The ability of immune-based cancer therapies to elicit beneficial CD8+ CTLs is limited by tolerance pathways that inactivate tumor-specific CD4 Th cells. A strategy to bypass this problem is to engage tumor-unrelated CD4 Th cells. Thus, CD4 T cells, regardless of their specificity per se, can boost CD8+ CTL priming as long as the cognate epitopes are linked via presentation on the same dendritic cell. In this study, we assessed the therapeutic impact of engaging tumor-unrelated CD4 T cells during dual costimulation with CD134 plus CD137 that provide help via the above-mentioned classical linked pathway, as well as provide nonlinked help that facilitates CTL function in T cells not directly responding to cognate Ag. We found that engagement of tumor-unrelated CD4 Th cells dramatically boosted the ability of dual costimulation to control the growth of established B16 melanomas. Surprisingly, this effect depended upon a CD134-dependent component that was extrinsic to the tumor-unrelated CD4 T cells, suggesting that the dual costimulated helper cells are themselves helped by a CD134+ cell(s). Nevertheless, the delivery of therapeutic help tracked with an increased frequency of tumor-infiltrating granzyme B+ effector CD8 T cells and a reciprocal decrease in Foxp3+ CD4+ cell frequency. Notably, the tumor-unrelated CD4 Th cells also infiltrated the tumors, and their deletion several days following initial T cell priming negated their therapeutic impact. Taken together, dual costimulation programs tumor-unrelated CD4 T cells to deliver therapeutic help during both the priming and effector stages of the antitumor response. The Journal of Immunology, 2015, 195: 5816–5826.
nations (29), costimulation with CD134 plus CD137 (dual costimulation [DCo]) acts synergistically to elicit robust CD8 T cell effector and tumoricidal activity (30–33). Importantly, DCo also induces cytotoxic CD4 Th1 cells that can directly kill MHC class II+ tumors (34) and provide linked help to CD8 T cells (35, 36). Thus, we reasoned that a tumor-unrelated helper epitope might augment DCo therapeutic efficacy by helping CD8 T cells responding to cross-presented tumor epitopes (37). However, because cross-presentation is biased toward highly abundant Ags (38, 39), this linked helper pathway may not boost CTLs specific to less abundant tumor epitopes. Nevertheless, this potential therapeutic gap might be bridged by an additional DCo-elicted nonlinked helper activity. Thus, dual costimulated Ag-responding CD4 T cells can also help cognate Ag-nonresponding T cells to develop CTL function (34). This nonlinked help might influence tumor-specific CTLs in draining lymph nodes that would otherwise not be cross-primed or alternatively boost CTLs encountering cognate tumor epitopes intratumorally (13).

To assess the potential of tumor-unrelated CD4 Th cells to augment DCo therapy, mice were challenged with tumors lacking an MHC class II–restricted peptide that was used as an immunogen with DCo. Thus, although dual costimulated CD4 T cells become cytolytic, they could only enhance tumor immunity by providing help. Notably, engagement of tumor-unrelated CD4 Th cells dramatically augmented DCo-mediated control of established B16 melanomas. Although these tumor-unrelated CD4 T cells were directly costimulated, their ability to provide therapeutic help depended, surprisingly, on an indirect CD134-dependent component. This suggested that the dual costimulated helpers are themselves helped by a CD134+ cell(s). Nevertheless, therapeutic help was associated with an increased frequency of tumor-infiltrating CD8+ CTLs and a reciprocal decrease in Foxp3+CD4+ cell frequency. The tumor-unrelated CD4 T cells also infiltrated the tumors, and their deletion several days following initial T cell priming negated their therapeutic impact. Taken together, DCo programs tumor-unrelated CD4 Th cells to

FIGURE 1. DCo induces cytotoxic CD4 Th1 T cells and controls lymphoma growth in BALB/c mice. HA-specific Thy1.1+ 6.5 TCR-Tg CD4 T cells were transferred into Thy1.2+ BALB/c recipients, followed by immunization with viral-HA or viral-HA plus DCo. Spleens were analyzed 5 d later. The transferred CD4 T cells were analyzed for intracellular IFN-γ versus TNF-α expression (A) and GzmB versus CD25 expression (B). The FACS plots are representative of an experiment containing three mice/group, as well as of two other experiments. BALB/c mice were inoculated with A20HA (C and D) or parental A20WT (E and F) lymphoma 4 d prior to receiving 6.5 CD4 T cells and subsequent immunization with rat Ig (Control), viral-HA, DCo, or viral-HA plus DCo (Both). (C and E) Tumor growth curves for individual mice. (D and F) Scatter plots of AUC values; horizontal lines indicate the means corresponding to the data in (C) and (E), respectively. The experiment in (C)–(F) contained six mice/group, and similar results were observed in another trial. *p < 0.05, **p < 0.01.
facilitate therapeutic efficacy, which relies on feedback from other CD134+ cells.

Materials and Methods

Mice, adoptive transfer, tumor challenge, and cell depletion

One million 6.5 TCR-transgenic (Tg) CD4 T cells specific to an I-Ed-restricted influenza hemagglutinin (HA) epitope (110SFERFEIFPKE120) on the Thy1.1+ BALB/c background (40) were prepared from CD8-depleted spleens plus lymph nodes of Tg donors and adoptively transferred into Thy1.2+ BALB/c recipients. TEa TCR-Tg CD4 T cells specific to an I-Ab-restricted I-Ed epitope (Ea, 52ASFEAQGALANIAVDKA68) on the Thy1.1+ C57BL/6 background (41) that were either wild-type (WT) or lacking CD134 (Tnfrsf4 null mutation) (42) (The Jackson Laboratory) were similarly prepared and transferred into WT or CD134-/- Thy1.2+ C57BL/6 recipients. Mice were treated i.p. with DCo (50 µg OX86 mAb [anti-OX40/CD134] plus 25 µg 3H3 mAb [anti-4-1BB/CD137]; Bio X Cell) or 75 µg control rat Ig (Sigma-Aldrich) 1 d following adoptive T cell transfer, recombinant vaccinia virus expressing HA (viral-HA; 106 PFU) 1 d prior to T cell transfer, or 250 µg soluble Ea peptide on the day of T cell transfer (35).

CD8+, CD4+, and Thy1.1+ cells were depleted by i.p. injection of 200 µg each the mAbs 2.43, GK1.5, and 19E12, respectively (Bio X Cell).

Flow cytometry

Transferred 6.5 and TEa TCR-Tg CD4 T cells were identified as CD4+Thy1.1+, whereas host-derived T cells were identified as Thy1.1+CD4+ or CD8+Thy1.1-. Ex vivo granzyme B (GzmB) and CD25 staining was performed following 5 h of in vitro stimulation with either 100 µg/ml HA peptide for 6.5 CD4 T cells or 2.5 µg/ml soluble anti-CD3 mAb (eBioscience) for TEa CD4 T cells and host-derived T cells (34). Intratumoral T cells were extracted by mechanical crushing, straining, and washing with
PBS. The frequency of CD4 and CD8 T cells is expressed as their percentage within the lymphocyte gate (determined by forward and side scatter using splenocytes as a reference).

Statistical analysis
The p values were calculated using an unpaired two-tailed t test. Asterisks located above horizontal lines indicate comparisons between circumscribed groups, whereas all other asterisks indicate differences relative to the control group at the far left of each graph. Sample sizes and the number of experimental trials are indicated in the figure legends.

Results
Tumor-unrelated CD4 T cell help augments DCo therapy

The BALB/c-derived MHC class II+ B cell lymphoma A20 and the A20HA subline (16) were initially used to assess the tumoricidal potential of dual costimulated CD4 T cells. A20HA presents an I-Eα–restricted HA epitope that can be recognized by adoptively transferred 6.5 TCR-Tg CD4 T cells (16), raising the potential for direct CD4 T cell–mediated tumor killing (34). In contrast, the potential of dual costimulated 6.5 CD4 T cells to control the parental A20WT lymphoma presumably depends entirely on their helper capacity.

Because the potential of DCo to elicit cytotoxic CD4 Th1 cells was established previously in the Th1-predisposed C57BL/6 and B10.D2 mouse strains (34), it was first necessary to assess the CD4 T cell response to DCo in BALB/c mice that are Th2 predisposed (44). Thy1.2+ BALB/c recipients infected with viral-HA and treated with DCo or control IgG (Fig. 1). In B10.D2 mice, viral-HA programs 6.5 CD4 T cells to become standard Th1 effectors that express IFN-γ and TNF-α, whereas the addition of DCo pushes these CD4+ effectors to also express GzmB (34). In BALB/c mice, viral-HA without DCo programmed the 6.5 CD4 T cells to express only modest amounts of IFN-γ and TNF-α (Fig. 1A); importantly, the addition of DCo pushed the viral-HA–specific CD4 T cells to express high levels of IFN-γ, TNF-α (Fig. 1A), and GzmB (Fig. 1B). DCo also increased CD25 expression (Fig. 1B), consistent with the essential role of IL-2 in programming GzmB expression (34). Taken together, DCo can program cytotoxic Th1 differentiation in Th2-predisposed BALB/c mice.

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BALB/c mice were inoculated with A20HA tumor, received transferred 6.5 CD4 T cells 4 d later, and were immunized with viral-HA, DCo, or both. Viral-HA alone had no impact on A20HA tumor growth compared with control IgG-immunized mice (Fig. 1C, 1D). DCo alone also had no impact on A20HA tumor growth; notably, however, DCo given with viral-HA (Both) reduced tumor growth 3-fold (p < 0.009) (Fig. 1C, 1D).

The ability of DCo plus viral-HA to control A20HA tumor growth (Fig. 1C, 1D) is consistent with the potential of HA–specific 6.5 cytotoxic CD4 Th1 cells to directly kill A20 cells presenting the cognate MHC class II–restricted HA epitope (34). We next used a similar experimental set-up, but with A20WT tumor, to assess whether the dual costimulated CD4 T cells can

FIGURE 3. CD8+ and CD4+ cells are both critical for the DCo therapeutic effect with tumor UH. B16-challenged mice received TEa CD4 T cells and were treated with Ea peptide (UH) and/or DCo. Mice treated with DCo plus UH (Both) were treated 3 d posttumor challenge with CD8, CD4, or no depleting Abs. (A) Tumor growth curves for individual mice. (B) AUC analysis. n = 9–10 mice/group. **p < 0.01, ***p < 0.001, ****p < 0.0001.
control tumor growth through their helper activity. Similar to the results with A20HA (Fig. 1C, 1D), immunization with viral-HA alone did not significantly impact A20WT tumor growth compared with nonimmunized controls (Fig. 1E, 1F). DCo alone significantly slowed tumor growth in some mice (2-fold, \( p = 0.03 \)), although a more substantial effect was observed in mice treated with both DCo plus viral-HA (4-fold, \( p = 0.005 \)).

That the priming of HA-specific CD4 T cells by viral-HA was associated with an enhanced ability of DCo to control A20 lymphoma growth, regardless of whether this tumor expressed HA (Fig. 1C–F), suggested that DCo tumor-unrelated CD4 T cells can augment tumor immunity by providing help. To rigorously test this possibility, we devised a second system in which C57BL/6 mice were challenged with the highly aggressive B16 melanoma and received transferred TEa CD4 T cells along with cognate I-A\(^d\)-restricted Ea\(^a\) peptide (41). Importantly, B16 melanoma does not express I-Ed from which the Ea\(^a\) epitope derives and, thus, it cannot be directly targeted by the TEa CD4 T cells.

Because our previous studies demonstrating the potential of dual costimulated CD4 T cells to acquire the cytotoxic Th1 phenotype and deliver nonlinked help were performed using different Ags and cognate CD4 T cells (34), it was first necessary to characterize the response of transferred TEa CD4 T cells to DCo in nontumor-bearing mice. TEa CD4 T cells induced by Ea peptide plus DCo expressed GzmB, CD25 (Fig. 2A, second row, far right panel), and IFN-\( \gamma \) and TNF-\( \alpha \) (Fig. 2A, bottom row, far right panel). Further, host-derived (Ag-nonresponding) T cells were also programmed to express GzmB, CD25, and IFN-\( \gamma \), although GzmB expression was greater in CD8 T cells compared with their CD4 counterparts (Fig. 2A, fourth column). Importantly, DCo given without transferred TEa CD4 T cells and Ea peptide induced lower amounts of these effector molecules in host-derived T cells (Fig. 2A, third column). TEa CD4 T cells plus Ea peptide, but without DCo, had no effect (Fig. 2A, second column). Thus, dual costimulated TEa CD4 T cells become cytotoxic and provide nonlinked unrelated help (UH).

C57BL/6 mice were challenged with B16 melanoma and received TEa CD4 T cells. Tumor growth in mice with TEa CD4 T cells and Ea peptide (UH), but without DCo, was comparable to IgG-treated controls (Fig. 2B, 2C). DCo given alone reduced tumor growth in some mice (Fig. 2B, 2C), but this trend did not reach statistical significance (\( p = 0.24 \), versus no treatment) (Fig. 2C). Nevertheless, DCo with Ea peptide (Both) markedly reduced tumor growth (3.5-fold versus controls, \( p < 0.0001 \)) (Fig. 2B, 2C).

To delineate the tumoricidal T cell subsets helped by the DCo tumor-unrelated TEa CD4 T cells, B16-challenged mice were given TEa CD4 T cells along with Ea\(^a\) peptide and DCo (Both, as in Fig. 2). Three days later, the mice were treated with anti-CD4 or anti-CD8 depleting mAbs (Fig. 3). Depletion of either CD4\(^+\) or CD8\(^+\) cells accelerated tumor growth (\( p < 0.0001 \), similar to the rate observed in mice treated with DCo only (Fig. 3A, 3B)); this suggested that both CD4 and CD8 T cells provide complementary tumoricidal functions. Consistent with this possibility, in a repeat experiment in which UH augmented DCo-mediated tumor growth control (Fig. 4A), the tumors controlled in mice treated with DCo plus UH exhibited an increased frequency of infiltrating CD8 T cells (Fig. 4B) that expressed greater amounts of GzmB (Fig. 4C) compared with mice treated with DCo only or not given DCo. DCo plus UH also boosted the frequency of intratumoral CD4 T cells (Fig. 4D), which primarily consisted of the DCo tumor-unrelated TEa CD4 T cells (Fig. 4E) and reciprocally di-

![FIGURE 4](http://www.jimmunol.org/) Intratumoral T cell subsets following treatment with DCo plus tumor UH. Tumor challenge and treatments were performed as in Fig. 2B. Tumor sizes were measured and lymphocytes were extracted on day 16. (A) Tumor size (\( n = 9 \) mice/group). (B) CD8 T cell frequency. (C) GzmB expression levels. (D) Total CD4 T cell frequency. (E) Percentage of CD4\(^+\) cells that is TEa. (F) Percentage of CD4\(^+\) cells that expresses Foxp3. \( n = 6 \) mice/group. Similar results were observed in another trial. *\( p < 0.05 \), **\( p < 0.01 \), ***\( p < 0.001 \), ****\( p < 0.0001 \).
minimized the frequency of CD4+Foxp3+ regulatory T cells (Tregs) (Fig. 4F).

To test the potential of tumor-unrelated CD4 T cell help to augment DCo in a therapeutic scenario, pre-established B16 tumors were allowed to grow for 5 d (reaching an average size of 25–50 mm²) prior to initiating treatment (Fig. 5). Under these stringent conditions, a single DCo dose failed to slow tumor growth compared with controls that did not receive DCo, although booster treatments given on days 8, 11, and 14 reduced tumor growth (DCo + Boost) \((p < 0.001)\) (Fig. 5A, 5B). In contrast, engagement of UH enabled a single dose of DCo to slow tumor growth (Both) \((p < 0.001)\), and provision of additional DCo boosters (Both + Boost) resulted in tumor regression between days 12 and 15 in three of six mice (dotted lines in Fig. 5A). These three mice were eventually euthanized on day 30 following resumption of tumor growth (Supplemental Fig. 1). All of the other mice were euthanized on day 15 (Fig. 5A). Taken together, engagement of UH substantially augments DCo therapeutic efficacy. Similar to the previous challenge study (Fig. 4), DCo with engagement of UH increased the frequency of intratumoral CD8 T cells (Fig. 5C) and their GzmB expression (Fig. 5D) in this therapeutic model. The increase in total (Fig. 5E) and TEa (Fig. 5F) CD4 T cell frequency was also associated with a reciprocally decreased Foxp3+ Treg frequency (Fig. 5G). Although DCo booster treatments did not further increase the frequency of intratumoral Foxp3+ T cells or further decrease CD4+Foxp3+ cells (Fig. 5C, 5F, 5G), they increased GzmB expression in CD8 T cells (Fig. 5D). Thus, therapeutic efficacy tracks with increased intratumoral effector T cell function.

**Delivery of therapeutic help requires CD134-dependent feedback that is extrinsic to the dual costimulated tumor-unrelated CD4 T cells**

To study the mechanistic basis of how tumor-unrelated CD4+ helpers augment the therapeutic effect of DCo, we first hypothesized that tumor-specific T cells receiving help require direct costimulation. Hence, the potential of DCo plus UH to control established B16 tumors was compared in WT versus CD134−/− mice. As before, DCo plus engagement of WT tumor–unrelated CD4 Th cells slowed tumor growth in WT recipients \((p < 0.05)\).
but, importantly, not in CD134<sup>−/−</sup> counterparts (Fig. 6A, 6B). The reduced intratumoral CD8<sup>+</sup> cell frequency (<i>p</i> < 0.001, Fig. 6C) and GzmB expression (<i>p</i> < 0.05, Fig. 6D) in CD134<sup>−/−</sup> mice compared with WT DCo + UH mice suggested that the therapeutic effect might require that the helped CD8<sup>+</sup> T cells receive direct CD134 costimulation. DCo + UH also failed to reduce the frequency of intratumoral CD4<sup>+</sup>Foxp3<sup>+</sup> cells in CD134<sup>−/−</sup> mice (Fig. 6F). This result was consistent with the known ability of CD134 agonist to deplete intratumoral Tregs (45) and could potentially contribute to the reduction in effector CD8<sup>+</sup> T cell accumulation (Fig. 6C, 6D). Nevertheless, DCo + UH CD134<sup>−/−</sup> mice also contained fewer intratumoral TEa helper CD4<sup>+</sup> T cells (Fig. 6G). This was unexpected given that these tumor-unrelated helper CD4<sup>+</sup> T cells were WT and, thus, presumed to express CD134 in response to specific Ag (46). Hence, it was also possible that the responding DCo CD4<sup>+</sup> T cells, with the CD8<sup>+</sup> T cells that they help, depend on CD134-dependent host cell feedback.

Thus, to directly assess how CD134 programs CD4 helper activity during DCo, the response of WT versus CD134<sup>−/−</sup> TEa CD4<sup>+</sup> T cells was compared in WT and CD134<sup>−/−</sup> nontumor-bearing recipients (Fig. 7). Consistent with our previous observation that CD134 plays a more dominant role than CD137 in driving CD4<sup>+</sup> T cell expansion and effector differentiation (34), CD134<sup>−/−</sup> TEa CD4<sup>+</sup> T cells transferred into WT recipients and recovered from spleens exhibited a greatly diminished expansion (Fig. 7A, 7B) and capacity to express IFN-γ and TNF-α (Fig. 7C, 7D) compared with WT. Importantly, WT TEa CD4<sup>+</sup> T cells transferred into CD134<sup>−/−</sup> recipients also underwent substantially reduced expansion (<i>p</i> < 0.05) (Fig. 7A, 7B) and expressed less IFN-γ and TNF-α (<i>p</i> < 0.05) (Fig. 7C, 7D) compared with WT → WT controls. Thus, although the transferred CD4<sup>+</sup> T cells responding to specific Ag and DCo were directly costimulated by CD134 agonist, their expansion and differentiation also required CD134-dependent feedback from the endogenous host cells. This result was similar to the previous observation that CD137 can impact CD8<sup>+</sup> T cell responses both directly and indirectly (31).

**Dual costimulated tumor-unrelated CD4 T cells provide therapeutic benefit beyond the T cell–priming phase**

CD8<sup>+</sup> T cells are essential for controlling tumor growth when tumor-unrelated CD4<sup>+</sup> helpers are engaged during DCo. Thus, depletion of CD8<sup>+</sup> cells accelerates tumor growth (Fig. 3), and increased
frequency of intratumoral GzmB+ CD8 T cells tracks with therapeutic efficacy (Figs. 4–6). CD4 help–mediated augmentation of CD8 T cell responses likely involves initial T cell priming in lymph nodes where both linked help (35, 36) and nonlinked help (34) (Fig. 2A) can be delivered. Nevertheless, DCo tumor-unrelated CD4 Th cells also accumulated at high frequency within tumors, suggesting that they might also deliver tumoricidal help during the effector phase of the antitumor response. To test this possibility, mice with pre-established B16 tumors were treated with DCo plus UH and were treated or not 3 d later with an anti-Thy1.1 mAb to deplete the unrelated Thy1.1+ TEa cells (Fig. 8). At this time point, the DCo TEa CD4 T cells have already delivered help to T cells undergoing initial priming in lymphoid organs (34); thus, their depletion should primarily impact the effector phase of the antitumor response. As expected, anti-Thy1.1 mAb treatment prevented total (Fig. 8A) and TEa (Fig. 8B) CD4 T cell accumulation within tumors. Importantly, Thy1.1 depletion accelerated tumor growth (Fig. 8C, 8D), diminished the accumulation of intratumoral CD8 T cells (Fig. 8E) and their expression of GzmB (Fig. 8F), and prevented the reduction in intratumoral Foxp3+CD4+ cell frequency (Fig. 8G). Taken together, these data are consistent with a role for the DCo tumor-unrelated CD4 T cells in helping CD8+ CTLs within the tumor microenvironment.

Discussion

T cell–based cancer therapies should elicit multipronged responses to prevent outgrowth of tumor escape variants. An advantage of DCo is that it generates potent tumor-reactive CD8+ CTLs (30, 31) and drives cytotoxic CD4 T cell–mediated tumor killing (34). This may be useful in treating tumors, such as melanoma, that can be induced to express MHC class II (6, 7).

A general issue limiting tumor vaccine efficacy is T cell–intrinsic tolerance mechanisms that limit the pool of tumor-reactive specificities (14, 15). A strategy to bypass tolerant tumor-specific CD4 Th cells is to add a class II–restricted tumor-unrelated Ag to vaccines composed of a mixture of class I–restricted tumor peptides (19). This is based on the ability of CD4 T cells to help CD8 T cells responding to an unrelated Ag, as long as both T cells are linked through priming by the same dendritic cell (20–22). Nevertheless, the repertoire of CTLs generated is limited by the choice of class I peptides included in the vaccine and, therefore, may exclude many therapeutically useful specificities. DCo, in contrast, does not target predetermined CD8 T cell specificities but, rather, can potentially augment the response of any tumor-reactive T cell. We reasoned that engaging tumor-unrelated CD4 T cells might augment DCo therapy through the combined effects of their ability to provide help in both linked (35, 36) and nonlinked (34) (Fig. 2A) manners.

Engagement of tumor-unrelated CD4 T cell help substantially enhanced DCo efficacy in treating established B16 melanomas (Fig. 5). This effect could be mediated through two distinct mechanisms. First, during initial T cell priming in lymph nodes, linked help (35, 36) could boost the response of CD8 T cells responding to dendritic cells cross-presenting abundant tumor epitopes (37), whereas nonlinked help (34) (Fig. 2A) might facilitate CTL function in T cells specific to tumor epitopes that are inefficiently cross-presented due to low abundance (38, 39). Second, dual costimulated tumor-unrelated CD4 T cells also appear to support CD8+ CTLs within tumors (Fig. 8). In a different immunization model, CD4 T cells help CD8+ CTLs within the tumor microenvironment, provided that these CD4+ helpers are tumor specific (13). This raises the intriguing question of how dual costimulated tumor-unrelated CD4 T cells are triggered to help CD8+ CTLs within tumors. One possibility, based on our recent observation that dual costimulated CD8 T cells can be triggered in the absence of TCR ligation by IL-12 or IL-2 plus IL-33 to secrete IFN-γ (47), is that cytokines in the tumor microenvironment
trigger dual costimulated CD4 T cells to elaborate helper functions. This help may be delivered to the CD8 T cells through a variety of mechanisms, such as dendritic cell licensing (5), or secretion of cytokines, such as IL-2 (11), that can facilitate TCR-independent triggering of fully differentiated effector CD8 T cells (47). Another question relates to the specificity of the helped CD8 T cells. Thus, some might exert tumoricidal function upon recognizing cognate Ag on the surface of tumor cells, although TCR-independent triggering of intratumoral effector CD8 T cells whose TCR do not recognize tumor Ags may also occur.

An unexpected observation in this study was that dual costimulated tumor-unrelated CD4 Th cells require an indirect CD134-dependent component to undergo maximal expansion and effector differentiation (Fig. 7) and deliver therapeutic help (Fig. 6). This suggests that the dual costimulated CD4 Th cells are themselves helped. A “help for helpers” mechanism was described previously: CD4 T cells responding to a strong foreign Ag engage otherwise tolerant tumor-specific CD4 Th cells (48). However, our model is distinct in that a CD4 Th cell responding to a strong foreign Ag appears to receive help from a cell(s) that expresses CD134, yet is not responding to a strong cognate Ag. Although the identity of this cell(s) is unknown, Foxp3+ Tregs represent a plausible candidate, in part because they were shown in other systems to undergo phenotypic reprogramming (49) and acquire helper capacity (50). Further, they also express CD134 constitutively (51), can lose their suppressive capacity in response to CD134 agonist (52), and are the only detectable lymphocyte subset in secondary lymphoid organs that expresses high levels of CD134 during steady-state conditions (Supplemental Fig. 2A). Finally, in response to DCo and UH help, Tregs gain the capacity to express the Tc1/cytotoxic Th1 lineage transcription factor eomesodermin (34, 53), as well as the effector molecules IFN-γ and GzmB (Supplemental Fig. 2B–F). Regardless of the identity of this helper facilitator cell, this observation suggests that the DCo helper response is a complex process. Importantly, although the current study defines this feedback mechanism as being CD134 dependent, it might be possible that CD137 is also involved. Thus, in addition to Ag-stimulated conventional T cells, CD137 is expressed on a variety of other cell types (54), including Foxp3+ Tregs (55).

DCo possesses powerful antitumor activity. This is likely due, in part, to the individual abilities of CD137 agonist to activate NK cells (56) and CD134 agonist to deplete intratumoral Tregs (45). Additionally, combining the two agonists elicits both cytotoxic...
CD8 (30) and CD4 (34) tumoricidal T cells. We found that enga
gaging tumor-unrelated CD4 T cell help substantially augments
the ability of DCs to control an inherently difficult-to-treat, highly
aggressive tumor. This approach has strong potential for human
translation. Thus, humanized agonists to both CD137 (57) and
CD137 (58) are being tested as monotherapies in human cancer
patients. Further, it was demonstrated that tumor-unrelated CD4
T cell help can be effectively engaged in human patients by adding
tetanus toxoid to vaccines containing MHC class I-restricted tu-
mor peptides (19). By taking advantage of the fact that most
people have CD4+ memory pools against pathogens that they have
been vaccinated against (e.g., tetanus), it should be possible to call
upon these responses to help fight cancer and perhaps provide a
lower risk for autoimmunity because they are directed against
foreign entities. In sum, triggering tumor-unrelated CD4 Th cells
with costimulation will likely be safe and effective in patients
suffering from cancer.

Disclosures
The authors have no financial conflicts of interest.

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