ability of scD70 to augment the proliferation of OT-I T cells following stimulation with different doses of the H-2Kb-restricted OVA257-264. Soluble CD70 enhanced the incorporation of [3H]thymidine by T cells, suggesting that T cells proliferate to a greater extent when stimulated by scD70 (Fig. 1B). To confirm that the increase in [3H]thymidine incorporation was due to an increase in the efficiency of cell division, OT-I T cells labeled with CFSE were stimulated with OVA257-264 in the presence of the absence of scD70, and cell division was measured by CFSE dilution. Soluble CD70 greatly increased the number of cells that had divided more than once during the 72-h incubation period (Fig. 1C). These results clearly demonstrate that CD27 signaling enhances the proliferation of CD8+ T cells. We also examined whether scD70 can stimulate T cells independently of CD28. We used blocking Abs directed against the CD28 ligands, B7-1 and B7-2, to block CD28 signaling. Incubation of OT-I splenocytes with OVA257-264 or with E.G7 cells that express OVA resulted in a dose-dependent proliferation of T cells that was inhibited significantly by the presence of anti-B7-1 and B7-2 mAbs (Fig. 1, D and E). Soluble CD70 enhanced T cell proliferation even in the presence of anti-B7-1 and B7-2 blocking mAbs, suggesting that scD70 triggers signaling independently of CD28 (Fig. 1, D and E). At low levels of OVA257-264 or low numbers of E.G7 cells, T cells proliferated to a greater extent when scD70 was present in the culture, and the B7-1/B7-2 interaction with CD28 was not blocked (Fig. 1, D and E). These results suggest that scD70 acts in a synergistic fashion with B7-1 and B7-2 to enhance the proliferation of T cells.

We then examined the effects of scD70 on the response of CD8+ T cells in vivo, we adoptively transferred OT-I T cells (10^6 cells) into normal syngeneic B6 mice, challenged them with OVA257-264 together with scD70 or hlgG as a control, and then monitored the kinetics of the response using Kb OVA257-264 tetramers. Experiments were initially conducted to determine the dose of OVA257-264 required to produce the maximum amount of clonal expansion (data not shown). After challenge with OVA257-264 and hlgG as a control, the percentage of OT-I T cells rose significantly, reaching a peak by day 4. When OVA257-264 was injected together with scD70, a much greater increase in the percentage of OT-I T cells was observed (Fig. 3A). When OVA257-264 was injected with

![FIGURE 2. Soluble CD70 enhances cytokine production. Naïve OT-I RAG1−/− splenocytes were stimulated with the indicated concentrations of OVA257-264 plus control hlgG or scD70 for 48 h. IFN-γ and IL-2 concentrations in culture supernatant were determined by ELISA. Viable T cells were enumerated, and this value was used to calculate the amount of cytokine produced per 10^6 T cells. The data are representative of two independent experiments, and error bars represent the SEM of triplicate cultures.](http://www.jimmunol.org/ether.php?his=6041)
sCD70, OT-I T cells expanded by >300-fold, which is in marked contrast with the 18-fold expansion observed in the absence of sCD70 (Table I). The majority of the OT-I T cells (>80%) at the peak of the response (day 4) were positive for the proliferation marker Ki67; however, the proliferation was not sustained, and by day 10 most cells had stopped dividing (Fig. 3B). Previous studies have demonstrated that the natural adjuvant LPS enhances the expansion and survival of activated T cells in vivo (27, 28).

We therefore compared the effects of sCD70 with those of LPS on expansion and survival of activated T cells in vivo (27, 28). We have demonstrated that LPS enhances the short term survival of OT-I T cells. This difference was observed after injection of sCD70 and OVA257–264, and CD8+ indicated (n = 3 mice/group, except the CD70 without Ag group, where n = 2). The data are representative of three similar experiments.

We therefore compared the effects of sCD70 with those of LPS on expansion and survival of activated T cells in vivo (27, 28). When sCD70 and LPS were injected together with OVA257–264, the magnitude of the response was similar to that observed after injection of sCD70 and OVA257–264 (Fig. 3A and Table I). The percentage of OT-I T cells at the peak of the response (day 4) in the group receiving the combination of LPS and sCD70 was not significantly different from that in the group receiving sCD70 alone. However, on days 6 and 10 the percentages of OT-I T cells in the mice that received OVA257–264 together with LPS and sCD70 were significantly (p < 0.003) higher than those that received OVA257–264 and sCD70 (Table I), suggesting that LPS enhances the short term survival of OT-I T cells. This difference was not maintained over time because the percentage of OT-I T cells in both groups was similar 23 days after antigenic challenge (Table

Table I. Primary expansion of naive OT-I CD8+ T cells is amplified by CD27 costimulation

<table>
<thead>
<tr>
<th>Days Post Primary Ag</th>
<th>CD70 (%)</th>
<th>OVA257-264 (%)</th>
<th>OVA257-264 + LPS (%)</th>
<th>OVA257-264 + CD70 (%)</th>
<th>OVA257-264 + CD70 + LPS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.13 ± 0.03</td>
<td>3.09 ± 0.86</td>
<td>6.37 ± 0.23</td>
<td>52.14 ± 5.93</td>
<td>64.24 ± 3.96</td>
</tr>
<tr>
<td>6</td>
<td>0.68 ± 0.20</td>
<td>0.25 ± 0.09</td>
<td>2.02 ± 0.45</td>
<td>10.63 ± 1.06</td>
<td>23.48 ± 0.35</td>
</tr>
<tr>
<td>10</td>
<td>0.10 ± 0.02</td>
<td>1.01 ± 0.45</td>
<td>4.31 ± 0.56</td>
<td>10.14 ± 0.72</td>
<td>23.48 ± 0.35</td>
</tr>
<tr>
<td>23</td>
<td>0.08 ± 0.01</td>
<td>1.38 ± 0.04</td>
<td>6.67 ± 0.1</td>
<td>6.99 ± 0.82</td>
<td>3.05 ± 0.68</td>
</tr>
<tr>
<td>Maximal fold expansion</td>
<td>0.8</td>
<td>18</td>
<td>37</td>
<td>307</td>
<td>378</td>
</tr>
</tbody>
</table>

*Experimental setup was as described in Fig. 3. Mean percentages of Ag-specific CD8+ T cells in peripheral blood over time with SE are indicated; n = 3 mice/group, except the sCD70 (without Ag) control group, where n = 2. Maximal fold expansion was calculated using the day 2 value of 0.17% (as the background) from mice given sCD70 only. The data are representative of three similar experiments.
I). Thus, the long term survival of CD70-stimulated OT-I cells was not enhanced by LPS.

**Effect of CD27 signaling on CD8⁺ T cell proliferation in vivo**

Administration of sCD70 together with OVA257–264 resulted in significant expansion of OT-I T cells in vivo (Fig. 3A and Table I). To address the mechanism by which sCD70 mediated its effects, we adoptively transferred CFSE-labeled OT-I T cells into C57BL/6 mice and examined their ability to proliferate after administration of OVA257–264 with sCD70 or OVA257–264 with hIgG as a control. Administration of OVA257–264 with sCD70 resulted in extensive proliferation of OT-I T cells as evident by the lower levels of CFSE compared with unstimulated cells (Fig. 4). The proliferation of OT-I T cells in mice that received OVA257–264 with hIgG was also efficient, although the levels of the CFSE label were consistently higher in these cells compared with cells stimulated with OVA257–264 and sCD70 (Fig. 4). The difference in CFSE levels between the two cell populations represents a difference of a single cell division. Thus, OVA257–264 plus sCD70-stimulated cells cycle only slightly faster than those stimulated with OVA257–264. As the numbers of OT-I T cells were 10-fold (day 3; data not shown) and 17-fold (day 4; Fig. 3A and Table I) higher in OVA257–264 plus sCD70-stimulated mice compared with mice stimulated with OVA257–264 and hIgG, our results show that sCD70 enhances T cell expansion only in part by enhancing T cell division. Although OT-I T cells stimulated with OVA257–264 and hIgG continued to proliferate between 56 and 68 h after injection of Ag, they failed to accumulate, most likely due to cell death, which is in contrast with OT-I cells stimulated with OVA257–264 and sCD70 (Fig. 4). Taken together these results suggest that CD27 signaling also promotes the survival of activated CD8⁺ T cells. Therefore, both enhanced proliferation and increased T cell survival contribute to the ability of sCD70 to promote clonal expansion of OT-I T cells in vivo.

**Soluble CD70 promotes the generation of CTLs**

To examine whether CD27 signaling in vivo enhances the generation of CD8⁺ effector T cells, we tested the ability of spleen cells from mice immunized with OVA257–264 or OVA257–264 and different combinations of LPS and sCD70, to kill target cells. To avoid any potential artifacts caused by in vitro restimulation we measured the activity of CTLs directly ex vivo. The ability of spleen cells to kill EL4 cells pulsed with OVA257–264 or EG7 cells, which express OVA, correlated well with the percentage of Ag-specific T cells present in the spleen 10 days after immunization (Fig. 5). Thus, specific cytotoxicity against target cells was easily measurable in spleen cells obtained from animals that were immunized with OVA257–264 and sCD70 or OVA257–264 with sCD70 and LPS (Fig. 5, A and B). In contrast, spleen cells obtained from animals that were immunized with OVA257–264 alone had no detectable cytotoxic activity, and those from animals immunized with OVA257–264 and LPS showed only low levels of cytotoxicity against target cells. Furthermore, when the percentage of cell killing was calculated on the basis of the actual ratio of OT-I T cells to target cells, no significant difference was observed among cells from mice that received LPS, sCD70, or sCD70 and LPS (data not shown). Overall these data suggest that sCD70 promotes the generation of CTLs most likely by enhancing the frequency of primed Ag-specific CD8⁺ T cells.

**CD27 signaling enhances secondary responses to Ag**

The massive expansion of OT-I T cells after injection of OVA257–264 and sCD70 was transient in nature and was followed by a rapid contraction phase. At the end of the contraction phase (day 10) OT-I T cells represented ~4% of the total lymphocytes, and this percentage remained the same on day 23 (Fig. 6A and Table I). Given that the number of cells that survive after the contraction phase is determined primarily by the magnitude of the primary response (29), it was not surprising that only low numbers of OT-I cells persisted in animals immunized with OVA257–264 in the absence of sCD70 (Fig. 6A and Table I). As injection of antigenic peptides can lead to T cell unresponsiveness (30–32), it was important to address whether the surviving OT-I T cell population is capable of eliciting an effective response after secondary challenge with Ag. Upon restimulation with OVA257–264 on day 24, mice that were previously immunized with OVA257–264 and sCD70 responded with a marked increase in the percentage of OT-I T cells in blood and secondary lymphoid organs (Fig. 6, A and B). An ~8-fold expansion of Ag-specific T cells was observed in the secondary response of mice that were previously immunized.

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**FIGURE 4.** The effect of CD27 signaling on T cell proliferation in vivo. Representative histogram plots of CFSE-labeled cells gated on CD8⁺ Kb OVA257–264 tetramer⁺ lymphocytes. CFSE-labeled OT-I T cells were transferred into C57BL/6 recipients, and mice were primed 24 h later by i.v. injection of OVA257–264 plus sCD70 (□) or OVA257–264 plus hIgG as a control (●). At various times after Ag administration (52 and 68 h) spleen cells were stained with Kb OVA257–264 tetramers and anti-CD8 and were analyzed by flow cytometry. Unstimulated mice (top histogram) were analyzed 4 days after adoptive transfer. The mean fluorescence intensity (MFI) of the CFSE peaks for unstimulated cells or cells stimulated with OVA257–264 plus hIgG (OVA257–264) or OVA257–264 plus sCD70 (OVA257–264 + sCD70) is indicated. The data are representative of two independent experiments.
with OVA<sub>257-264</sub> and sCD70 (Fig. 6C). In contrast, Ag-specific T cells from animals immunized with OVA<sub>257-264</sub> alone or OVA<sub>257-264</sub> and LPS showed minimal expansion (<2-fold) after secondary challenge with OVA<sub>257-264</sub> (Fig. 6C). Thus, in addition to increasing the frequency of primed Ag-specific T cells, CD27 signaling during the primary response programs naive T cells to

FIGURE 5. CD27 stimulation enhances the number of cytotoxic CD8<sup>+</sup> effectors generated during the primary response. The experimental setup was as described in Fig. 3. Ex vivo cytotoxicity was measured on day 10 post-Ag administration by <sup>51</sup>Cr release from target cells. Pooled splenocytes (n = 3 mice/group) from each group were incubated at the indicated ratios with OVA<sub>257-264</sub>-pulsed EL4 (A) or E.G7 (B) targets. □, Nonspecific killing of nonpulsed EL4 targets. Error bars represent the SEM of triplicate cultures.

FIGURE 6. CD27 stimulation during the primary response enhances the responsiveness of CD8<sup>+</sup> T cells after secondary challenge with Ag. The experimental setup was described in Fig. 3. A. The percentage of Ag-specific CD8<sup>+</sup> T cells in peripheral blood was tracked over time by double staining with K<sup>b</sup> OVA<sub>257-264</sub> tetramers and anti-CD8. Twenty-four days after primary Ag stimulation all groups were given a secondary stimulation of OVA<sub>257-264</sub> by i.v. injection, and Ag-specific CD8<sup>+</sup> T cells were tracked for an additional 20 days. The arrow indicates OVA<sub>257-264</sub> Ag administration. Error bars represent the SEM (n = 3–6 mice/time point). B, Percentage of Ag-specific CD8<sup>+</sup> T cells in the spleen and lymph nodes (inguinal and brachial) on day 44 (20 days after secondary Ag exposure; n = 3–6 mice). C, The maximal fold expansion of Ag-specific CD8<sup>+</sup> T cells during the secondary response was calculated using the mean percentage of Ag-specific CD8<sup>+</sup> T cells in peripheral blood on day 23 after primary Ag stimulus as the background. The response in mice that were given sCD70 or hIgG alone during the primary stage of stimulation and are therefore naive is also shown (−). The data are representative of two similar experiments.
overcome T cell unresponsiveness during secondary exposure to Ag.

Discussion

The data presented in this study demonstrate a dual role for the CD27-CD70 interaction in the generation of CD8+ T cell responses in vivo. CD27 signaling enhanced both the magnitude of the T cell response, by augmenting the proliferation/survival of CD8+ T cells (Figs. 3 and 4), and the quality of the response, such that CD8+ T cells that respond effectively upon secondary Ag encounter are generated (Fig. 6). It has been suggested that the differentiation of naive CD8+ T cells into effector and memory cells is a progressive process that requires multiple stimulatory signals (33). Although it is generally accepted that activated APCs, such as mature DCs, are capable of delivering signals that drive the progressive differentiation of T cells (33, 34), the molecular nature of these signals is not fully known. Activation of naive CD8+ T cells in the presence of B7-1 and ICAM-1 is not sufficient to drive their complete differentiation. Thus, stimulation of naive CD8+ T cells via TCR, CD28, and LFA-1 results in clonal expansion; however, after 3–4 days the cells lose their capacity to proliferate in response to Ag, but retain effector functions, such as IFN-γ production and CTL activity (35–38). This form of split anergy is referred to as activation-induced unresponsiveness to distinguish it from the classical anergy observed in T cells that receive TCR stimulation in the absence of costimulation (35–38).

In this study we have shown that stimulation via CD27 during the initial activation of CD8+ T cells leads to the effective generation of cytotoxic effector cells that are capable of killing target cells ex vivo (Fig. 5). This enhanced response, compared with stimulation by Ag alone or Ag together with LPS as an adjuvant, correlates well with the significant increase in the number of activated Ag-specific CD8+ T cells that results from CD27 stimulation (Table I and Figs. 3 and 5). Increased numbers of activated T cells have been previously observed in transgenic mice that constitutively express membrane-bound CD70, presumably as a result of amplification of responses to environmental Ags (39, 40). In these mice, T cell proliferation is sustained due to chronic stimulation via CD27, which eventually leads to exhaustion of the T cell pool (40). In contrast, administration of sCD70, which is cleared relatively quickly from the circulation, results in a short period of rapid T cell proliferation, which then declines (Fig. 3B). Thus, the transient proliferative response obtained using sCD70 is likely to be more representative of the response induced by native CD70, because expression of the latter is tightly controlled under physiological conditions (20).

In addition to augmenting CD8+ T cell immune responses by quantitative means, our study demonstrates that CD27 triggering instills a program of differentiation that allows CD8+ T cells to overcome a state of unresponsiveness. A number of previous studies have shown that injection of soluble antigenic peptides leads to CD8+ T cell anergy (30–32). Consistent with this, the secondary response of CD8+ T cells was diminished in animals that received OVA257–264 alone during the primary immunization stage (Fig. 6). In contrast, animals that received OVA257–264 and sCD70 generated effective recall responses (Fig. 6). In this in vivo model, we believe that CD27-mediated programming of CD8+ T cells occurs during the primary response, because sCD70 was only administered during the initial immunization phase, and we could not detect sCD70 in the blood before secondary immunization with Ag (data not shown). Previous studies have shown that CD8+ T cells become anergic even in the presence of costimulatory signals delivered by CD28, and these anergic cells display defects in the activation of mitogen-activated protein kinases, extracellular signal-regulated kinase, c-Jun N-terminal kinase, and p38 (36, 37). Our results demonstrating that CD27 signaling prevents induction of anergy in CD8+ T cells suggest that the costimulatory signals delivered by CD27 may be distinct from those generated by CD28. It is also possible that both CD27 and CD28 signaling are required to prevent the induction of anergy in CD8+ T cells.

The inhibitory receptor CTLA-4, which regulates the induction of anergy in CD4+ T cells (41), is not directly involved in the induction of anergy in CD8+ T cells (42). However, blockade of this receptor on CD4+ T cells prevents the induction of anergy in OT-I T cells, suggesting a role for CD4+ T cells in the regulation of CD8+ T cell anergy (43). It has recently been established that CD4+ T cells are required for effective secondary responses by CD8+ T cells (44–46). However, the nature of the help provided by CD4+ T cells is incompletely understood. IL-2 has been shown to constitute at least one form of CD4-mediated help that could prevent the induction of anergy in CD8+ T cells (38, 43). CD40 ligand, which is expressed on activated CD4+ T cells, is also required for the generation of optimal secondary responses by CD8+ T cells (47). The induction of anergy in OT-I T cells in our model system after the injection of OVA257–264 alone is likely to have resulted from the lack of CD4+ T cell help, as OVA257–264 does not activate CD4+ T cells (48, 49). Our results suggest that, at least in this model, sCD70 can substitute for the lack of CD4+ T cell help. The full mechanism by which CD27 signaling prevents CD8+ T cell unresponsiveness remains to be elucidated. However, it is possible that the substantial increase in IL-2 production triggered by CD27 signaling in CD8+ T cells (Fig. 2) is sufficient to prevent the induction of anergy. Aside from IL-2 (38, 43) and CD27 stimulation (current study), the administration of IL-12 has recently been shown to prevent T cell anergy induced by the injection of OVA257–264 (32). Therefore, it appears that there are multiple signaling pathways capable of preventing the induction of anergy in CD8+ T cells.

The results of this study highlight the potential of using sCD70 in a vaccination regimen to boost weak CD8+ T cell responses such as those generated by tumor Ags. Current immunization strategies for the generation of antitumor immunity include the administration of tumor-derived CD8+ T cell epitopes with the capacity to stimulate CD8+ T cells (50). However, in the absence of CD4-mediated T cell help, CD8+ T cell responses are likely to be suboptimal, and these cells may become refractory to secondary antigenic stimulation (51, 52). Our results imply that vaccination with tumor-derived CD8+ T cell epitopes could be improved by concurrent provision of CD27 stimulation.

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References


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