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Adenovirus-Specific Immunity Following Immunization with an Ad5 HIV-1 Vaccine Candidate in Humans

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Abstract

The immunologic basis for the potential enhanced HIV-1 acquisition in Ad5 seropositive individuals who received the Merck rAd5 HIV-1 vaccine in the STEP study remains unclear. Here we show that baseline Ad5-specific neutralizing antibodies are not correlated with Ad5-specific T lymphocyte responses and that Ad5 seropositive subjects do not develop higher vector-specific cellular immune responses as compared with Ad5 seronegative subjects following vaccination. These findings challenge the hypothesis that activated Ad5-specific T lymphocytes were the cause of the potential enhanced HIV-1 susceptibility in the STEP study.

In the phase 2b efficacy study (the STEP study) evaluating the Merck recombinant adenovirus serotype 5 (rAd5) vector-based HIV-1 vaccine, vaccinees with baseline Ad5-specific neutralizing antibodies (NAbs) exhibited a 2.3-fold increased rate of HIV-1 acquisition as compared with controls1,2. This potential increased HIV-1 susceptibility appeared to be durable for >52 weeks of follow-up and remains unexplained. These findings largely paralyzed the HIV-1 vaccine field and emphasized the importance of evaluating vector-specific immunity in the context of HIV-1 vaccine studies. A hypothesis has been proposed in which baseline Ad5-specific NAbs may have been surrogate markers for Ad5-specific T lymphocyte responses, and anamnestic Ad5-specific $CD4^+$ T lymphocytes following vaccination in Ad5 seropositive subjects may have served as increased targets for HIV-1 infection.

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Author Contributions

K.L.O. and J.L. performed and analyzed the cellular immunologic assays. S.L.K. and Y.S. performed and analyzed the humoral immunologic assays. J.E.S., M.A.L., N.A.H., and M.R.B. provided reagents and performed flow cytometric assays. S.A.D., J.W.S., M.N.R., and D.R.C. provided clinical samples and participated in the design of the study. J.G. provided vectors and participated in the design of the study. D.H.B. led the design and conduct of the study. All coauthors discussed the data and contributed to writing the manuscript.

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To explore the immunologic basis of this hypothesis, we analyzed Ad vector-specific immunity in 116 subjects vaccinated with 10^{10} or 10^{11} viral particles (vp) of the Merck rAd5-Gag vaccine in the phase 1 studies that preceded the STEP study3. Serum and peripheral blood mononuclear cells (PBMC) at week 0 (baseline) and week 8 (4 weeks following the second vaccination) were utilized to assess vector-specific humoral and cellular immunity before and after vaccination.

We assessed baseline Ad5-specific NAb titers by virus neutralization assays4 and and Ad5-specific T lymphocyte responses by IFN-γ ELISPOT assays5 following Ad5 virus stimulation (Supplementary Methods). Ad5-specific cellular immune responses were detected in >90% of subjects at baseline (Fig. 1a), and these data were confirmed by ELISPOT assays using pooled Ad5 hexon peptides (data not shown). We speculate that the remarkably high frequency of Ad5-specific T lymphocyte responses reflect cross-reactive hexon-specific responses among multiple common subgroup C Ads (data not shown). We observed no correlation between baseline Ad5-specific ELISPOT responses and NAb titers (Fig. 1a, $P = 0.83$, Spearman rank-correlation test), demonstrating that Ad5-specific NAbs are not simply surrogate markers for Ad5-specific T lymphocyte responses.

We next evaluated the evolution of Ad5-specific NAbs following vaccination (Fig. 1b). Individuals who were Ad5 seronegative at baseline developed high titers of Ad5-specific NAbs at week 8. Subjects who were Ad5 seropositive at baseline developed supraphysiologic titers of Ad5-specific NAbs at week 8 that were significantly higher than those that developed in Ad5 seronegative subjects ($P = 5.4\times10^{-4}$, Wilcoxon rank-sum test, 10^{11} vp dose). In contrast, rAd5 vaccination did not elicit NAbs to the rare serotype Ad26, Ad35, and Ad48 viruses6–8, indicating minimal cross-reactive NAbs among these Ad serotypes (Supplementary Fig. 1, P = NS).

Ad5-specific cellular immune responses at baseline were comparable between Ad5 seronegative and Ad5 seropositive subjects (Fig. 1c, open symbols). In Ad5 seronegative subjects, rAd5 vaccination resulted in a 2-fold (10^{10} vp dose, $P = 4.2\times10^{-3}$) or a 3-fold (10^{11} vp dose, $P = 8.9\times10^{-5}$) increase of Ad5-specific ELISPOT responses at week 8 (Fig. 1c, circles). In Ad5 seropositive subjects, vaccination resulted in only marginal increases in Ad5-specific ELISPOT responses (Fig. 1c, inverted triangles; 10^{10} vp dose, $P = 0.53$; 10^{11} vp dose, $P = 0.043$), which were significantly lower than those observed in Ad5 seronegative subjects (10^{10} vp dose, $P = 8.3\times10^{-3}$; 10^{11} vp dose, $P = 2.3\times10^{-3}$). These data suggest that baseline Ad5-specific NAbs partially neutralized the rAd5 vaccine vector following vaccination and resulted in a lower effective dose of the vaccine in Ad5 seropositive subjects. Gag-specific antibody and T lymphocyte responses were similarly blunted by baseline Ad5-specific NAbs as expected2,3,9 (Supplementary Fig. 2). Baseline Ad5-specific NAbs did not impact the cytokine profile (IFN-γ, IL-2 ≫ IL-4, IL-10) or IgG subtype (IgG1, IgG3 ≫ IgG2, IgG4) of Ad5-specific cellular or humoral immune responses following vaccination (data not shown).

We next evaluated the capacity of rAd5 vaccination to induce T lymphocyte responses to rare serotype Ads. Ad26-, Ad35-, and Ad48-specific ELISPOT responses were common (Fig. 1d–f) despite their low seroprevalence6,7,10. Nevertheless, these responses were not

detectably augmented following rAd5 vaccination (Fig. 1d–f, *P* = NS). These data indicate that cellular immune responses to the homologous Ad5 virus were substantially more potent than cross-reactive responses to these rare Ad serotypes.

We confirmed these findings in $CD4^+$ and $CD8^+$ T lymphocyte subpopulations by multiparameter intracellular cytokine staining (ICS) assays5,11 (Supplementary Fig. 3; Supplementary Methods) in 23 subjects who received the 10^{11} vp dose of the vaccine. Vaccination resulted in clear increases in Ad5-specific IFN-γ^+CD4^+ and IFN-γ^+CD8^+ T lymphocyte responses in Ad5 seronegative subjects, but less convincing responses were observed in Ad5 seropositive individuals (Fig. 2a). Similar results were evident for Ad5-specific IL-2^+ T lymphocyte responses (data not shown). In contrast, no clear changes in Ad26-specific $CD4^+$ and $CD8^+$ T lymphocytes were observed following rAd5 vaccination. We also found no perturbation of total, $CCR5^+$, $CD45RO^+CD27^+$ central memory (CM), and $CD45RO^+CD27^-$ effector memory (EM) $CD4^+$ T lymphocyte subpopulations (Fig. 2b, *P* = NS) and no sustained $Ki67^+$ activation of any of these $CD4^+$ T lymphocyte subpopulations (Fig. 2c, d, *P* = NS) at week 8. We also did not detect any differences in Ki67 expression on Ad5-specific $CD4^+$ T lymphocytes between Ad5 seronegative and Ad5 seropositive subjects (Supplementary Fig. 4a). Short-term cellular immune activation for 1–2 weeks following rAd5 vaccination has been reported12, but these transient effects do not likely explain the durable increase in HIV-1 susceptibility for >52 weeks in the STEP study1,2.

In summary, Ad5-specific NAbs at baseline are not surrogate markers for Ad5-specific cellular immune responses, and Ad5-specific T lymphocyte responses following rAd5 vaccination were lower in Ad5 seropositive as compared with Ad5 seronegative subjects. We also observed no evidence of sustained modulation or activation of $CD4^+$ T lymphocyte subpopulations following rAd5 vaccination. These data challenge the hypothesis that anamnestic Ad5-specific $CD4^+$ T lymphocyte responses following rAd5 vaccination were responsible for the potential enhancement of HIV-1 acquisition in Ad5 seropositive subjects in the STEP study. A caveat is that we were unable to assess mucosal immune responses in the present study, although we did not detect differences between Ad5 seronegative and Ad5 seropositive subjects in $\alpha 4$ and $\beta 7$ integrin expression on total or Ad5-specific peripheral $CD4^+$ T lymphocytes (Supplementary Fig. 4b). An alternative model has been suggested in which the potential enhancement of HIV-1 susceptibility in Ad5 seropositive subjects may have been due to immune complex formation following rAd5 vaccination13. If this model proves correct, then the use of vaccine vectors that evade baseline vector-specific NAbs may offer a potential solution to this problem.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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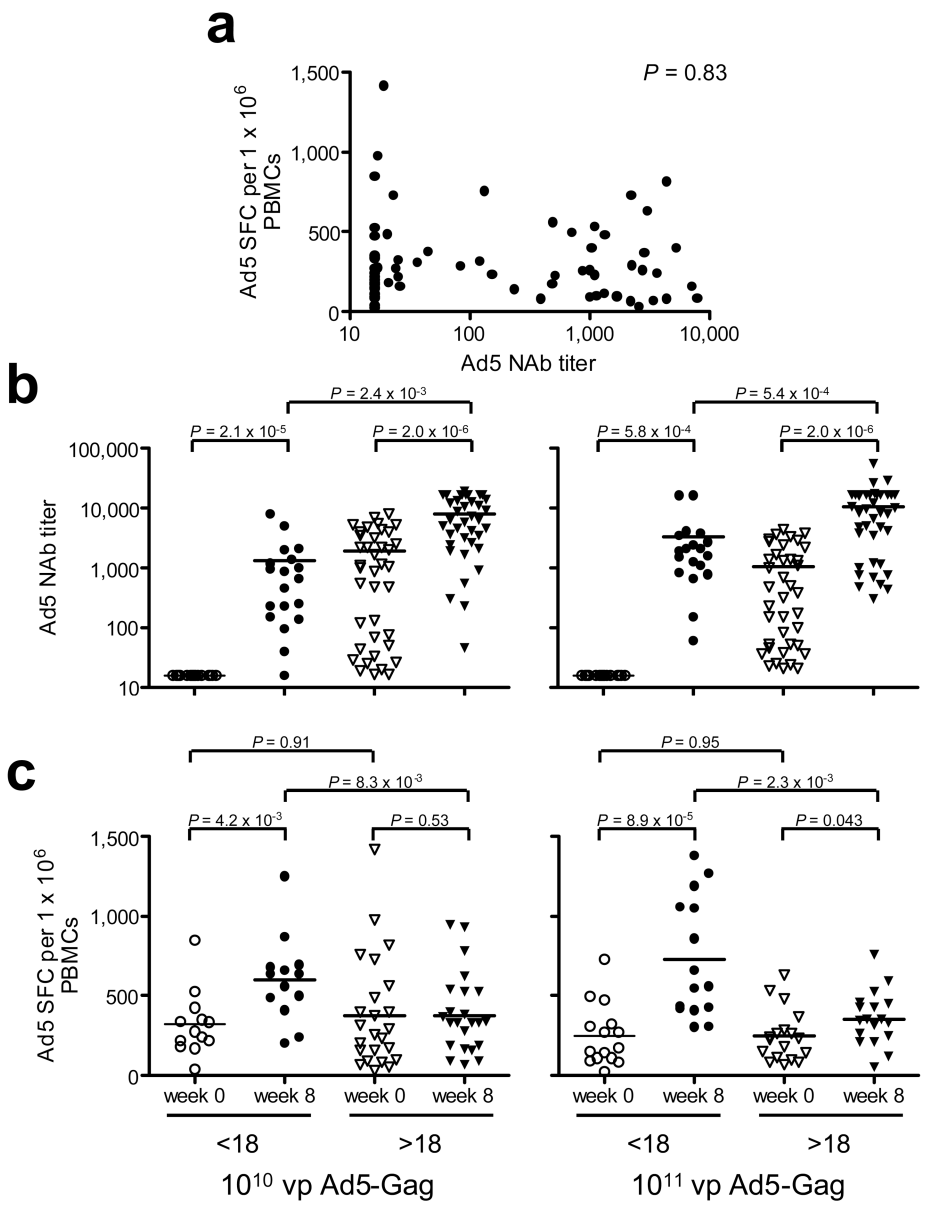

Fig. 1a-c

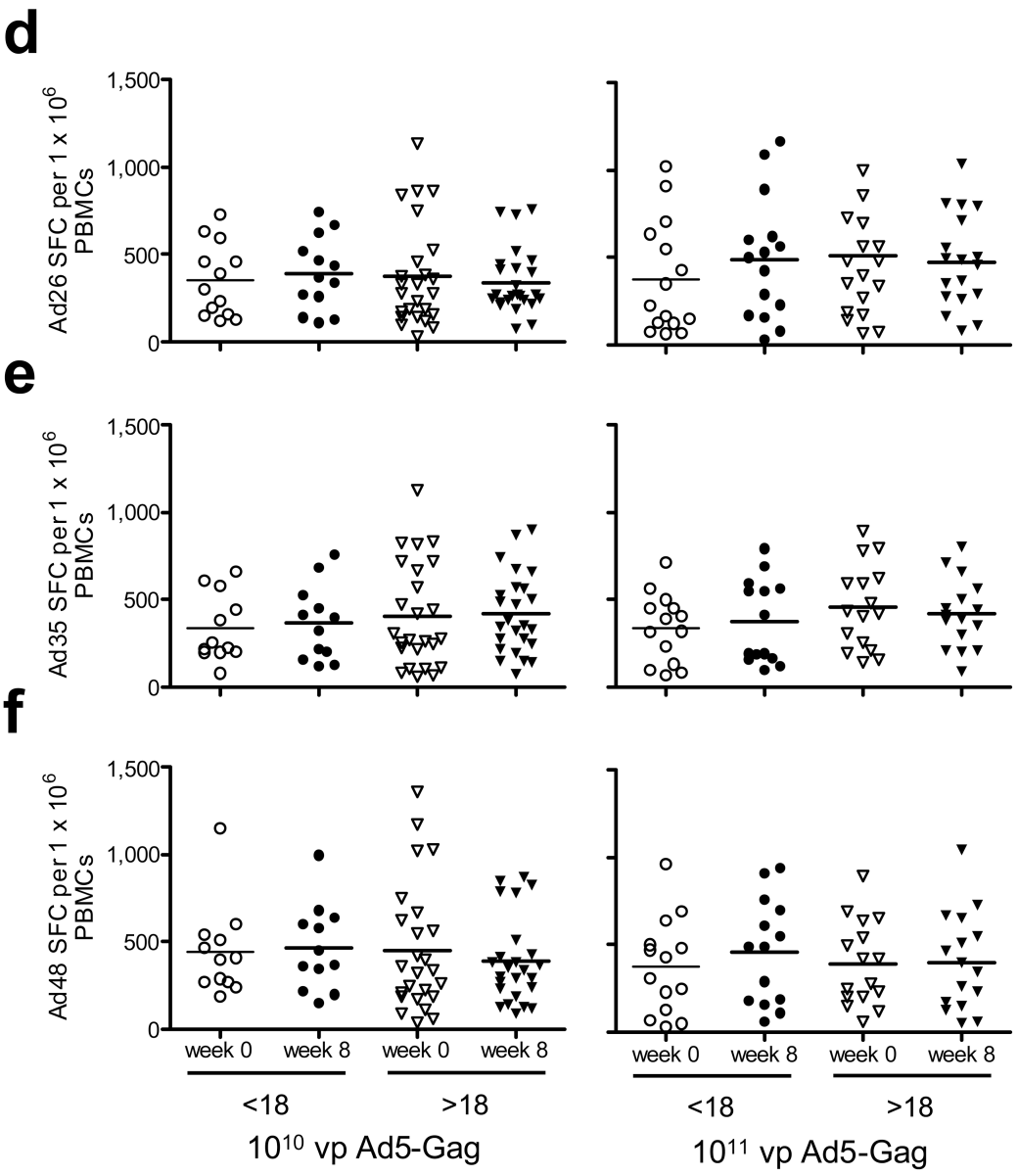

Fig. 1d-f

Figure 1.

Ad-specific humoral and cellular immune responses before and after rAd5-Gag vaccination. (**a**) Correlation between Ad5-specific IFN-γ ELISPOT responses and Ad5-specific NAb titers at baseline. (**b**) Ad5-specific NAb titers and (**c**) Ad5-specific IFN-γ ELISPOT responses as stratified by vaccine dose (10^{10} vp, 10^{11} vp), baseline Ad5 titer (<18, >18), and study timepoint (week 0, week 8). (**d**) Ad26-, (**e**) Ad35-, and (**f**) Ad48-specific IFN-γ ELISPOT responses.

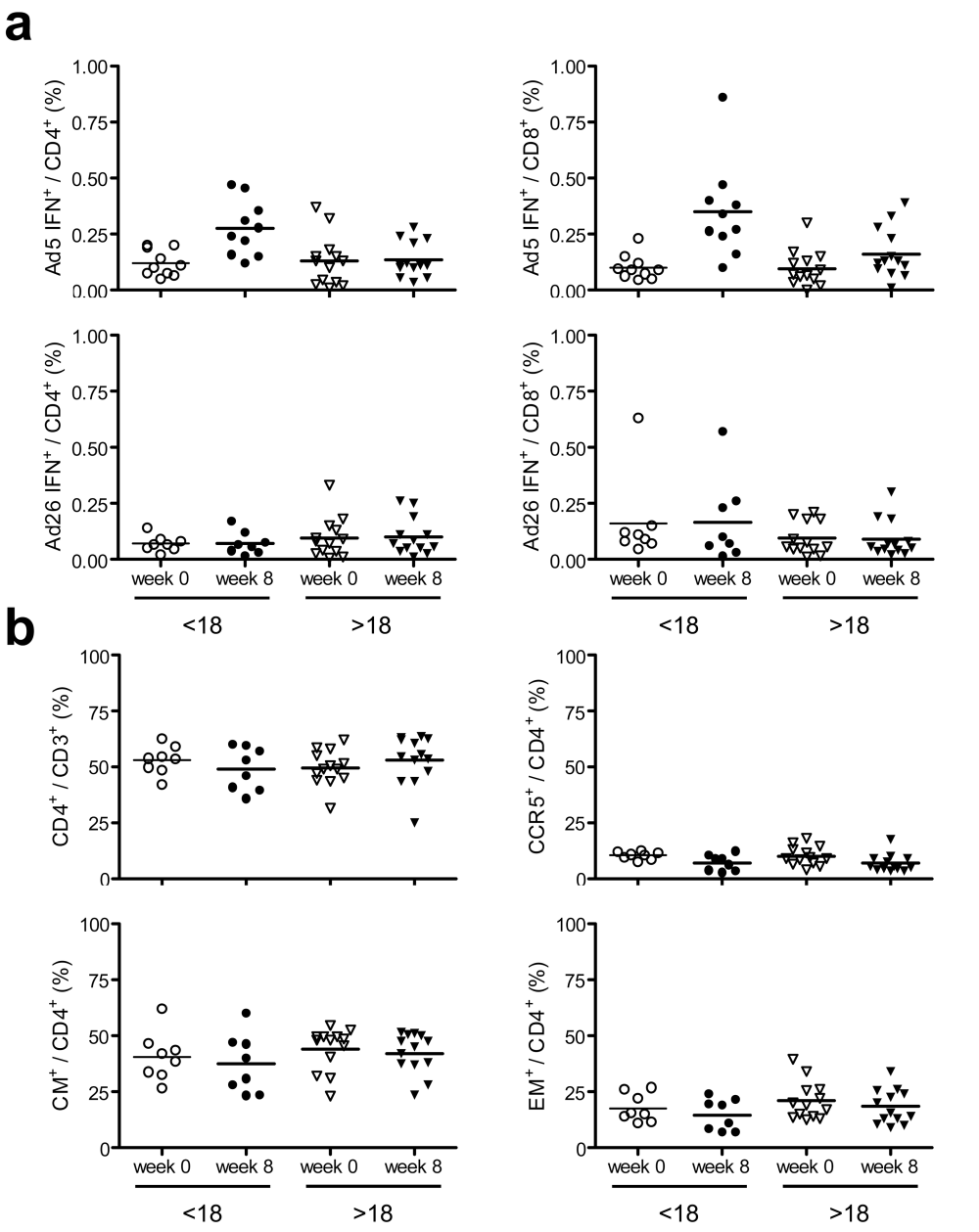

Fig. 2a-b

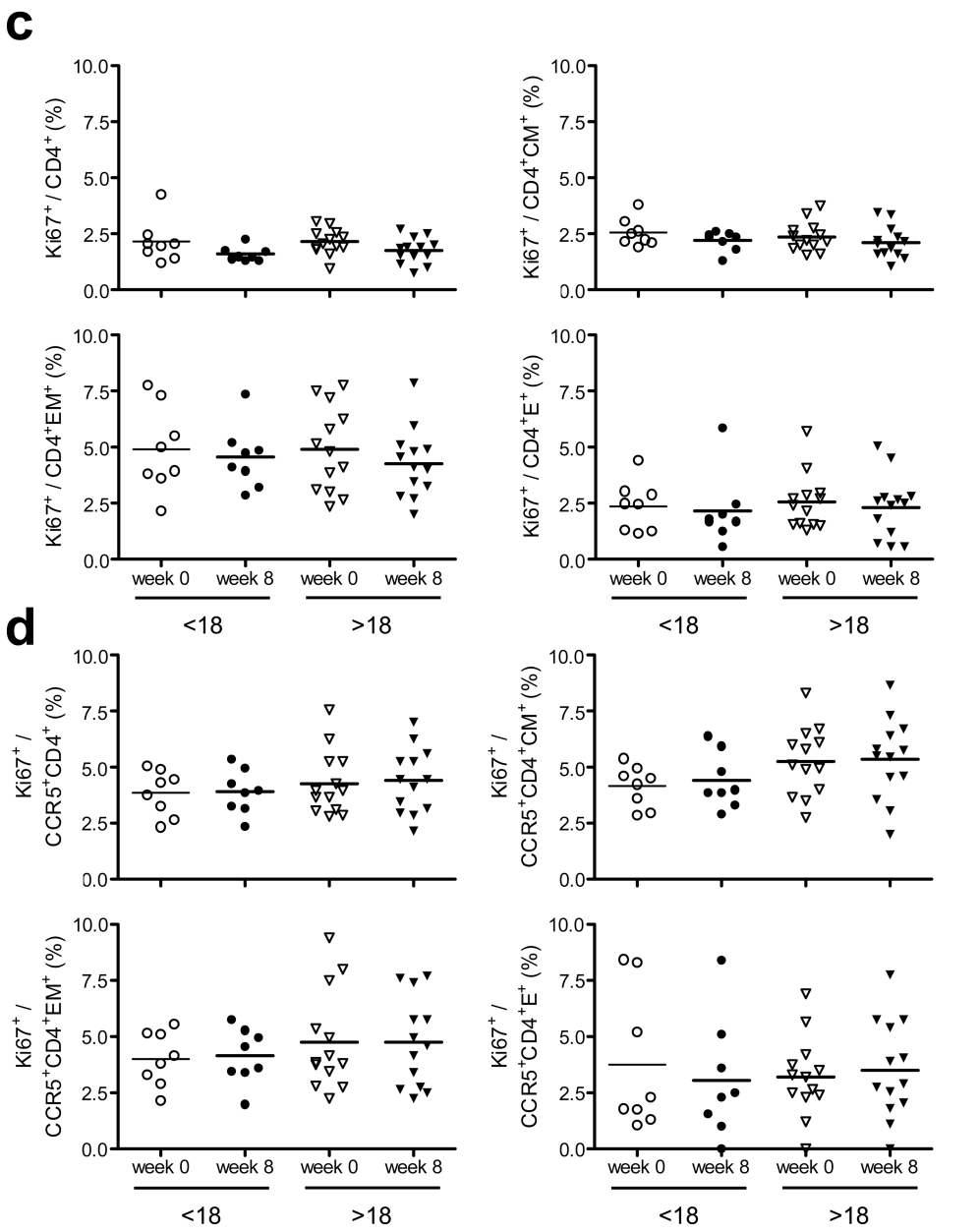

Figure 2.

Magnitude and phenotype of Ad-specific and total $CD4^+$ T lymphocyte subpopulations before and after 10^{11} vp rAd5-Gag vaccination. (**a**) Ad5- (top panels) and Ad26- (bottom panels) specific IFN-γ^+CD4^+ (left panels) and IFN-γ^+CD8^+ (right panels) ICS responses as stratified by baseline Ad5 titer (<18, >18) and study timepoint (week 0, week 8). (**b**) Total, $CCR5^+$, $CD45RO^+CD27^+$ central memory (CM), and $CD45RO^+CD27^-$ effector memory (EM) $CD4^+$ T lymphocyte subpopulations. (**c**) $Ki67^+$ activation of total, CM, EM, and

CD45RO^{-}CD27^{-} effector (E) CD4^{+} T lymphocyte subpopulations. (**d**) Ki67^{+} activation of total, CM, EM, and E CCR5^{+}CD4^{+} T lymphocyte subpopulations.