Anti-CD25 Antibody Enhancement of Vaccine-Induced Immunogenicity: Increased Durable Cellular Immunity with Reduced Immunodominance

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Anti-CD25 Antibody Enhancement of Vaccine-Induced Immunogenicity: Increased Durable Cellular Immunity with Reduced Immunodominance

Anne C. Moore,2* Awen Gallimore,† Simon J. Draper, Katherine R. Watkins,* Sarah C. Gilbert,* and Adrian V. S. Hill*

An efficacious vaccine strategy must be capable of inducing strong responses of an appropriate phenotype that are long lasting and sufficiently broad to prevent pathogen escape mechanisms. In the present study, we use anti-CD25 mAb to augment vaccine-induced immunity in mice. We demonstrate that coformulation of Ab and poxviral- or adenoviral-vectored vaccines induces significantly increased T cell responses to a malaria Ag; prior anti-CD25 Ab administration was not required for this effect. Furthermore, this vaccination approach subverts immunodominant epitope hierarchies by enhancing responses to subdominant epitopes induced by recombinant modified vaccinia virus Ankara immunization. Administration of anti-CD25 with a vaccine also induces more durable immunity compared with vaccine alone; significantly higher T cell responses were observed 100 days after the primary immunization. Enhanced immunogenicity is observed for multiple vaccine types with enhanced CD4+ and CD8+ T cell responses induced by bacillus Calmette-Guérin and a recombinant subunit protein vaccine to hepatitis B virus and with multiple Ags of tumor, viral, bacterial, and parasitic origin. Vaccine strategies incorporating anti-CD25 lead to improved protection against pre-erythrocytic malaria challenge. These data underpin new strategies for the design and development of more efficacious vaccines in clinical settings. The Journal of Immunology, 2005, 175: 7264–7273.

Vaccination presents a cost-effective strategy to relieve the burden on human health caused by infectious pathogens. However, sufficiently efficacious vaccines that induce primarily cell-mediated immunity to pathogens generally remain elusive. We have developed heterologous prime-boost vaccine strategies against a range of diseases, including malaria (1–5) and tuberculosis (6, 7), that are based on the use of plasmid DNA, recombinant protein particles, or nonreplicating virus vectors that express the Ag of interest. These are highly efficacious in preclinical malaria and tuberculosis models, where IFN-γ secretion by CD8+ or CD4+ T cells correlates with protection (8–10), as it does in humans (11). With respect to malaria vaccines, a priming vaccination with Fowlpox strain 9 (FP9)1, a nonreplicating avipoxvirus, or recombinant DNA followed by a boosting immunization with recombinant modified vaccinia virus Ankara (MVA) is highly efficacious in mice, and in humans, this strategy induces sterile immunity in some individuals and, overall, a 92% reduction in liver stage parasite numbers (1–5, 12, 13). However, further improvements are required to develop higher level durable protective efficacy.

We examine here methods of enhancing vaccine efficacy in mice to foreign Ags using anti-CD25 Ab. A subset of T cells, primarily identified as CD4+CD25+ T cells and termed regulatory T cells (Tregs), are crucial to the regulation of the magnitude and specificity of immunity to self and foreign Ags (14–16). Functional Tregs have been shown to be a barrier to cancer immunotherapies. A common method of depleting these T cells is by injecting an Ab to the CD25 molecule that is constitutively expressed on these cells, and this has been demonstrated to significantly improve the clearance of injected tumor cells (17–20).

We initially postulated that the induction of an immune response to a vaccine Ag would be improved when the Treg population is temporarily depleted using anti-CD25 Ab at the time of the primary immunization. We demonstrated that vaccine immunogenicity is significantly increased when anti-CD25 Ab is administered to mice at the same time as vaccination. Although previous studies have suggested that this strategy may be useful for improving DNA priming (21) or boosting (22) vaccination or for improving immunity to chronic viral infections (23, 24) or to tumor peptides (17), we have extensively examined the role of anti-CD25 Ab in optimizing multiple vaccine strategies to multiple Ags. In contrast to previous studies, where the Ab was injected a number of days before immunization, we coadministered the Ab with the vaccine or gave it simultaneously at a separate site to the vaccination site. We demonstrate that anti-CD25 Ab is a broad spectrum enhancer of all nonreplicating virus vector, bacterial based, or recombinant protein vaccines tested. Both CD4+ and CD8+ T cell responses to the encoded Ag were significantly augmented when anti-CD25 Ab is included in the vaccine regime. Furthermore, we describe an enhanced single-dose vaccination approach, comprising anti-CD25 mAb coformulated with various vaccines that subverts immunodominant epitope hierarchies and induces superior durable
immunity compared with Ab-free vaccines. This strategy leads to improved protection against murine malaria parasite challenge. The safe clinical use of licensed mAbs to human CD25 suggests that this approach could provide a novel simple strategy for enhancing the cellular immunogenicity of a broad range of vaccines.

Materials and Methods

Vaccines and anti-CD25 Ab

The construction, design, and preparation of DNA, MVA, FP9, and adenovirus (ADV) expressing *Plasmodium berghei* circumsporozoite protein (PcCSF) and MVA expressing *Mycobacterium tuberculosis* Ag 85A (MVA85A) have been described previously (1, 5, 6, 25). MVA-T expressing (PbCSP) and MVA expressing VSV-G (MVA85A) have been described previously (1, 5, 6, 25). MVA-T expressing (PbCSP) and MVA expressing VSV-G (MVA85A) have been described previously (1, 5, 6, 25).

Female BALB/c or C57/BL6 mice 4–6 wk old (Biomedical Services, John Radcliffe Hospital) were used in all experiments under the terms of the U.K. Home Office Animals Scientific Procedure Act Project Licence. Mice and immunizations

Animals and immunizations

Female BALB/c or C57/BL6 mice 4–6 wk old (Biomedical Services, John Radcliffe Hospital) were used in all experiments under the terms of the U.K. Home Office Animals Scientific Procedure Act Project Licence. Mice were immunized intradermally (i.d.) with 10^6 PFU of poxvirus, 10^7 PFU of U.K. Home Office Animals Scientific Procedure Act Project Licence. Mice and immunizations

administration of anti-CD25 Ab, clone GL113, were prepared by the ammonium sulfate precipitate of hybridoma culture supernatants. Endotoxin levels were assessed in all Ab preparations using the Limulus amebocyte lysate assay (BioWhittaker) and, in all cases, were shown to be below the level of detection in this assay (0.1 EU/ml).

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Administration of anti-CD25 Ab significantly enhances immune responses induced by recombinant MVA immunization

In vivo IFN-γ assays were conducted as previously described (1, 5–7, 25), using coating and detecting Abs from Mabtech. To examine IFN-γ-secreting T cells in the blood, lymphocytes were prepared from tail vein blood as previously described (1) and were restimulated in duplicate in vitro with peptide-pulsed target cells (0.25 × 10^5/well) that were prepared by pulsing naïve lymphocytes with test peptide or were left unstimulated. For responses to PhCSP, the H-2K^d^-restricted epitope, P9, SYPSSAEKI, was used. To examine the breadth of the immune response, PBMCs or spleen from immunized mice were restimulated with pools of 15-mer peptides that span the length of PhCSP and overlap by 10 aa. The 66 peptides of PhCSP were tested in 6 pools of consecutive peptides, and each peptide was used at a final concentration of 5 μg/ml. Peptide PhCS51, which contains the dominant P9α-sequence, was excluded from these pools.

The sequences of other peptides used are as follows: p15 from Ag85A; TFLTSELPGWQLQARHVKPT, and the dominant H-2K^d^-restricted epitope from HBsAg; IQPSLDSWWTSL. Epitopes in the tumor epitope string expressed by MVA-T are as follows: H-2L^d^-restricted epitopes, LPYLGLWLVP from the P1A Ag of P815 cells and SPSTYVYHQF from gp70 of CT26 cells, and the H-2L^d^-restricted epitope from melanoma cell line A375 and KHYLFRNL. To examine the responses to MVA in C57/BL6 mice, the H-2K^d^-restricted epitope in the proteins BSR (aa 20–27, sequence TSYKKEVS), A47L (aa 138–146, sequence AAFEFINSL), and A19L (aa 47–55 sequence VSLDYINTM), as described by Tscharke et al. (26), were used. All peptides were used at a final concentration of 1 μg/ml. In all ELISPOT assays, cells were also stimulated with medium alone or with nonspecific peptide. In all cases, responses in these control wells were <80 spot-forming cell (SFC)/million cells. After overnight incubation, spots were developed as previously described and counted using an ELISPOT counter (AID).

P. berghei sporozoite challenge

Challenge was conducted with *P. berghei* (ANKA strain clone 234) sporozoites as described previously (1, 5, 25). In brief, mice were challenged by i.v. injection in the tail vein with sporozoites dissected from the salivary glands of infected female *Anopheles stephensi* mosquitoes and homogenized in RPMI 1640 medium. Mice were challenged at 28, 42, or 52 days after they had received anti-CD25 with or without vaccination. Infection was determined by the presence of ring forms in Giemsa-stained blood smears taken 7–14 days after challenge. Animals were challenged with 1000 sporozoites to provide a stringent liver stage challenge.

P. berghei blood stage challenge

Naive or anti-CD25 treated BALB/c mice were infected with 10^8 parasitized erythrocytes of *P. berghei* (ANKA strain clone 234) by the i.v. route. Parasitemia was monitored daily by microscopic examination of Giemsa-stained blood smears. Levels of parasitemia were calculated as the percentage of parasitized erythrocytes.

Statistical analysis

Statistical analysis was performed using SPSS for Windows, version 10 (SPSS). Unpaired Student’s t tests were performed to detect significant differences in the mean frequencies of T cells between animals that received the same vaccine in the presence or in the absence of anti-CD25 Ab. Differences between groups in the sporozoite challenge assay were determined using a χ^2 test with or without Mantel-Haenszel stratification, as appropriate. A value of p ≤ 0.05 was considered significant in all cases.

Results

Administration of anti-CD25 Ab significantly enhances immune responses induced by recombinant MVA immunization

IFN-γ-secreting CD8^+ T cell responses to the immunodominant MHC class I-restricted epitope, P9, of PhCSP (27) were examined in peripheral blood of BALB/c mice that had been immunized with MVA-CSP with or without i.p. injection of anti-CD25 Ab. Ab-treated, MVA-CSP-vaccinated mice had significantly higher Ag-specific CD8^+ T cells circulating in the blood compared with control animals that had been vaccinated with MVA-CSP alone (Fig. 1a). To simplify delivery of this vaccination approach, we examined immune responses induced when the anti-CD25 was coformulated with the recombinant vaccine vector and coinjected by the i.d. route (labeled “aCD25+ MVA-CSP ji.d.”). This strategy significantly increased the immune responses induced by MVA-CSP, compared with immunizing with MVA-CSP alone (Fig. 1a). Coinjecting the Ab and vaccine also demonstrates that prior binding of Ab to the CD25 molecule is not necessary to mediate this effect. No responses were observed in mice that received Ab alone without vaccine (data not shown).

Anti-CD25 Ab administration augments homologous prime-boost vaccine strategies

To examine the effect of anti-CD25 on homologous prime-boost virus vector-based vaccines, BALB/c mice that had received MVA-CSP with or without anti-CD25 by the i.p. or i.d. route were boosted with MVA-CSP 2 wk after this prime (Fig. 1, b and c). Analysis of P9-specific CD8^+ T cells in the blood or spleen after boosting demonstrated significantly greater immune responses in vaccinated mice that had received anti-CD25. Again, coformulating anti-CD25 mAb with the vaccine prime also resulted in a significant increase in the immune response. Unlike responses observed in peripheral blood, administration of anti-CD25 by the i.p. route resulted in slightly

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higher IFN-γ responses in the spleen compared with coformulating the Ab with the vaccine.

The enhancing effect of anti-CD25 administration on vaccine-induced immunity is not limited to MVA- or orthopoxvirus-based vaccines. When MVA is replaced by recombinant FP9 or ADV expressing PbCSP (FP-CSP or ADV-CSP respectively) (d-f), or it was mixed with MVA-CSP and co-administered by the i.d. route (labeled “[aCD25+MVA-CSP]id”). Control BALB/c mice were immunized with the corresponding vaccine. CD8+ T cell IFN-γ responses to the dominant Pb9 epitope were assessed in PBMCs on day 10 postvaccination (a and d). Mice were boosted with MVA-CSP on day 14. T cell responses in the blood were measured 10 days after this boost (b and e) by ELISPOT. T cell responses to Pb9 were assessed in the spleen 2 wk postboost (c and f). Columns represent the mean number of IFN-γ SFCs per million cells ± SEM (n = 14–30 mice/group), *, p < 0.05, and ***, p ≤ 0.001 compared with Ab-untreated vaccinated mice. Similar results were obtained in five independent experiments.

**FIGURE 1.** Anti-CD25 Ab enhances immune responses induced by homologous recombinant virus-vector prime-boost immunization. Anti-CD25 Ab was administered by an i.p. route at the same time as immunization with MVA-CSP (a–c), or with FP9 or ADV expressing PbCSP (FP-CSP or ADV-CSP respectively) (d–f), or it was mixed with MVA-CSP and co-administered by the i.d. route (labeled “[aCD25+MVA-CSP]id”). Control BALB/c mice were immunized with the corresponding vaccine. CD8+ T cell IFN-γ responses to the dominant Pb9 epitope were assessed in PBMCs on day 10 postvaccination (a and d). Mice were boosted with MVA-CSP on day 14. T cell responses in the blood were measured 10 days after this boost (b and e) by ELISPOT. T cell responses to Pb9 were assessed in the spleen 2 wk postboost (c and f). Columns represent the mean number of IFN-γ SFCs per million cells ± SEM (n = 14–30 mice/group), *, p < 0.05, and ***, p ≤ 0.001 compared with Ab-untreated vaccinated mice. Similar results were obtained in five independent experiments.

Administration of anti-CD25 Ab augments heterologous prime-boost vaccine strategies

Heterologous prime-boost vaccine regimens, where an alternative vaccine vector is used in the boost, generally induces stronger and more protective immunity compared with homologous prime-boosting (1–3, 5, 7). Ab-treated or control BALB/c mice were immunized with DNA-CSP or FP-CSP or ADV-CSP, and 2 wk later, all groups were boosted with MVA-CSP (Fig. 2). Anti-CD25 treatment in combination with vaccination significantly increased CD8+ T cell responses in the blood and the spleen compared with untreated vaccinated mice (Fig. 2, a and b). Administration of a control rat Ab (GL113) with FP/MVA immunization, instead of anti-CD25, did not significantly alter the immune responses compared with Ab-untreated vaccinated mice (data not shown).

Changes in levels of Foxp3+ cells in anti-CD25-treated, vaccinated mice

As CD25 is expressed on effector T cells, generated by immunization, as well as on CD4+CD25+ Tregs, it is not possible to examine the effect of anti-CD25 Ab on Tregs by surface phenotype. Therefore, we analyzed changes on the level of Foxp3 protein, the critical transcription factor of Treg cells, in CD4+ cells as a measure of fluctuations in Treg populations after mice were immunized with vaccine in the context of anti-CD25. It can be seen from Table I that anti-CD25 Ab administration results in a 44–65% decrease in the levels of Foxp3 expressing CD4+ cells compared with expression in CD4+ cells from naive mice. Interestingly, i.d. administration of anti-CD25 depletes more Foxp3-expressing cells in the lymph node compared with i.p. injection. In contrast, immunizing with vaccine alone resulted in a 7–8% decrease in Foxp3 expression.

Enhanced vaccine-induced CD4+ responses to M. tuberculosis and CD8+ responses to tumor epitopes

We used MVA expressing MVA85A (6) to determine whether anti-CD25 administration had an effect on CD4+ T cell responses to MHC class II-restricted epitopes, such as the Ag85A epitope, p15. Anti-CD25 Ab administration before MVA85A immunization resulted in a significant increase in the frequency of circulating p15-specific CD4+ T cells compared with the control immunized group (Fig. 3a). Furthermore, anti-CD25 administration before an immunization with MVA-T, which expresses a string of MHC class I-restricted tumor epitopes, resulted in significant increases in the circulating CD8+ T cell
responses to the CT26 carcinoma cell gp70 epitope (SPSYVHQF) and to the mastocytoma P815-specific epitope (LPYLGWLVF) when responses were examined 2 wk after a second immunization with MVA-T (Fig. 3).

It has been demonstrated previously that replicating vaccinia virus provides persistent TLR signals required for bypassing T cell-mediated tolerance to tumor epitopes (20). To examine if the effects of anti-CD25 treatment was specific to virus vector-based vaccines, we immunized mice depleted of CD25 with the Mycobacterium tuberculosis vaccine, BCG. As the kinetics of the induction of immune responses to Ag85A following BCG vaccination are slower than responses induced by MVA85A (7), BCG-immunized animals were boosted with MVA85A 4 wk, instead of 2 wk, after priming. Mice that were administered anti-CD25 Ab displayed an almost 3-fold increase in the levels of MHC class II-restricted CD4\(^+\)/H11001 T cells, specific for the p15 peptide, compared with control immunized mice (Fig. 4).

Table I. Reduction in Foxp3\(^+\)/H11001 CD4\(^+\)/H11001 levels in anti-CD25 Ab-treated mice

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>% Reduction in Foxp3(^+)/CD4(^+)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>FP i.d.</td>
<td>8.6</td>
<td>6</td>
</tr>
<tr>
<td>[0.5 mg of anti-CD25+FP] i.d.</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>MVA i.d.</td>
<td>7.4</td>
<td>6</td>
</tr>
<tr>
<td>[0.5 mg of anti-CD25+MVA] i.d.</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>ADV-CSP</td>
<td>7.4</td>
<td>6</td>
</tr>
<tr>
<td>[0.5 mg of anti-CD25+ADV] i.d.</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>0.5 mg of anti-CD25 i.d.</td>
<td>44 (a)</td>
<td>6</td>
</tr>
<tr>
<td>0.5 mg of anti-CD25 i.d.</td>
<td>64 (b)</td>
<td></td>
</tr>
<tr>
<td>1 mg of anti-CD25 i.p.</td>
<td>44 (a)</td>
<td>6</td>
</tr>
<tr>
<td>1 mg of anti-CD25 i.p.</td>
<td>53 (b)</td>
<td></td>
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</table>

\(p \leq 0.05\), \(p \leq 0.01\), and \(p \leq 0.001\) compared with Ab-untreated mice that were immunized with the same vaccine regime. Similar results were obtained in two independent experiments.

It has been demonstrated previously that replicating vaccinia virus provides persistent TLR signals required for bypassing T cell-mediated tolerance to tumor epitopes (20). To examine if the effects of anti-CD25 treatment was specific to virus vector-based vaccines, we immunized mice depleted of CD25 with the M. tuberculosis vaccine, BCG. As the kinetics of the induction of immune responses to Ag 85A following BCG vaccination are slower than responses induced by MVA85A (7), BCG-immunized animals were boosted with MVA85A 4 wk, instead of 2 wk, after priming. Mice that were administered anti-CD25 Ab displayed an almost 3-fold increase in the levels of MHC class II-restricted CD4\(^+\)/H11001 T cells, specific for the p15 peptide, compared with control immunized mice (Fig. 4).
ter this priming immunization. CD4+ vaccinated mice were boosted with MVA85A 4 wk after this priming immunization. CD4+ T cell IFN-γ responses to the p15 epitope were assessed in spleen cells 2 wk postboost. Columns represent the mean number of IFN-γ SFCs per million splenocytes ± SEM (n = 4 mice/group). *, p < 0.05, compared with Ab-untreated vaccinated mice. Similar results were obtained in four independent experiments.

Therefore, anti-CD25 treatment enhances both CD4+ and CD8+ T cell responses to Ags of tumor, bacterial, and parasitic origins.

Anti-CD25 treatment enhances T cell responses induced by a recombinant subunit protein vaccine

To examine if the augmentation by anti-CD25 was restricted to live or vectored vaccines, we assessed the immune response induced when a licensed recombinant subunit hepatitis B virus vaccine, Engerix-B (GSK Biologicals), was administered to mice in the presence or in the absence of anti-CD25 Ab. Administration of anti-CD25 before immunization with Engerix-B, which is composed of the HBsAg formulated with alum, significantly increased the MHC class I-restricted response (Fig. 5). This demonstrates that the enhancing effect of anti-CD25 treatment also works on immune response to foreign Ags through a mechanism that is independent of viral or bacterial infection and stimulation of known TLRs.

Anti-CD25 treatment enhances the T cell response induced by vaccination to subdominant epitopes

It has been suggested that a T cell response to a broad range of epitopes may reduce or avoid selection of pathogen escape mutants and may provide increased protection against viral or parasitic challenge (28–30). Using pools of overlapping peptides spanning the entire sequence of PbCSP, but omitting the peptide containing the dominant Pb9 epitope, we examined the breadth of the immune response induced by different prime-boost vaccine regimes in the presence or in the absence of anti-CD25 (Fig. 6). Coadministration of anti-CD25 in the prime with MVA-CSP significantly increased the T cell responses to subdominant epitopes present in all peptide pools (Fig. 6). Increased responses were observed to all six peptide pools in Ab-treated compared with untreated vaccinated mice for both heterologous (FP/MVA) and homologous (MVA/MVA) prime boost regimes. Injection of an isotype control Ab, GL113, had no significant effect on broadening the immune response (Fig. 6 and Table II). Furthermore, no T cell responses were observed in mice that received Ab without vaccine, and responses were not observed when cells from immunized mice were stimulated with irrelevant peptides (data not shown). We also observed a 3- to 4-fold increase in the breadth of the immune responses to PbCSP in the spleen of mice that were homologously immunized twice with MVA, ADV, or FP (data not shown). Total responses to PbCSP, which were not due to recognition of Pb9, were analyzed as a frequency of the responses to the entire protein (including Pb9 responses) (Table II). It can be seen that coadministration of anti-CD25 with an MVA-CSP prime significantly increases the frequency of T cells responding to subdominant epitopes from 8 to 32% of the total T cell response, and as such, results in a less focused response to the immunodominant epitope, Pb9. Mice that received anti-CD25 i.p. and MVA-CSP i.d., or anti-CD25 mixed with FP-CSP, had increased responses to subdominant epitopes as well as Pb9, and the frequency of non-Pb9 to Pb9 responses were maintained, demonstrating that there was no further skewing toward the dominant epitope in the presence of anti-CD25. In no vaccine regimen tested was there preferential enhancement of the Pb9 response compared with subdominant epitopes.

We also examined the broadening effect of anti-CD25 Ab on immune response to subdominant epitopes in MVA proteins using MVA-T in C57/BL6 mice. In this case, we looked at the fold increase in responses when anti-CD25 was included in the vaccination because the entire response to a protein was not being investigated. Coadministration of anti-CD25 with MVA-T doubled the responses in the spleen to the dominant epitope in the B8R protein; however, it resulted in a 4- to 8-fold increase in the T cell responses to the subdominant epitopes in the A19L and A47L protein and to the weak tumor epitopes (AQHPNAELL and KHYLFRNL) expressed by the recombinant MVA-T (Table III). The fold increases in these responses were more significant in the lymph nodes draining the immunization site compared with the spleen.
Anti-CD25 treatment increases T cell response induced by vaccination to subdominant epitopes. On day 0, anti-CD25 Ab was administered by the i.p. route to BALB/c mice before FP-CSP or MVA-CSP immunization, or it was coadministered with MVA-CSP and injected by the id route. Ab-treated or control vaccinated mice were boosted with MVA-CSP on day 14. Circulating T cell responses in PBMCs to PfCSP were assessed 2 wk after the second immunization using six pools of 15-mer peptides overlapping by 10 aa. Responses to individual pools are shown in a. The response to the entire protein (excluding Pb9) was assessed by summing the responses to each pool (b). Columns represent the mean number of IFN-γ SFCs per million cells ± SEM (n = 5 mice/group). **, p ≤ 0.01, and ***, p ≤ 0.001, compared with Ab-untreated vaccinated mice. Similar results were obtained in three independent experiments. No responses were observed in control wells that were not stimulated with Ag.

Anti-CD25 treatment maintains the effector CD8+ T cell response induced by vaccination at a higher level over time compared with untreated homologous or heterologous prime boost vaccination

We examined the immune response induced by combining anti-CD25 treatment with homologous MVA-CSP or heterologous FP/MVA-CSP immunization over the course of 100 days. We also assessed the effect of the dose of Ab on the longevity of the induced response by administering only 0.5 mg of anti-CD25, either by the i.p. or i.d. route. Finally, we assessed the effect of coadministering the Ab with the vaccine, compared with giving it by the i.p. route on the maintenance of the immune response. It has been demonstrated that anti-CD25 is undetectable in the serum 21 days postinjection (19). The peak of the immune response in the periphery was observed when responses were examined 28 days after priming (Fig. 7). In control immunized animals, heterologous FP-CSP/MVA-CSP induced a higher CD8+ T cell response for longer compared with two MVA-CSP immunizations. Treatment with anti-CD25 Ab significantly increased all vaccine-induced responses at 28 and 42 days after the priming immunization compared with Ab-untreated, vaccinated controls. Analysis of circulating CD8+ T cell responses in peripheral blood at days 69 and 100, when the immune system is fully replete after Ab administration, demonstrated that anti-CD25 in combination with FP-CSP/MVA-CSP induced the strongest response that was sustained. Mice that had received 1 mg of anti-CD25 and were immunized twice with MVA-CSP still had significantly higher CD8+ T cell responses compared with untreated immunized controls at days 69 and 100. However, the T cell responses in mice that had only received 0.5 mg of Ab had returned to the same level as that observed in control MVA-CSP prime-boosted mice by day 69. Overall, the results demonstrate that addition of 1 mg of anti-CD25 Ab to the priming vaccine not only enhances the immunogenicity at the peak time point, but it also augments the durability of this increased response.

Anti-CD25 administration in conjunction with vaccination improves protective efficacy against a malaria sporozoite challenge

To assess whether the augmented immunogenicity observed by anti-CD25 treatment and immunization resulted in increased protective efficacy, Ab-treated or -untreated mice that were primed with FP-CSP and boosted with MVA-CSP immunization were challenged with P. berghei sporozoites (Table IV). Interestingly, we observed that administration of anti-CD25 alone resulted in

Table II. Frequency of T cells responding to subdominant epitopes in the total population of IFN-γ-secreting cells

<table>
<thead>
<tr>
<th>Immunization</th>
<th>Pb9 (SFC/Million PBMCs) ± SE</th>
<th>Summed Responses to OLPs (SFC/Million PBMCs) ± SE</th>
<th>OLP as % of total IFN-γ-responding cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FP/MVA</td>
<td>22,660 ± 2,772</td>
<td>3,372 ± 854</td>
<td>13 ± 2.3</td>
</tr>
<tr>
<td>[Anti-CD25 + FP] i.d./MVA</td>
<td>46,488 ± 11,260</td>
<td>6,291 ± 1,857</td>
<td>12 ± 2.4</td>
</tr>
<tr>
<td>MVA/MVA</td>
<td>8,448 ± 1,772</td>
<td>625 ± 126</td>
<td>8 ± 1.6</td>
</tr>
<tr>
<td>Anti-CD25 i.p. MVA i.d./MVA</td>
<td>16,423 ± 2,482</td>
<td>876 ± 144</td>
<td>5 ± 1.2</td>
</tr>
<tr>
<td>[Anti-CD25 + MVA] i.d./MVA</td>
<td>16,334 ± 3,475</td>
<td>7,222 ± 863</td>
<td>32 ± 3.9</td>
</tr>
<tr>
<td>GL113 i.p. FP/MVA</td>
<td>34,706 ± 5,375</td>
<td>3,293 ± 536</td>
<td>9 ± 1.4</td>
</tr>
</tbody>
</table>

*Responses to individual overlapping peptide pools (OLPs) were summed, and the frequency of these responses in the total population of IFN-γ-secreting T cells (that respond to Pb9 and OLP) were calculated as a percentage of the total responding population.
significantly enhanced the strength, breadth, and duration of the and, more interestingly, coformulated with the priming vaccine, ministering this Ab around the time of the primary vaccination of C57/BL6 mice that had been immunized with MVA-T alone or with MVA-T coadministered with anti-CD25 Ab.

The enhanced durability of immunity induced by FP/MVA vaccination compared with Ab-untreated immunized mice (Table IV). The enhanced durability of immunity induced by this strategy approached the level induced by heterologous FP/MVA prime-boost vaccine strategies using vaccines based on virus vectors, plasmid DNA, bacteria, as well as recombinant subunit vaccines and increased protection against pathogen challenge. We also demonstrate that this approach works for multiple Ags of diverse non-self-origin. This is the first study to demonstrate that administration of anti-CD25 Ab significantly enhances CD4+ T cell responses to foreign pathogen Ags and to Ags that are presented by nonreplicating, attenuated viral vectors or whole protein subunit vaccines that are applicable to human use.

The finding that blocking CD25 can substantially enhance numbers of vaccine-induced activated T cells is perhaps surprising given that CD25 is widely regarded as a marker of activated T cells. Indeed, monoclonal anti-CD25 is in clinical use to reduce rates of acute graft rejection after transplantation, presumably by reducing activated T cell effects. One resolution may be that the levels of CD25 appear higher and more stable on Tregs than activated effector cells (31, 32). Enhancement of immunity to a vaccine based on a subunit foreign Ag (HBsAg) in alum also demonstrates that this effect appears independent of TLR signaling.

We find that homologous MVA prime-boost regimes are significantly enhanced when anti-CD25 is used in the prime. Efficacious homologous prime-boost vaccines may be easier to administer and deploy than heterologous regimes and lead to increased uptake in the field. The immune response induced by this strategy approached the level induced by heterologous FP/MVA prime-boost at the peak of the immune response and was maintained at approximately the same level as FP/MVA up to 100 days after

### Table III. Coadministration of anti-CD25 with MVA-T broadens the immune response to MVA subdominant epitopes and to the encoded Ag

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>MVA-T</td>
<td>1561 ± 4 SFC/10^6</td>
<td>23 ± 4 SFC/10^6</td>
<td>45 ± 4 SFC/10^6</td>
<td>27 ± 2 SFC/10^6</td>
<td>85 SFC/10^6</td>
</tr>
<tr>
<td>[Anti-CD25 + MVA-T]</td>
<td>4549 ± 169 SFC/10^6</td>
<td>111 ± 14 SFC/10^6</td>
<td>201 ± 25 SFC/10^6</td>
<td>212 ± 22 SFC/10^6</td>
<td>269 SFC/10^6</td>
</tr>
<tr>
<td>Fold increase: spleen</td>
<td>2.9</td>
<td>4.8</td>
<td>4.5</td>
<td>8.0</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Lymph nodes (pooled)

| MVA-T  | 580 SFC/10^6 | 45 SFC/10^6 | 17 SFC/10^6 | 18 SFC/10^6 | 46 SFC/10^6 |
| [aCD25 + MVA-T] | 1280 SFC/10^6 | 307 SFC/10^6 | 217 SFC/10^6 | 192 SFC/10^6 | 256 SFC/10^6 |
| Fold increase: LN | 2.2 | 6.8 | 12.8 | 10.7 | 5.6 |

a The fold increase in IFN-γ T cell responses to epitopes in the presence or absence of anti-CD25 was calculated. Responses (IFN-γ SFC/10^6 cells) were examined in spleens of C57BL6 mice that had been immunized with MVA-T alone or with MVA-T coadministered with anti-CD25 Ab.
priming. However, the vaccine itself must be capable of inducing strong immunity; homologous FP- or ADV-based immunization with anti-CD25 was significantly stronger compared with Ab-untreated vaccinated controls, but the strength of these responses still remained lower compared with heterologous prime-boost immunization. So the enhancement provided by anti-CD25 appears to be proportionate to the magnitude of the natural immunogenicity of the vaccination approach without Ab.

Immunodominance is a hurdle to vaccine development as narrow responses may favor the generation of pathogen escape mutants (33–35). We observed that anti-CD25 treatment also significantly enhanced the breadth of the immune responses induced by prime-boost immunization. Of the vaccine regimens tested, the greatest enhancement occurred when anti-CD25 was used in conjunction with MVA/MVA immunization. Coadministering the Ab with the priming vaccine by the i.d. route resulted in significantly greater peripheral T cell responses to subdominant epitopes compared with injecting the Ab at a distal site (Fig. 6 and Table II). We confirmed this finding by examining the T cell responses to dominant and subdominant H-2Kb epitopes in MVA proteins and to the encoded epitope string (Table III), demonstrating that the broadening effect of anti-CD25 is not limited to a malaria vaccine model. This suggests that the peripheral T cell response to weaker epitopes is favored when CD25 molecules are bound by anti-CD25 at the local lymph node or immunization site where the immune response is being generated by vaccination. If anti-CD25 Ab is functioning solely through depleting Tregs, it suggests that the Treg population is at least partially responsible for the epitope dominance hierarchy induced by vaccination. This potential new role for CD4+ CD25+ Tregs may thus provide an additional mechanism contributing to the selection of immunodominant epitopes and has been discussed in a recent study (36). In this study, it was proposed that dominance ranking was maintained, but in their replicating recombinant vaccinia virus model, anti-CD25 treatment enhanced the difference between T cells responding to dominant and subdominant epitopes, as Ab treatment selectively enhanced responses to the dominant epitope. We demonstrate that this is not a universal phenomenon; it depends on the regime of Ab and vaccine administration and assessing responses to the entire protein compared with examining a selection of known epitopes within the protein. We observed that administering a single mixed formulation of Ab and vaccine significantly enhances the frequency of T cells that respond to subdominant epitopes, therefore inducing a stronger response that is less focused on Pb9. This contrasts with findings in the vaccinia virus model (36). The significance of this effect is at least partly dependent on the vector used in the priming immunization. When FP-CSP is used instead of MVA-CSP, responses to subdominant epitopes are moderately enhanced when anti-CD25 is included in the vaccine, with a skewing toward increased responses to peptide pool 6. However, the proportion of T cells that recognize subdominant regions of the protein is the same in Ab-treated and -untreated, FP/MVA-immunized animals.

![FIGURE 8. Anti-CD25 treatment has no effect on P. berghei blood stage infection. BALB/c mice were injected with 1 mg of anti-CD25 Ab by the i.p. route the day before i.v. infection with 10^4 parasitized RBCs. Levels of parasitemia were monitored daily in these mice and in control, naive mice as described in Materials and Methods.](http://www.jimmunol.org/)
This demonstrates that there is not a selective effect of anti-CD25 on T cells responding to dominant epitopes; in this system, T cell responses to all epitopes are enhanced to equivalent proportional extents. A possible reason for the contrasting findings may relate to the replication competence of the viral vectors used in the different studies. Thus, anti-CD25 treatment at the time of vaccination provides a strategy for preventing a narrow immune response to dominant epitopes, and broader immunity induced in this way may result in increased efficacy for vaccines against some poly-morphic pathogens such as HIV (28) and *P. falciparum*.

A single shot prime would be preferable to multiple injections of Ab and vaccine by different routes or over the course of several days. We demonstrate that this is achievable with this coadministered Ab and vaccine formulation, and the single shot vaccine is effective at inducing responses to subdominant epitopes. This contrasts with recent reports suggesting that the enhancing effect of anti-CD25 was only observed when it was given days before immunization (21). In contrast to other studies that focused on Treg control of memory T cells (22, 37, 38), our study focuses on the effect of anti-CD25 Ab and a possible role for its effects on CD4^+^CD25^+^ Tregs on the induction of a primary immune response in a normal, naive host and demonstrates the powerful regulation by Treg or CD25 in a normal, naive animal of effector and memory CD4^+^ and CD8^+^ T cell induction by diverse vaccine types. Removal of this suppression at the same time as the prime significantly increases the vaccine-induced immune response. The lack of dependence on giving the Ab by an i.p. route days before immunization permits the use of a single formulation.

The precise mechanisms involved in the enhancement of vaccine-induced immunity by anti-CD25 Ab treatment remain to be fully elucidated. Administration of certain anti-CD25 Abs to mice has become an accepted method of depleting CD4^+^CD25^+^ Tregs (39). CD4^+^CD25^+^ Treg function through cytokine-dependent and -independent methods to suppress CD4^+^ and CD8^+^ T cell responses to self-Ags and thus to maintain tolerance and prevent autoimmunity (39). It has been shown in vitro that activated Tregs are Ag independent in their suppressive function (40). If anti-CD25 is solely functioning by depleting these cells, we demonstrate here that in a nondepleted normal animal, Tregs exert a suppressive effect on vaccine immunogenicity during immunization that affects a broad range of vaccine types. This regulatory response dampens the strength and duration of the effector T cell response and inhibits the induction of strong T cell responses to subdominant epitopes. However, the removal of this subset of T cells may not be the only reason for the enhancement of immune responses observed when mice are vaccinated in the presence of anti-CD25 Ab. The anti-CD25 Ab used here, PC61, blocks IL-2 binding to CD25 (41, 42) and may function by preventing IL-2-induced, activation-induced cell death of effector T cells specific for the vaccine Ag during the contraction phase of the immune response (43, 44). Alternatively, blocking IL-2 stimulation of Tregs may be of greater importance. Tregs are more dependent on IL-2 for their survival and function than effector T cells (45). As IL-2 transcription is severely inhibited in Tregs, these cells depend on paracrine IL-2 for their growth and survival (45, 46). It is conceivable that the IL-2 produced locally by vaccine-induced effector T cells stimulates Treg activation and proliferation and supports their suppressive function. Preventing IL-2, the main Treg survival factor, from binding to its receptor may prevent the expansion of this population and thus reduce the magnification of an inhibitory signal. We demonstrate that coadministration of anti-CD25 with a vaccine results in an approximate 60% reduction in the level of Foxp3^+^CD4^+^ cells in peripheral blood (Table I). This suggests that complete depletion of Tregs, defined by expression of Foxp3, is not required to mediate significant enhancement by anti-CD25 of vaccine-induced immunity. This may suggest that the level of Treg depletion is not solely responsible for anti-CD25 enhancement of the breadth and duration of the immune response. The relative importance of anti-CD25 effects on Tregs compared with IL-2 binding and signaling is currently under investigation.

As further evidence of the enhancing effect of anti-CD25 on vaccine-induced immunity, we observed increased protection against parasite challenge in groups that were immunized in the context of anti-CD25. At an early time after Ab treatment (day 28), pre-erythrocytic protection was also observed in the Ab-treated, nonvaccinated animals. We observed identical parasite growth curves in anti-CD25-treated or untreated naive mice that were challenged with blood-stage *P. berghei* parasites, in agreement with a previous study (47), demonstrating that anti-CD25-mediated protection occurs at the liver stage of *P. berghei* infection. However, when challenge is performed at 42 or 52 days after Ab treatment, at a time when the anti-CD25 Ab is undetectable in the serum (19) and immune responses in immunized, Ab-treated animals are still at a high level (Fig 7), greater protection against malaria challenge is observed in mice who were earlier vaccinated in the context of anti-CD25. Vaccine-induced protection against *P. berghei* sporozoite challenge correlates with immune responses to the Pb9 epitope (5), and the broader responses to subdominant epitopes does not seem to further increase this efficacy. However, it remains to be tested if the broader responses induced by the inclusion of anti-CD25 Ab to a vaccine enhances protective efficacy against other pathogens.

The anti-CD25 mAbs, daclizumab and basiliximab, have been licensed for clinical use in the context of renal transplantation (48) and appear effective in the treatment of uveitis and multiple sclerosis (49). The side effects of this treatment appear to be minimal, and the success of this treatment in reducing acute rejection episodes following solid organ transplantation has been remarkable (48). In contrast to mAbs to CTLA4 (50), there appears to be no evidence that depletion of Tregs with anti-CD25 can lead to any autoimmune phenomena in humans. Thus, a combination of anti-CD25 and vaccination may provide a successful immunotherapeutic approach for infectious diseases and tumors and, if initial evidence of safety is confirmed, could provide an improved strategy for prophylactic as well as therapeutic vaccination against multiple pathogens.

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**Disclosures**

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**References**


