CD4 T-cell help influences the magnitude and quality of the CD8 T-cell response. In some systems, the primary CD8 T-cell responses primed in the absence of CD4 T-cell help are deficient relative to those primed in the presence of T-cell help (1, 4, 12). In other experimental systems, CD4 cells are not required for effective primary CD8 responses (7, 9). However, more profound and consistent deficiencies are observed in memory CD8 T-cell populations when they are generated in the absence of CD4 T-cell help (4, 7, 9, 10). During a primary immune response, CD4 and CD8 T cells expand and differentiate simultaneously, acquiring an effector function 2 to 5 days following antigenic challenge, suggesting that the delivery of help by Th cells may be delayed. In contrast, memory CD4 T cells respond rapidly in response to antigenic challenge, potentially allowing the immediate delivery of help to CD8 T cells, which could influence early events in CD8 T-cell responses. Here we used a system in which CD8 T-cell responses are primed in the presence of T-cell help from preexisting memory CD4 T cells. Specifically, mice primed with dendritic cells (DCs) pulsed with a major histocompatibility complex class II (MHC-II)-restricted peptide, LLO190 (NEKYAAYPNVS), from the listeriolysin O protein of Listeria monocytogenes, which generates an LLO190-specific Th response, are subsequently challenged with recombinant L. monocytogenes expressing the MHC-I-restricted epitope of ovalbumin (Ova257-264) (rLMOVa) (8). This experimental system allows analysis of the primary CD8 T-cell response to Ova in a setting where the LLO190-specific memory CD4 T-cell population has been engaged. In this study, we showed that engaging memory Th helper cells at the time of naive CD8 T-cell priming enhances CD8 T-cell responses. The enhanced responses are characterized by increased numbers of cytokine-producing, antigen-specific cells. These findings suggest that engaging endogenous memory Th1 cells may increase cellular responses in an immunotherapy or vaccination setting.
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Mice were immunized with DCs pulsed with LLO190 plus heat-killed *P. acnes* (DC/P. acnes/LLO190) to generate LLO190-specific Th1 CD4 T cells (6). Seven days later, splenocytes were harvested, stimulated with the LLO190 peptide, and stained for intracellular IFN-γ. In line with previous findings (6), DC/P. acnes/LLO190-immunized mice generated Th1 responses as LLO190-specific CD4 T cells produced IFN-γ and IL-2 but not IL-4 (Fig. 1A and data not shown).

To determine whether DC immunization generates functional memory T cells, mice were immunized with DCs pulsed with *P. acnes* alone or with *P. acnes* and LLO190 and 60 days later were challenged with rLMova. Seven days following challenge, splenocytes were harvested and stimulated with the LLO190 peptide. Compared to mice immunized with DCs pulsed with *P. acnes*, mice which had been immunized with DC/P. acnes/LLO190 exhibited a significantly enhanced Th1 response upon challenge with rLMova (Fig. 1B), demonstrating that DC immunization generates functional CD4 memory T cells, which are capable of responding to subsequent *L. monocytogenes* infection.

To examine the effects of engaging CD4 memory during the CD8 primary response, mice were first immunized with DCs pulsed with *P. acnes* or with DC/P. acnes/LLO190 and then 60 days later challenged with rLMova. Seven days following challenge, splenocytes were harvested, the numbers of Ova257-specific CD8 T cells were determined by tetramer staining, and IFN-γ- and IL-2-producing cells were identified by intracellular staining (7). We found that the size of the primary CD8 T-cell response, as assessed by each of these parameters, was significantly greater in mice with LLO190-specific memory Th1 cells than in mice that had no CD4 memory for *L. monocytogenes*. percentages and numbers of IFN-γ-producing cells/spleen (three mice per group). Data from one of five experiments are shown. An asterisk indicates that the *P* value is <0.05 as determined by Student’s *t* test.

FIG. 1. DC immunization generates peptide-specific Th1 effector cells in vivo. (A) Mice were immunized with DCs pulsed for 18 h with *P. acnes* with or without LLO190 peptide, as indicated. Seven days following immunization, splenocytes were harvested from immune mice and restimulated ex vivo for 6 h with medium or LLO190. IFN-γ production was determined by intracellular staining and FACS. A live splenocyte gate was used, and the numbers indicate the percentages of lymphocytes that were CD4+ and producing IFN-γ. The results are representative of three experiments in which there were three mice per group. (B to D) Mice were immunized with DCs pulsed with *P. acnes* with or without LLO190. More than 60 days following DC immunization, the mice were each challenged with 3 × 104 CFU of Listeria (rLMova). Seven days following infection, splenocytes were harvested from immune mice and restimulated ex vivo with LLO190. IFN-γ and IL-2 production was determined by intracellular staining and FACS. A live (B) or live/CD4+ (C) gate was used. (D) Bar graphs indicating the
genes (Fig. 2). These data demonstrate that CD4 memory T cells enhance the primary CD8 T-cell response. Differentially activated DCs can induce either Th1 or Th2 effector differentiation. DCs pulsed with Toll-like receptor ligands promote differentiation of CD4 T cells to the Th1 lineage (5, 6). Conversely, DCs pulsed with helminth antigens, such as soluble SEA, promote the differentiation of CD4 T cells to the Th2 lineage (6). We took advantage of this system to determine whether the ability of CD4 memory T cells to help the CD8 primary responses was characteristic of CD4 T cells in general or specifically a quality of memory Th1 cells and not Th2 cells. Mice were immunized with DC/P. acnes with or without LLO190 and challenged 60 days later with rLMova. Splenocytes were analyzed for Ova antigen-specific cells by K\(^{b}/\)Ova tetramer staining. Cytokine production was determined as described in the legend to Fig. 1. (A) Cells gated on live CD8\(^{+}\) splenocytes. The numbers indicate the percentages of gated cells which are tetramer positive (top panels) or produce the cytokines indicated (bottom panels). (B) Percentages of CD8\(^{+}\) splenocytes which are Ova specific or produce IFN-\(\gamma\) or IFN-\(\gamma\) and IL-2. The results for three mice from one of three experiments are shown. An asterisk indicates that the \(P\) value is <0.05 as determined by Student’s \(t\) test. IMM, immunization; CHG, challenge; PA, P. acnes.

FIG. 2. CD4 memory T cells enhance the CD8 primary response. Mice were immunized with DCs pulsed with P. acnes with or without LLO190 and challenged 60 days later with rLMova. Splenocytes were analyzed for Ova antigen-specific cells by K\(^{b}/\)Ova tetramer staining. (A) Cells gated on live CD8\(^{+}\) splenocytes. The numbers indicate the percentages of gated cells which are tetramer positive (top panels) or produce the cytokines indicated (bottom panels). (B) Percentages of CD8\(^{+}\) splenocytes which are Ova specific or produce IFN-\(\gamma\) or IFN-\(\gamma\) and IL-2. The results for three mice from one of three independent experiments. DC/PA/LLO values differed significantly (\(P < 0.05\), Student’s \(t\) test) from DC/PA or DC/SEA/LLO values. IMM, immunization; CHG, challenge; PA, P. acnes.

FIG. 3. Th2 memory CD4 T cells do not enhance the primary CD8 response. Mice were immunized with DCs pulsed with P. acnes with or without SEA and LLO190 and challenged 60 days later with rLMova. Splenocytes were analyzed for Ova antigen-specific cells by K\(^{b}/\)Ova tetramer staining. (A) Cells gated on live CD8\(^{+}\) splenocytes. The numbers indicate the percentages of gated cells which are tetramer positive. (B) Total number of Ova-specific CD8 T cells. The data are the data for three mice from one of three independent experiments. DC/PA/LLO values differed significantly (\(P < 0.05\), Student’s \(t\) test) from DC/PA or DC/SEA/LLO values. IMM, immunization; CHG, challenge; PA, P. acnes.

One impediment in vaccination research is the difficulty of eliciting the magnitude of response that is necessary to establish protection. For example, the immunogenicity of nonreplicating Ad can be compromised by preexisting immunity to Ad or the toxicity of high viral loads (11). To determine whether primary CD8 T-cell responses to Ad could be enhanced by existing memory CD4 T cells, mice immunized with DCs pulsed with P. acnes or with DC/P. acnes/LLO190 >60 days previously were vaccinated with Ad.LLO.Ova. The number of Ova190-specific CD8 T cells induced by Ad.LLO.Ova was increased approximately 3-fold when LLO190-specific memory Th1 cells were present at the time of vaccination (Fig. 4A and B). To examine whether preexisting immunity elicited by infection might be utilized to enhance vaccine efficacy, mice were infected with L. monocytogenes and >60 days later were vaccinated with Ad.LLO.Ova. Similarly, when LLO190-specific memory CD4 T cells were generated by L. monocytogenes infection, the primary Ova190-specific CD8 T-cell responses were >5-fold greater than those in mice that had never been infected with L. monocytogenes (Fig. 4C). Importantly, in response to immunization with lower doses of Ad, the CD8 T-cell responses in the presence of preexisting Th1 help were comparable to those elicited by a 10-fold-greater Ad titer, indicating that priming CD8 T cells in the presence of Th1 memory could significantly reduce the viral load required to
A live gate. The numbers indicate the percentages of CD8 antigen-specific cells by Kb/Ova tetramer staining. (A) Cells gated on Seven days following challenge, splenocytes were analyzed for Ova or with DC/lenge infection. Mice were immunized with DCs pulsed with specific Th1 cells could confer a protective advantage during chal-

generate productive CD8 T-cell responses (Fig. 4C and data not shown).

Lastly, we asked whether the enhanced primary CD8 T-cell response elicited in mice that have preexisting pathogen-spe-
cific Th1 cells could confer a protective advantage during challenge infection. Mice were immunized with DCs pulsed with P. acnes or with DC/P. acnes/LLO190 or were infected with L. monocytogenes and >60 days later were challenged with L. monocytogenes. The bacterial numbers in the spleens of mice immunized with DC/P. acnes/LLO190 were significantly lower than those in the spleens of mice that did not have preexisting Th1 memory (Fig. 5). As expected, mice preinfected with L. monocytogenes were completely protected against challenge infection.

CD4 T-cell responses are known to influence the magnitude and quality of CD8 T-cell responses. Here we show that en-
gaging CD4 memory T cells at the time of naïve CD8 T-cell activation enhances the number of antigen-specific CD8 cells capable of producing IFN-γ and IL-2. Interestingly, Th1, but not Th2, memory cells are capable of providing this help to

FIG. 4. CD8 T-cell responses to Ad immunization can be enhanced by activating endogenous memory CD4 T cells. (A and B) Mice were immunized with DCs pulsed with P. acnes with or without LLO190 and challenged 60 days later with recombinant Ad expressing a modified GFP which contained the Ova257 and LLO190 epitopes (Ad.LLO/Ova). Seven days following challenge, splenocytes were analyzed for Ova antigen-specific cells by K6/Ova tetramer staining. (A) Cells gated on a live gate. The numbers indicate the percentages of CD8+ cells which are tetramer positive. (B) Total numbers of Ova-specific cells from three mice from one of two independent experiments. An asterisk indicates that the P value is <0.05 as determined by Student’s t test. (C) Mice were infected with 3 × 10^7 CFU L. monocytogenes. More than 60 days later, mice were challenged with 10^7 PFU of Ad.LLO/Ova. Seven days following challenge, splenocytes were analyzed for Ova antigen-specific cells by K6/Ova tetramer staining. An asterisk indicates that the P value is <0.05 as determined by Student’s t test. IMM, immunization; CHG, challenge; PA, P. acnes.

FIG. 5. Enhanced CD8 T-cell responses are protective. Mice were immunized with DCs pulsed with P. acnes with or without LLO190 and challenged 60 days later with a 50% lethal dose of rLMova (1 × 10^5 CFU). Four days following challenge, spleens were harvested, and the numbers of bacteria in the spleens were determined by serial dilution. The double line indicates the limit of detection. Each data point represents the total number of CFU/spleen for one mouse (three mice per group). The data are representative of three independent experiments. The asterisk indicates that the P value is <0.05 as determined by Student’s t test n.d., not detected; IMM, immunization; PA, P. acnes.

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