CD40 Signaling Converts a Minimally Immunogenic Antigen into a Potent Vaccine Against the Intracellular Pathogen *Listeria monocytogenes*

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CD40 Signaling Converts a Minimally Immunogenic Antigen into a Potent Vaccine Against the Intracellular Pathogen Listeria monocytogenes

Michael S. Rolph and Stefan H. E. Kaufmann

Conventional vaccination strategies have failed for numerous pathogens, and the development of novel approaches to vaccine development is a major public health priority. Killed or subunit vaccines represent an attractive approach due to their safety, but they suffer from low immunogenicity and generally require adjuvants. In this study, the possibility of harnessing CD40 signaling for enhancing the immunogenicity of killed vaccines was investigated. Intravenous immunization of C57BL/6 mice with heat-killed Listeria monocytogenes (HKL) induced minimal immunity, but HKL administered together with an agonistic anti-CD40 mAb induced high levels of both CD4+ and CD8+ T cells capable of producing IFN-γ following in vitro HKL stimulation. HKL/anti-CD40 vaccination elicited robust protection against subsequent Listeria challenge. Approximately 1000-fold fewer bacteria were detected in the liver and spleen of vaccinated mice, and vaccinated mice were also able to resist a normally lethal Listeria challenge. CD40-mediated adjuvant activity required endogenous IL-12 at the time of vaccination, and protection was mediated by both CD8+ and CD4+ T cells. Thus, CD40 signaling can deliver potent adjuvant activity for vaccination against intracellular pathogens and is particularly effective for pathogens requiring both CD4+ and CD8+ T cells for effective control. The Journal of Immunology, 2001, 166: 5115–5121.

Vaccination is the most cost-effective measure for the control of infectious disease. Many infectious diseases can be prevented by vaccination, yet a large number of diseases remain for which an effective vaccine is not available. It has proved particularly difficult to develop effective vaccines against pathogens such as intracellular bacteria, for which effector T cells are a necessary part of the adaptive immune response (1).

Listeria monocytogenes is a Gram-positive facultative intracellular bacterium that has been used extensively for studying cell-mediated immunity against intracellular bacteria (reviewed in Ref. 2). L. monocytogenes causes an acute infection in mice characterized by early activation of macrophages, neutrophils, and NK cells. Subsequently, CD4+ and CD8+ T cells are activated, and these cells are required for sterile clearance of L. monocytogenes (3). Infection with a sublethal dose of L. monocytogenes induces potent T cell-mediated protection against subsequent infection. In contrast, immunization of mice with nonviable Listerial Ag preparations induces very limited immunity. Indeed, most studies have found that immunization with heat-killed L. monocytogenes (HKL) elicits no protection against subsequent Listeria challenge (4–6). An exception was reported by Szalay et al., (7) who described some protection against Listeria using an unconventional HKL immunization protocol.

CD40, a member of the TNFR family, was initially characterized as a B cell surface Ag critically involved in T cell-dependent humoral immune responses. It is now known to be expressed on a wide range of cell types, including APCs such as monocytes/macrophages and dendritic cells (DCs) (8). CD40 signaling in APCs leads to a wide range of phenotypic changes, including up-regulation of adhesion and costimulatory molecules such as CD80/CD86 and ICAM-1, as well as induction or increased expression of chemokines and cytokines such as IL-12, IL-18, EBI1-ligand chemokine and macrophage inflammatory protein-1α (9–13). The end result of these changes is to markedly enhance T cell priming and to favor the generation of Th1 cells. The effect of these changes can be observed in vivo using agonistic anti-CD40 mAb. Anti-CD40 mAb can convert the characteristic Th2 response in BALB/c mice infected with Leishmania major to a protective Th1 response (14), and CD8+ T cell responses to normally nonimmunogenic tumor cells (15) or tolerogenic peptides (16) can be induced using anti-CD40 mAb.

We hypothesized that a nonimmunogenic, nonviable L. monocytogenes preparation could be converted to an immunogenic vaccine by concurrent delivery of agonistic anti-CD40 mAb. Immunization of mice with HKL together with anti-CD40 mAb treatment induced a strong Th1 and CD8+ T cell response, and the mice were protected against subsequent challenge with a normally lethal dose of L. monocytogenes. Our results indicate that directly targeting CD40 is a promising approach for the development of novel subunit vaccines, particularly for those pathogens that require both CD4+ and CD8+ T cells for effective control.

Materials and Methods

Mice

C57BL/6 mice were raised under specific pathogen-free conditions at the Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin (Berlin, Germany) and used when they were between 6 and 10 wk of age. All mouse experiments were performed in accordance with German and institutional animal care guidelines.
**Bacteria**

L. monocytogenes (strain EGD) was grown overnight in tryptic soy broth. Wild-type Salmonella typhimurium (strain SL1344) was grown overnight in Luria-Bertani medium. Salmonella and Listeria stocks were stored at –80°C. For preparation of HKL and heat-killed S. typhimurium (HKS), bacteria were washed twice in LPS-free PBS (Biochrom, Berlin, Germany) at 80°C. For preparation of HKL and heat-killed S. typhimurium was used as a control. Abs were purified from culture supernatant or rat serum using protein G chromatography, and were biotin- or fluorochrome-conjugated according to standard protocols. Endotoxin-anti-CD40 mAb (clone 3/23) was purchased from BD Pharmingen (San Diego, CA).

**HKL stimulation in vitro**

Single spleen cell suspensions were prepared by passing the spleens through a metal siever. Erythrocytes were lysed by treatment with NH$_4$Cl lysis buffer (8.29 g/L NH$_4$Cl, 1.0 g/L KHCO$_3$, and 0.037 g/L EDTA). The cells were washed in RPMI 1640 containing 10 mM HEPES, and resuspended in RPMI 1640 supplemented with 10% FCS (Sigma), 1 mM l-glutamine, 5 × 10$^{-5}$ M 2-ME, 10 mM HEPES, 1 mM sodium pyruvate, 100 μg/ml penicillin, and 100 U/ml streptomycin. This medium was designated RPMI 1640. Except where indicated, all tissue culture reagents were obtained from Biochrom. The cells were cultured for 72 h at 2 × 10$^5$ cells/well in 200 μl RPMI 10 in a round-bottom 96-well plate (Nunc, Naper- ville, IL). The cells were left unstimulated or stimulated with 10$^8$/ml HKL. In some experiments, the cells were also stimulated with 10$^5$/ml HKL as an irrelevant Ag.

**Enzyme-linked immunospot assay (ELISPOT)**

ELISPOT plates (Millipore, Bedford, MA) were coated with 4 μg/ml anti-IFN-γ mAb (R4-6A2) or 2 μg/ml anti-IL-4 mAb (BVD4-1D1; BD Pharmingen) in 0.05 M carbonate buffer (pH 9.6) at 4°C overnight. The plates were washed once and blocked with PBS-1% BSA for 2 h at 37°C. After two washes, 10$^6$ spleen cells were added and either cultured with 10$^8$/ml HKL or left unstimulated in RPMI 10 for 24 h. The plates were washed extensively, and 0.25 μg/ml biotinylated anti-IFN-γ mAb (XMGI.2) or 0.25 μg/ml anti-IL-4 mAb (BVD6-24G2) was added. After 2 h incubation at 37°C, the plates were washed and alkaline phosphatase-conjugated streptavidin (Dianova, Hamburg, Germany) was added for a further 1 h incubation. The plates were washed, and color was developed for 15 min by addition of 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium alkaline phosphatase substrate (Sigma). Spots were counted under a dissecting microscope.

**ELISA**

IFN-γ in tissue culture supernatant was measured using a standard ELISA protocol. Briefly, ELISA plates were coated for 1 h at 37°C with 2 μg/ml of anti-IFN-γ mAb (R4-6A2). The plates were washed four times in wash buffer (PBS-0.1% Tween 20) and then blocked at 37°C for 2 h with PBS-1% BSA-0.1% Tween 20. After washing, appropriate dilutions of sample and rIFN-γ standard were added, and the plates were incubated overnight at 4°C. The plates were washed and incubated sequentially for 1 h at 37°C with 2 μg/ml biotinylated anti-IFN-γ (XMGI.2) and streptavidin-alkaline phosphatase (Dianova), with washing in between steps. Color was developed with p-nitrophenyl phosphate (Sigma), and the plates were read at 405 nm.

**Intracellular IFN-γ staining**

Spleen cells were cultured for 20 h in a 48-well plate (Costar, Cambridge, MA) at 3 × 10$^5$ cells/ml in 1 ml RPMI, either unstimulated or with 5 × 10$^7$/ml HKL. Brefeldin A (10 μg/ml; Sigma) was added during the last 4 h of incubation. The cells were washed in PBS, blocked for 10 min at room temperature with mAb to FcγRIII/II and rat serum (1/200; Jackson ImmunoResearch, West Grove, PA) and stained with Cy5-conjugated anti-CD4 mAb (BVD4-1D1; BD Pharmingen), Tri-Color-conjugated anti-CD8 mAb (XMG1.2), and anti-CD40 mAb to provide adjuvant activity is not restricted to the other groups (Fig. 1C). In this experiment, the adjuvant effect of a second anti-CD40 mAb (clone 3/23; BD Pharmingen) was found to be equivalent to the 1C10 Ab. Thus, the ability of anti-CD40 mAb to provide adjuvant activity is not restricted to the 1C10 clone. The 3/23 mAb was a commercially available "low-endotoxin" preparation (<0.1 ng/μl). A previous study found that the BD Pharmingen anti-CD40 preparations did not contain detectable levels of endotoxin and, when administered alone, did not induce IL-12 or IFN-γ production in mice (20). Therefore, these results argue against the involvement of endotoxin in our system, although we cannot fully exclude a possible contribution by very low levels of endotoxin.

The response to HKL in the HKL/anti-CD40 group (and the live L. monocytogenes group) was specific for HKL, as the IFN-γ response of spleen cells from this group to HKL was no different to that seen by the other experimental groups (data not shown).
and *Listeria*-infected mice produced IFN-γ (Fig. 2). In contrast, no HKL-specific IFN-γ production was detected in spleen cells from the control HKL or anti-CD40 groups. *L. monocytogenes* is known to induce a strong CD8⁺ T cell response by virtue of its capacity to escape from the phagosome into the cytoplasm (21). However, the strength of the CD8⁺ T cell response in the HKL/anti-CD40 group was unexpected, because exogenous Ags generally stimulate CD8⁺ T cell responses weakly or not at all.

**HKL/anti-CD40 vaccination completely protects mice against *L. monocytogenes* challenge**

Production of IFN-γ is critical for effective control of a primary *Listeria* infection. This suggests that HKL-specific IFN-γ-producing cells induced by HKL/anti-CD40 treatment may protect mice from *L. monocytogenes* challenge. C57BL/6 mice were immunized as described above, except that, on day 20 following the second immunization, mice were challenged i.v. with 5 × 10⁴ CFU *L. monocytogenes* (≈5 × LD₅₀) and survival was monitored for a 14-day period (Fig. 3). All mice treated with control IgG ± HKL or anti-CD40 mAb alone succumbed to *Listeria* infection. In contrast, all mice vaccinated with either HKL/anti-CD40 or live *Listeria* survived (Fig. 3).

Successful vaccination was associated with a reduction in bacterial load. C57BL/6 mice were vaccinated as described above and challenged i.v. with 5 × 10⁵ CFU *L. monocytogenes*. Three days later, bacterial load in the liver and spleen was determined. High bacterial loads were detected in mice treated with control IgG, IgG plus HKL, or anti-CD40 mAb. In contrast, HKL/anti-CD40-treated mice had almost 1000-fold fewer bacteria in both the liver (p < 0.01) and spleen (p < 0.05; Fig. 4).

Protection mediated by HKL/anti-CD40 vaccination was specific for *L. monocytogenes*, as mice vaccinated with HKL/anti-CD40 were not protected against challenge with *S. typhimurium* (Table I). Similarly, mice vaccinated with HKS and anti-CD40 mAb were protected against *S. typhimurium* challenge but not against *L. monocytogenes* (Table I). These results also underline the broad utility of anti-CD40 mAb as a vaccine adjuvant by demonstrating that HKS/anti-CD40 is an effective vaccine against *S. typhimurium* infection.

**IL-12 is essential for anti-CD40-mediated adjuvant activity**

To test the role of IL-12 in anti-CD40-mediated adjuvant activity, mice were vaccinated and immediately treated with 500 μg anti-IL-12 mAb or rat IgG. A second anti-IL-12 mAb treatment was given on day 2. Fourteen days after HKL/anti-CD40 immunization, one group of mice was killed and HKL-specific cytokine production by spleen cells was measured (Fig. 5A). Another group of mice was challenged i.v. with 5 × 10⁴ CFU *L. monocytogenes*. Bacterial load in the spleen was measured 3 days later (Fig. 5B). Neutralization of IL-12 at the time of vaccination abrogated the induction of IFN-γ-producing HKL-specific T cells (Fig. 5A; p < 0.05) compared with the rat IgG-treated group) and protective immunity (Fig. 5B; p < 0.01). In contrast, neutralization of TNF at the time of vaccination had no effect on vaccine efficacy (data not shown).

**CD8⁺ and CD4⁺ T cells mediate protection in HKL/anti-CD40 vaccinated mice**

To identify the cells responsible for protection, CD4⁺ and/or CD8⁺ T cells were depleted from HKL/anti-CD40-vaccinated mice immediately before i.v. challenge with 5 × 10⁵ CFU *L. monocytogenes*. Bacterial load in the liver and spleen was determined 3 days later. Depletion of either CD4⁺ or CD8⁺ T cells markedly reduced vaccine efficacy, whereas depletion of both...
CD4$^+$ and CD8$^+$ T cells fully abrogated vaccine-mediated protection (Fig. 6). In general, depletion of CD8$^+$ T cells had a greater effect than that of depleting CD4$^+$ T cells, although a significant difference between the two treatments was not found in all experiments (e.g., in the experiment shown in Fig. 6, $p < 0.05$ in the liver and $p = 0.056$ in the spleen for anti-CD4 vs anti-CD8 treatment). We conclude that CD4$^+$ and CD8$^+$ T cells are stimulated by HKL/anti-CD40 vaccination and that both T cell populations contribute to protection.

Duration of vaccine-induced protection

In an experiment to examine the longevity of protection, C57BL/6 mice were vaccinated with HKL/anti-CD40 on day 0 and day 14. Control mice received no treatment. Three mice per group were killed on days 34 and 85 for analysis of HKL-specific IFN-$\gamma$ production by their spleen cells. The amount of HKL-induced IFN-$\gamma$ produced by spleen cells from vaccinated mice was similar at days 34 and 85 (Table II). Additionally, five mice per group were challenged i.v. with $5 \times 10^3$ CFU $L$. monocytogenes on days 34 and 85. Three days following challenge, bacterial load in the spleens of vaccinated mice was substantially reduced in comparison to control mice (Table II). The extent of vaccine-mediated protection at days 34 and 85 was the same. Thus, a fully effective long-term memory response that lasted at least 10 wk following the booster vaccination was induced by HKL/anti-CD40 vaccination.

Discussion

Infectious diseases are a leading cause of morbidity and mortality worldwide. Killed or subunit vaccines offer promise as safe vaccine candidates against a wide range of infectious agents. However, they suffer from low immunogenicity and, in most cases, are only effective when given together with an adjuvant. To date, alum is the only adjuvant widely used for human vaccines, but this is not appropriate for the effective induction of cell-mediated immunity. Thus, the development of improved adjuvant strategies is a key component of vaccine research.

The results in this study indicate that targeting costimulatory molecules such as CD40 may be a promising approach for the development of improved vaccine adjuvants. HKL is weakly immunogenic in vivo (4–6), although transient protection has been reported in mice repeatedly vaccinated with HKL (7). In the present study, HKL was converted to a highly immunogenic vaccine when given together with anti-CD40 mAb. HKL/anti-CD40 vaccination was IL-12-dependent, induced an exclusively type 1 cytokine response (IFN-$\gamma$ but not IL-4), and stimulated both CD4$^+$ and CD8$^+$ T cells. Most importantly, vaccinated mice were protected against listeriosis. This is the first demonstration of the potential of anti-CD40 mAb as an adjuvant for vaccination against an intracellular pathogen.

CD40 is expressed on a wide range of immune cells, including B cells, macrophages, DCs, and mast cells, as well as on many cells not considered to be part of the immune system (8, 22). Reflecting its broad cellular distribution, the CD40-CD154 pathway is involved in many immune and inflammatory processes. We have identified CD4$^+$ and CD8$^+$ T cells as the effector cells mediating vaccine-induced protection against $L$. monocytogenes, but we have
FIGURE 4. Vaccinated mice have reduced bacterial load after *Listeria* challenge. C57BL/6 mice were vaccinated as described in Fig. 3 and challenged on day 34 with $5 \times 10^3$ CFU *L. monocytogenes* i.v. Bacterial load in the liver and spleen was measured 3 days later. Each point represents the bacterial load from one mouse, and a bar indicating the median value is shown for each group. HKL/anti-CD40-vaccinated mice had fewer bacteria in the liver ($p < 0.01$) and spleen ($p < 0.05$). This experiment was performed three times with similar results.

not defined the immediate target cell of anti-CD40 mAb treatment. A likely candidate for at least some of the CD40-mediated adjuvant activity is the DC, which undergoes a broad range of phenotypic changes in response to CD40 signaling, the end result of which is to markedly enhance the DC’s ability to productively prime T cells (23). This involves alteration of proteasome composition (24), up-regulation of costimulatory and adhesion molecules such as CD80/CD86 and ICAM-1, and the induction or increased expression of numerous cytokines and chemokines, including IL-12, IL-18, EBI1-ligand chemokine and macrophage-inflammatory protein-1α (9–13). Together, the characteristics of DCs, including their response to CD40 signaling, points to DCs as a central target cell for the anti-CD40-mediated adjuvant activity. However, a range of other cell types such as macrophages or endothelial cells may also be involved. A small proportion of T cells can express CD40 (25), although, in a similar system, it was shown that anti-CD40 mAb did not act directly on T cells (26).

The T cell response induced by HKL/anti-CD40 vaccination was notable for its extreme Th1 bias. Indeed, no HKL-specific IL-4 production could be detected using a sensitive ELISPOT assay. The potency with which anti-CD40 mAb treatment promotes a Th1 response was also demonstrated by therapeutic treatment of *L. major*-infected BALB/c mice, in which administration of anti-CD40 mAb was associated with a Th1, rather than the characteristic Th2, response normally observed in this mouse strain (14). This ability of anti-CD40 mAb to potentiate Th1 responses is an attractive feature for vaccination against intracellular bacteria and contrasts with alum, the only adjuvant widely used in human vaccines that predominantly enhances Th2 responses.

A second notable feature of the T cell response in HKL/anti-CD40-vaccinated mice was the strong CD8$^+$ T cell response. In recent years, increasing numbers of papers have described exceptions to the classical dogma that states that exogenous Ag does not enter the MHC class I pathway. However, the rules that determine when and how exogenous Ags can be presented via MHC class I to CD8$^+$ T cells are still under study (27, 28). The development of new strategies to enhance CD8$^+$ T cell responses is an area of considerable interest for vaccine research (29).

CD40 signaling is...
necessary for induction of Th-dependent CD8\(^+\) T cell responses, (30–32), and a recent study demonstrated that anti-CD40 mAb treatment greatly enhanced the CD8\(^+\) T cell response to i.p. administration of the model Ag OVA (33). Furthermore, anti-CD40 mAb treatment converted a tolerogenic CTL epitope into an immunogenic one (16), and CD8-mediated tumor eradication could be stimulated by treatment of mice with anti-CD40 mAb (15). Although it remains to be established how HKL-derived Ags are introduced into the MHC class I pathway, it is clear that targeting CD40 is a promising approach for enhancing CD8 T cell responses to exogenous Ags.

IL-12 was essential for CD40-mediated priming of IFN-\(\gamma\)-producing HKL-specific T cells. The requirement for IL-12 may reflect the failure of HKL to induce substantial IL-12 production in vivo, in contrast to the robust IL-12 production associated with live \textit{Listeria} infection (34, 35). Indeed, it has previously been shown that coadministration of IL-12 with HKL results in the development of a protective immune response, albeit one that predominantly involves CD4\(^+\) T cells (4). However, bolus delivery of immunostimulatory cytokines is sometimes associated with unacceptable toxicity (18). Direct targeting of APCs via cell surface molecules such as CD40 may be a superior approach with potentially fewer side effects. Anti-CD40 mAb treatment alone is associated with generalized immune activation (36, 37), but promising data has been reported for generation of T cell-independent Ab responses in which directly linking anti-CD40 mAb to the vaccine Ag allowed ~500-fold less Ab to be used (37). An alternative approach has been described by Gurunathan et al., (38) who used a trimeric CD40 ligand DNA construct to enhance cellular immunity to coadministered naked DNA vaccines. Furthermore, coadministration of the CD40 ligand construct with soluble Leishmania Ag was able to induce protective immunity (38).

Our inability to induce immunity after vaccination of mice with HKL alone is consistent with numerous previous studies (4–6). In contrast, an earlier study from this laboratory using an unconventional immunization protocol (three doses of HKL i.v. at 5-day intervals) demonstrated CD8\(^+\) T cell-mediated protection against subsequent \textit{Listeria} challenge. However, the protection achieved was weak and relatively short-lived, having already declined substantially at 4 wk after vaccination. Indeed, effective long-term protection by killed or subunit vaccines can be difficult to achieve. For example, vaccination with \textit{Mycobacterium tuberculosis} culture filtrate proteins in incomplete Freund’s adjuvant gave appreciable protection 1–3 mo following vaccination, but was markedly reduced by 5 mo (39). In the present study, the strength of vaccine-mediated protection was the same 34 or 85 days after vaccination, indicating that a genuine state of memory was obtained by this vaccination strategy. In a preliminary experiment, we also found substantial protection 5 mo after immunization (data not shown).

It is interesting to note that although anti-CD40 mAb treatment converts HKL to an effective vaccine, CD40 signaling is not required for effective immunity against \textit{L. monocytogenes} infection (40). This highlights the principle that rational vaccine development need not restrict itself to mimicking the natural immune response but may profitably follow alternative strategies.

Targeting CD40 is a promising approach to vaccination against intracellular pathogens and may be especially valuable for pathogens requiring both CD4\(^+\) and CD8\(^+\) T cells for control. This includes not only viral, but also bacterial (e.g., \textit{M. tuberculosis}) and protozoal (e.g., \textit{Plasmodium} sp.) pathogens. To this end, we have recently initiated studies in the mouse model of \textit{M. tuberculosis} infection; anti-CD40 mAb treatment greatly enhances the immunogenicity of \textit{M. tuberculosis} culture filtrate proteins (M. R., unpublished observations). Finally, we consider that our results

Table II. HKL/anti-CD40 vaccine-induced immunity is maintained for at least 10 wk\(^a\)

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<th>Day 34</th>
<th>Day 85</th>
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<tr>
<td></td>
<td>IFN-(\gamma) (U/ml)</td>
<td>Log(_{10}) CFU</td>
</tr>
<tr>
<td>Control</td>
<td>14 ± 9</td>
<td>7.0 ± 0.1</td>
</tr>
<tr>
<td>HKL + anti-CD40</td>
<td>438 ± 104</td>
<td>4.4 ± 0.8</td>
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\(^a\) C57BL/6 mice were vaccinated on days 0 and d14 with HKL/anti-CD40. Control mice received no treatment. On days 34 and 85, groups of mice were divided into two. Spleen cells were collected from one group and stimulated for 72 h with 10\(^6\)/ml HKL. IFN-\(\gamma\) was measured in the supernatant. The second group was challenged i.v. with 5 \times 10\(^3\) CFU \textit{L. monocytogenes}, and bacterial load in the spleen was determined 3 days later. Data are presented as mean ± SD; \(n = 3\) mice/group for spleen cell cultures 5 mice/group for \textit{Listeria} challenge.

FIGURE 6. Protection induced by HKL/anti-CD40 is mediated by both CD4\(^+\) and CD8\(^+\) T cells. C57BL/6 mice were immunized on days 0 and 14 with HKL/anti-CD40 and challenged with 5 \times 10\(^3\) \textit{L. monocytogenes} i.v. on day 34. CD4\(^+\) and CD8\(^+\) T cells were depleted by treatment on days 31 and 34 with 300 \(\mu\)g anti-CD4 and/or anti-CD8 mAb i.p. Each point represents the bacterial load from one mouse (\(n = 5\) mice/group) 3 days following challenge. A median bar is shown for each group. This experiment was performed three times with similar results. \(*, p < 0.05\) compared with undepleted mice. \(**, p < 0.01\) compared with undepleted mice, and \(p > 0.1\) compared with unvaccinated mice.
reflect a general and underappreciated paradigm for vaccine development, that directly targeting APCs is one of the most effective approaches for enhancing and tailoring an immune response.

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