

Daclizumab Treatment Reduces Activated T Cells: Results from the CHOICE Multiple Sclerosis Study

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Abstract

OBJECTIVE: To examine the effect of daclizumab (DAC), a humanized antibody against CD25, on activated T cells.
BACKGROUND: Aberrant T-cell activation is common in multiple sclerosis (MS) patients. DAC binds to CD25 and blocks proinflammatory signaling dependent on the high affinity IL-2 receptor.
METHODS: CHOICE was a randomized, double-blind, placebo-controlled study involving 230 relapsing MS patients. Patients received DAC 2 mg/kg every 2 weeks (high dose), DAC 1 mg/kg every 4 weeks (low dose), or placebo subcutaneously added to interferon beta therapy for 24 weeks. Among patients who participated in a pharmacodynamic sub-study, 63 had evaluable baseline and post-dose samples for analysis using flow cytometry. Blood was collected longitudinally, and absolute levels of activated T cells were determined using antibodies specific to HLA-DR, CD62L, and CD25.
RESULTS: Rapid reductions in absolute activated T cells were observed among patients in both DAC dose groups, but not in placebo-treated patients. At the last sampling at the end of the treatment period (week 22), mean levels in HLA-DR⁺CD4⁺ T cells were significantly lower in both DAC dose groups compared with placebo (DAC low dose vs placebo: 22.5 vs 37.4 cells/ μ L, $p=0.006$; DAC high dose vs placebo: 23.1 vs 37.4 cells/ μ L, $p=0.009$). Reductions in HLA-DR⁺CD4⁺ T cells reversed following discontinuation of DAC treatment. Similar changes in activated T cells among DAC-treated patients were seen when assessed by CD62L and CD25 expression.
CONCLUSIONS: DAC therapy produced sustained yet reversible reductions in activated T cells during treatment. This modulation of T-cell activation may contribute to DAC efficacy in MS.

Background

- Inadequate control of autoreactive, activated T cells is a hallmark of autoimmune diseases, including multiple sclerosis (MS)
- Activated T cells require the high affinity interleukin-2 (IL-2) receptor to respond to IL-2
- Daclizumab (DAC) is a humanized monoclonal antibody that blocks IL-2 from interacting with the alpha chain subunit (CD25) of the high affinity IL-2 receptor, selectively inhibiting high affinity IL-2 receptor-mediated responses
- The CHOICE study was a Phase II, randomized, double-blind, placebo-controlled, multicenter study of subcutaneous (SC) DAC added to interferon beta (IFN) for treatment of active and relapsed forms of MS
 - Primary efficacy endpoint was total number of new or enlarged (> 50% increase if < 5 mm diameter lesion, and \geq 20% increase for a lesion \geq 5 mm) gadolinium contrast enhancing lesions (Gd CELs) on monthly brain magnetic resonance imaging between weeks 8 and 24
 - Three dose arms were evaluated
 - IFN + placebo, q2wk x 16, SC (n=77)
 - IFN + DAC low-dose arm: 1 mg/kg q4wk x 8, SC (n=78)
 - IFN + DAC high-dose arm: 2 mg/kg q2wk x 16, SC (n=75)
 - Only DAC high-dose arm showed significant reduction in Gd CELs between weeks 8 and 24
- ~30% of CHOICE subjects consented to participate in optional pharmacokinetic and pharmacodynamic assessments

Methods

- CD25 receptor occupancy by DAC was monitored via flow cytometry (FACS) using a competitive anti-CD25 antibody (clone 2A3) on whole blood samples freshly analyzed within 24–48 hours of collection. Complete loss of 2A3 binding indicates CD25 saturation by DAC
- CD25 receptor expression was monitored using an anti-CD25 antibody (MA251) that does not compete with DAC for binding to CD25
- Daclizumab effects on absolute T cell counts were determined using fluorescently labeled antibodies, TruCOUNT™ and FACS
- Parametric (paired t test) and non-parametric (Wilcoxon) analyses were performed comparing levels of immune subset cell counts between dosing groups
- Individual DAC exposure characteristics (results of *post hoc* analysis) from a subset of subjects, including steady-state trough ($C_{ss,min}$), and AUC_{ss} , were used, separately, as predictors to model changes from baseline level in individual immune subsets or changes over time (calculated area under the change from baseline-time curve [AUC]). Relationship between changes from baseline level of putative activated T cell subsets and total new or enlarged Gd CELs between weeks 8 and 24 was also evaluated using linear correlation, negative binomial correlation, analysis of variance or Kruskal-Wallis Tests statistical analysis approaches

Results

Rapid, reversible, reductions in CD25⁺ T cells during the DAC treatment phase for both dosing groups

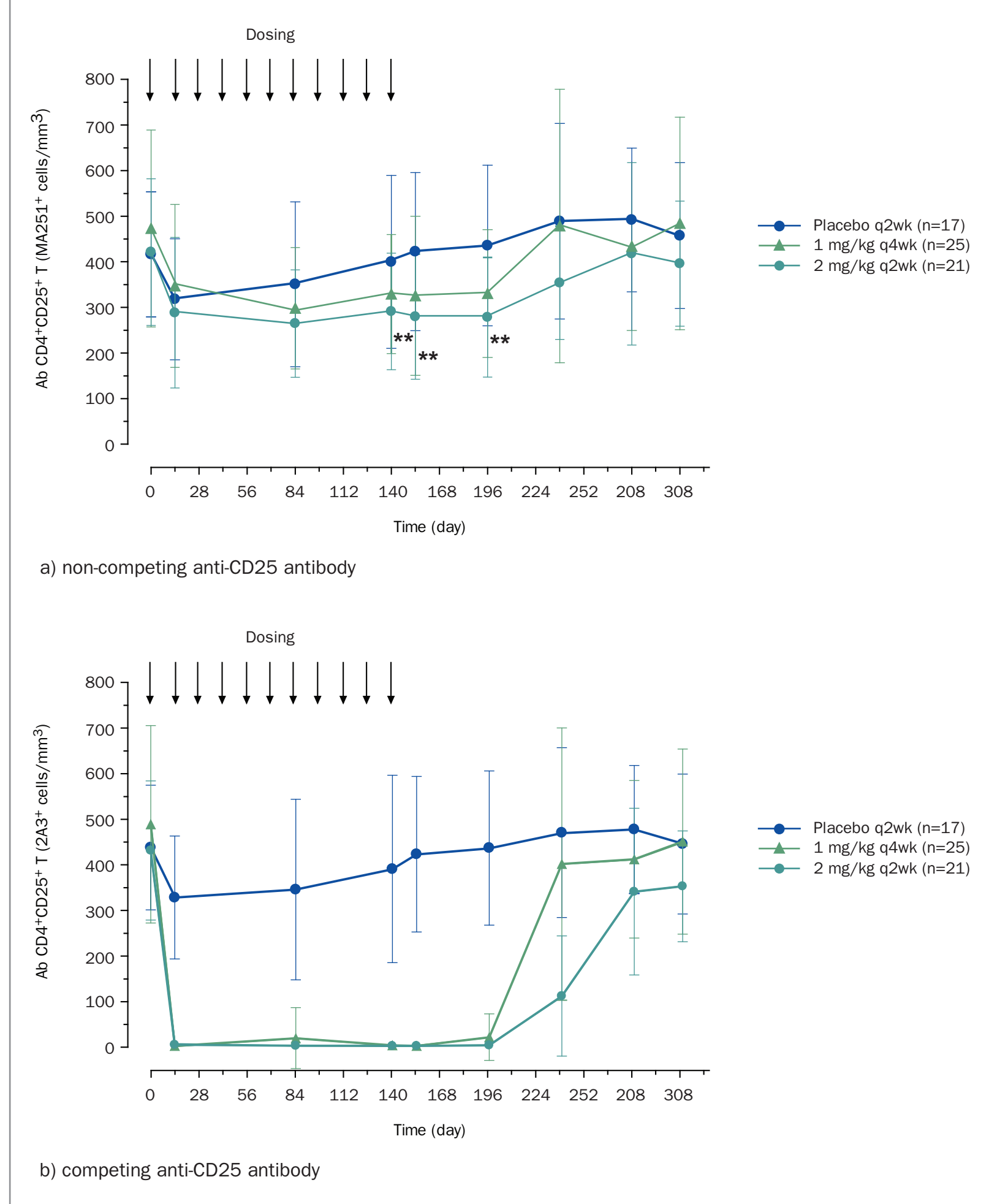


Figure 1. Decreases from baseline in levels of absolute CD25⁺ T cells (a) were observed by flow cytometry for both daclizumab (DAC) dosing groups when using an antibody that does not compete with DAC for CD25 binding, clone MA251. Statistically significant reductions ($p < 0.05$) in comparison to placebo were only observed for the DAC high-dose group and are indicated (**). Kinetics of CD25⁺ T cell saturation and desaturation (b) were determined using an antibody that competes with DAC for CD25 binding, clone 2A3. DAC has previously been shown to directly down-regulate CD25 expression on activated T cells. Absolute levels of CD25 positive staining T cells returned to approximate baseline levels at the time of CD25 de-saturation and mean recovery time was longer in the DAC high dose group, consistent with its longer time to desaturation

L-selectin (CD62L) negative CD4⁺ T cell

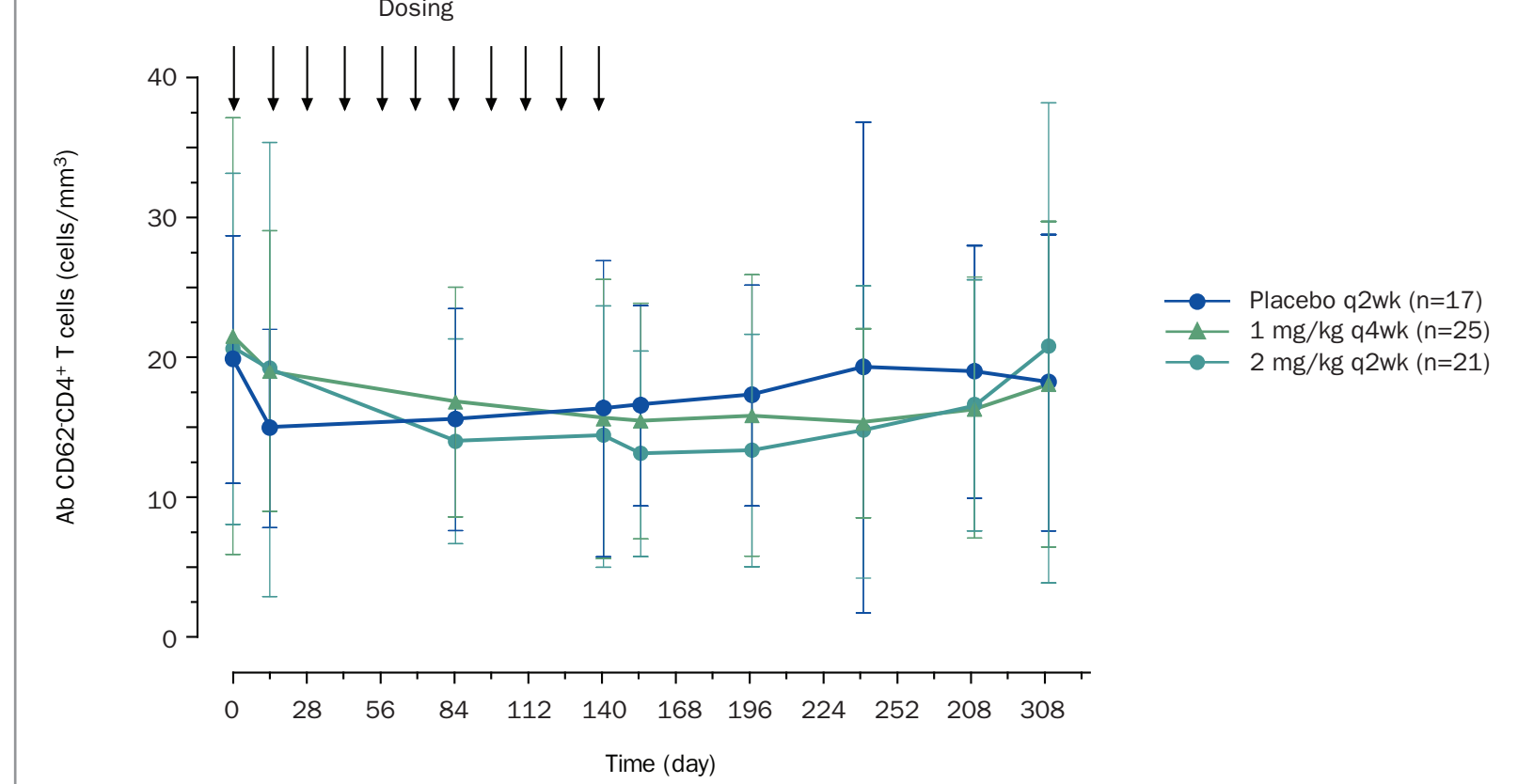


Figure 2. Absolute levels of L-selectin negative (CD62L-) staining CD4⁺ T cells were monitored using flow cytometry. No statistically significant reductions were observed in L-selectin staining CD4⁺ T in comparison to placebo levels

Rapid, reversible, reductions in HLA-DR⁺ activated T cells during the DAC treatment phase

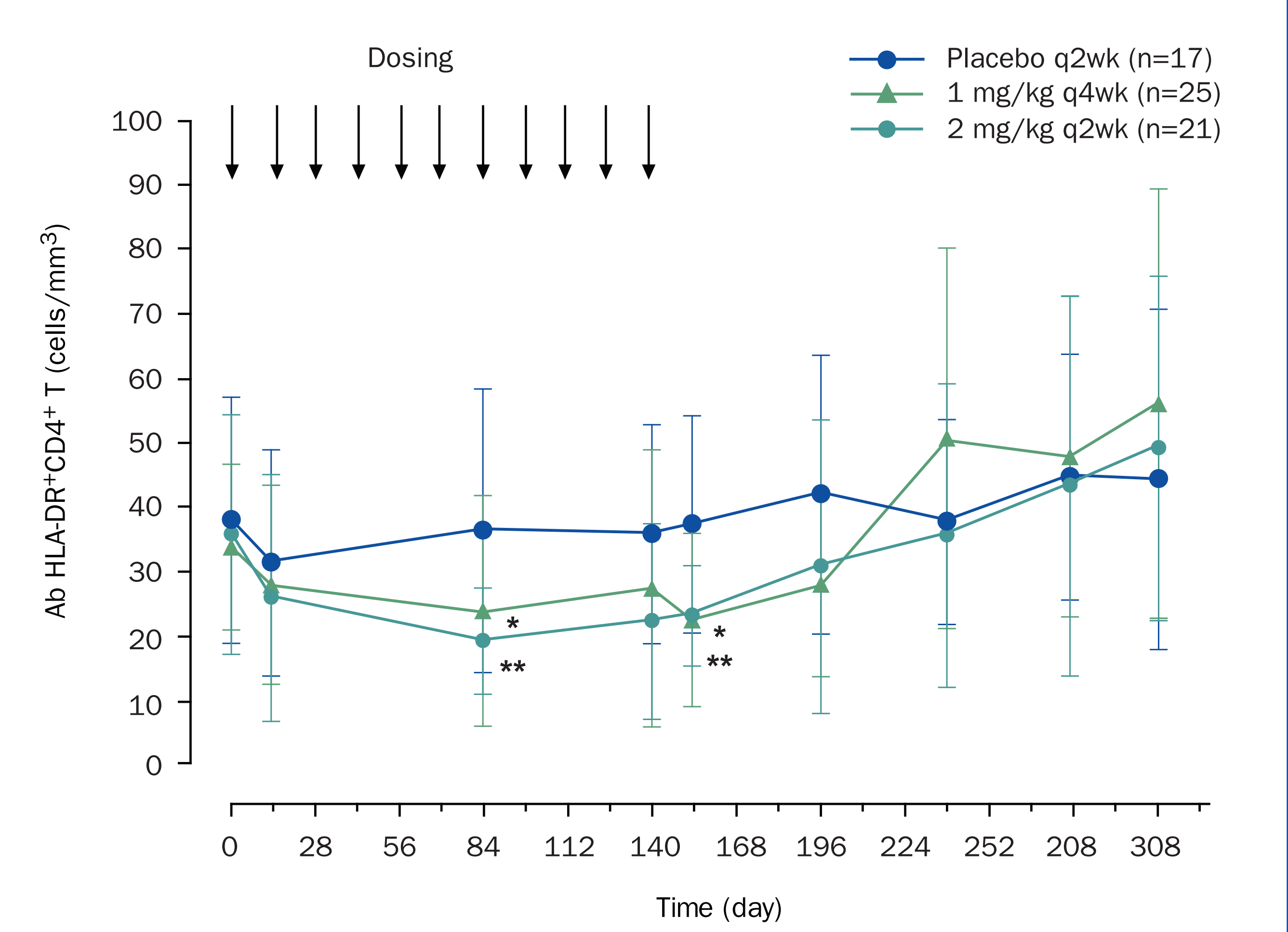


Figure 3. Decreases from baseline in levels of absolute HLA-DR⁺ activated CD4⁺ T cells were observed for both daclizumab (DAC) dosing groups. Statistically significant reductions ($p < 0.05$) in comparison to placebo are indicated (* DAC low dose and ** DAC high dose). Absolute levels of HLA-DR⁺ activated CD4⁺ T cells generally returned to approximate baseline levels at the time of CD25 de-saturation between days 196 and 280

Relationship between DAC steady-state AUC or trough levels ($C_{min,ss}$) and reductions in HLA-DR⁺ activated T cell counts when expressed as AUC over time

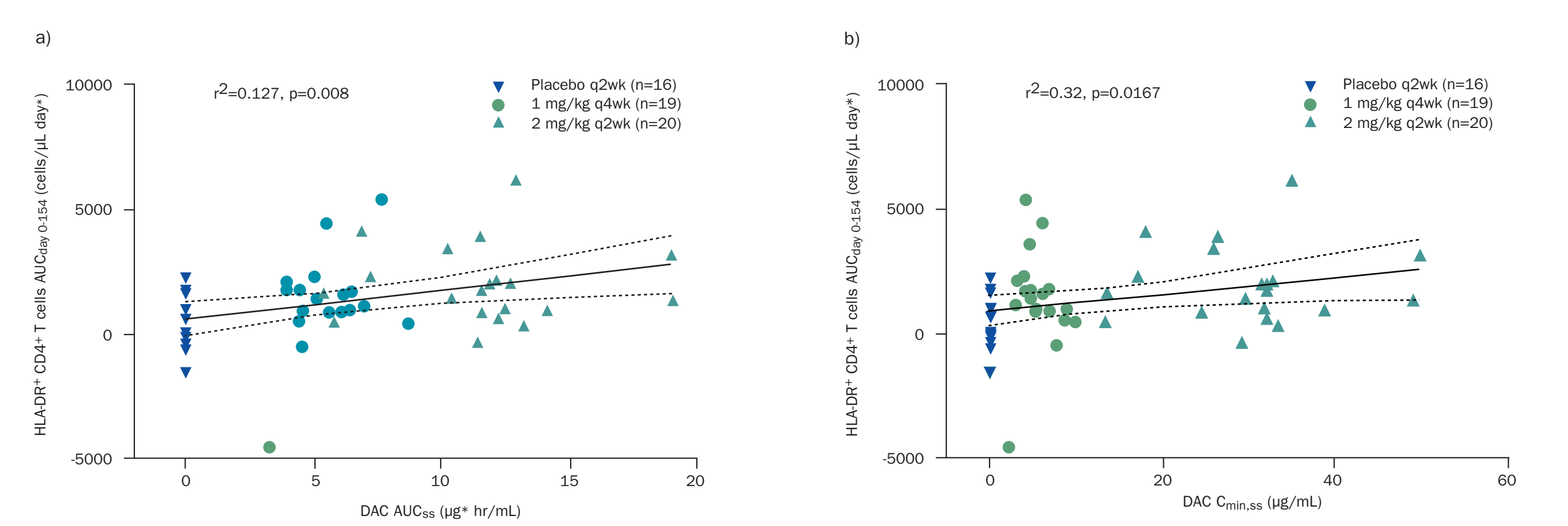


Figure 4. A statistically significant ($p=0.008$) positive linear relationship was observed (a) between individual daclizumab (DAC) exposure at steady state and subject's reductions from baseline in levels of absolute HLA-DR⁺CD4⁺ T cell counts over time (within the dosing period), when each factor was expressed in terms of area under the curve (AUC). When examining the effect of DAC treatment on HLA-DR⁺CD4⁺ T cell counts AUC using analysis of variance, a treatment effect was only significant for DAC high-dose group (DAC high dose vs placebo: $p=0.0103$ whereas DAC low dose vs placebo: $p=0.1044$). The exposure-response relationship between HLA-DR⁺CD4⁺ T cell count reductions over time vs steady state DAC trough levels ($C_{ss,min}$), as well as the Pearson correlation are shown (b)

Conclusions

- CD25 levels, a marker of immune cell activation, were rapidly reduced on T cells in both DAC dosing groups. Levels generally recovered to baseline at the time of CD25 de-saturation by DAC, between days 196 and 280 (longer recovery time in DAC high-dose group)
- Rapid, reversible, reductions in HLA-DR⁺ activated T cells were observed during the DAC treatment phase for both dosing groups
- A significant, dose-dependent, positive relationship was observed between DAC exposure (AUC_{ss}) and reductions in HLA-DR⁺ CD4⁺ T cell counts (AUC) over time
- Exposure-dependent reductions in activated T cell numbers were consistent with the exposure-dependent efficacy response for new Gd lesion formation that was seen in the CHOICE trial
- Further study is warranted to define whether the effects of DAC in reducing activated T cells may be an effect-mediator or efficacy biomarker of DAC therapy