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Harnessing the immunostimulatory properties of oncolytic reovirus for anticancer immunotherapy

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ADDENDUM



**CD4⁺ T-cell depletion abrogates
NAb production and improves the
efficacy of reovirus monotherapy**

BACKGROUND

Although NABs hamper the efficacy of reovirus (Reo) when used as oncolytic agent, they are required to prevent Reo-induced weight loss and viremia. Indeed, B-cell deficient mice succumb around 2 weeks after reovirus therapy (**Chapter 5, Figure S2**) (1). We also demonstrated that complete abrogation of NAB production by CD4⁺ T-cell depletion improved reovirus infection (**Chapter 5, Figure 3G**), but the lack of NABs in these mice did not coincide with weight loss. Therefore, we explored the depletion of CD4⁺ T cells as a strategy to study the effects of NABs on the clinical efficacy of reovirus while separating unwanted Reo-induced viremia from the desired Reo-induced antitumor effects.

RESULTS & DISCUSSION

We preexposed immunocompetent C57BL/6J mice intravenously to Reo. CD4⁺ T-cell depletion was initiated either before (B-exp) or after the first Reo preexposure (A-exp), or before the first intratumoral Reo injection and was continued for the duration of the experiment (**Figure 1A**). Depletion of CD4⁺ T cells was efficient (**Figure 1B**) and subsequent absence of NABs was sustained (**Figure 1C**), but only when CD4⁺ depletion was initiated before the first Reo exposure (**Figure 1C**). The complete removal of NABs, mediated by CD4⁺ T-cell depletion during Reo preexposure or Reo administration, was not associated with changes in body weight (**Figure 1D**), but significantly delayed outgrowth of KPC3 tumors upon intratumoral Reo administration (**Figure 1E**), resulting in smaller tumors at later time points (**Figure 1F**). Combined, these results show that abrogation of Reo-specific NAB responses by CD4⁺ T-cell depletion can improve the antitumor efficacy of Reo monotherapy, without the concomitant weight loss due to uncontrolled Reo replication that is normally observed when NABs are absent.

CD4⁺ T cells are essential for mounting effective B-cell responses. Especially the interactions between B cells and a specific type of CD4⁺ T cell, named follicular helper CD4⁺ T cell (Tfh), are important. Tfh cells release cytokines such as IL-2, IL-4, and IL-21 that contribute to the formation of germinal centers, where they promote the generation of antibody-producing plasma cells (2). Similar as what we observed for Reo, it is demonstrated that CD4⁺ T cells are absolutely required for the generation of optimal antibody responses after infection with coronavirus (3), vaccinia virus (4), or vesicular stomatitis virus (5). It has also been observed earlier that CD4⁺ T-cell depletion abrogates antibody responses, for instance after intramuscular immunization with an Adenovirus vector or a trimeric SIV Env gp140 protein (6). However, it is unclear why a complete lack of Reo-specific NABs after CD4⁺ T-cell depletion does not coincide with weight loss and viremia, which contrasts the observations in B-cell deficient μ MT and NSG mice that succumb after Reo exposure.

The immune system can be considered a dynamic system where certain immune cells can 'take over' when other cells are absent or dysfunctional. In the absence of NABs, it is possible that Reo-specific CD8⁺ T cells might have provided protection from Reo-induced pathology. Indeed, depletion of CD4⁺ T cells during Reo preexposure did not preclude the mounting of Reo-specific CD8⁺ T-cell responses (**Figure 2A**). However, Reo-specific CD8⁺ T cells could also be found in μ MT mice (**Figure 2B**), but these mice still succumbed quickly after Reo exposure. This suggests that CD8⁺ T cells might not be involved in the clearance of Reo-infected cells (and thus the clearance of Reo itself), or at least not in all settings. Thus, it would be very interesting to identify which other cell types or proteins might be able to provide protection against Reo-induced pathology in settings where NABs are absent.

These observations provide exciting avenues for further research, especially in the context of preclinical research investigating the role of NABs on OV therapy. Transient depletion of CD4⁺ T cells during OV therapy is not applicable as a therapeutic strategy for cancer patients since this would increase the susceptibility to opportunistic pathogens and malignancies, but specific depletion or inhibition of follicular helper CD4⁺ T cells (Tfh) cells (7), for example by inhibition of Tfh-specific transcription factor B-cell lymphoma 6 (Bcl-6) (8), might be a strategy to consider. It would also be interesting to investigate the specific depletion of regulatory CD4⁺ T cells (Tregs), since it has been shown in the context of infection with influenza or respiratory syncytial virus that depletion of Tregs leads to reduced virus-specific B-cell responses (9,10). Furthermore, the depletion of Tregs might also result in stronger virus-specific CD8⁺ T-cell responses that could contribute to protection against virus-induced pathology (11). However, as the effects of CD4⁺ T-cell depletion on NABs only work when applied before Reo exposure, this might only be of interest for the minority of patients that do not present with preexisting NABs (**Chapter 5, Figure 1**). But, we also observed a small delay in tumor outgrowth in mice that received CD4⁺ T-cell-depleting antibodies after Reo preexposure (A-exp group), even though these mice presented with similar NAB levels as preexposed mice that did not receive CD4⁺ T-cell-depleting antibodies (**Figure 1C**). We cannot explain this observation, but it indicates that removal of CD4⁺ T cells might also be employed to enhance the anticancer efficacy of Reo in preexposed individuals, in a NAB independent manner. Furthermore, it would also be interesting to investigate the effect of CD4⁺ T-cell depletion on the efficacy of Reo&CD3-bsAb therapy, since this might result in increased viral persistence but simultaneously limit the number of T cells that can be employed by CD3-bsAbs and thereby impair, and not improve this combination therapy.

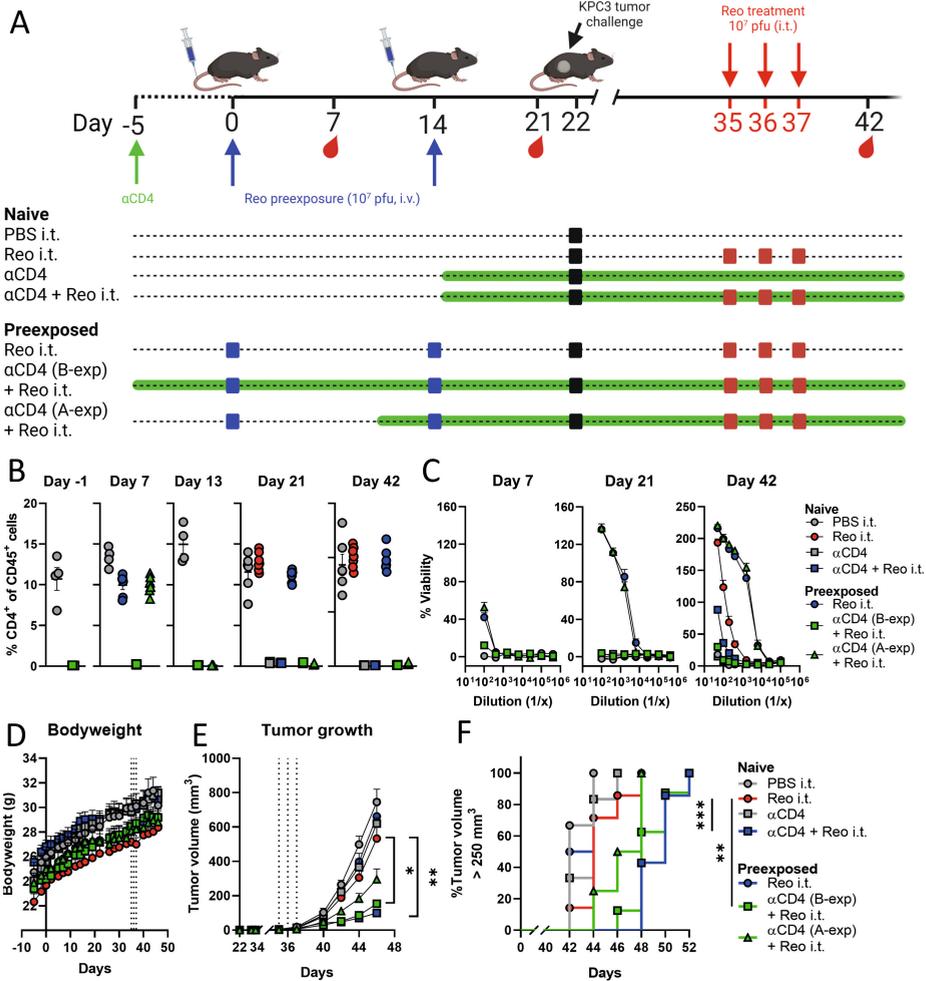


Figure 1. Abrogated production of Reo-specific neutralizing antibodies by CD4⁺ T-cell depletion improves Reo monotherapy efficacy without inducing weight loss. (A) Overview of experiment described in (B-F). Male C57BL/6J mice (n=6-8/group) were preexposed by intravenous injection of Reo (10⁷ plaque-forming units (pfu)/injection) on days 0 and 14. Depletion of CD4⁺ T cells (αCD4, 100 μg/injection, intraperitoneally) was initiated either before preexposure 1 (B-exp), after preexposure 1 (A-exp), or before KPC3 tumor challenge, and was maintained by weekly injections of αCD4. Blood was drawn on indicated days for interim analysis. After preexposure, mice were inoculated with KPC3 cells (1x10⁵/mouse) and received intratumoral Reo injections (10⁷ pfu/injection) and tumor growth and body weight were monitored during the experiment. (B) Frequency of CD4⁺ T cells in the circulation. (C) Reo neutralization assay. Average dilution curves using plasma harvested on indicated days. (D) Average body weight curves. (E) Average tumor volume curves. (F) Kaplan-Meier graph showing accumulation of animals reaching tumor volume > 250 mm³. Data represent mean±SEM. IC₅₀ values were calculated using non-linear regression analysis. Differences between groups in (E) were determined using an ordinary two-way analysis of variance (ANOVA) with Tukey's post hoc test, and Mantel-Cox Log-rank tests were used to calculate significant differences between groups in (F). Significance levels: *p<0.05, **p<0.01, and ***p<0.001.

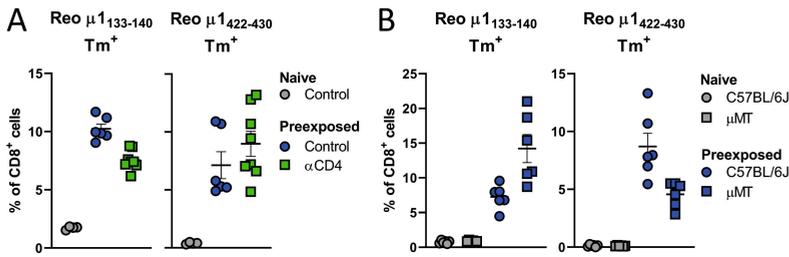


Figure 2. Reo-specific CD8⁺ T-cell responses are present after CD4⁺ T-cell depletion or in B-cell deficient μ MT mice. (A) Frequency of Reo-specific CD8⁺ T cells in blood. Male immunocompetent C57BL/6J mice (n=6-8/group) were preexposed by intravenous (i.v.) injection of Reo (10^7 plaque-forming units (pfu)/injection) on day 0. Depletion of CD4⁺ T cells (α CD4, 100 μ g/injection, intraperitoneally (i.p.)) was initiated before preexposure. Blood was harvested on day 7 for flow cytometry analysis. (B) Frequency of Reo-specific CD8⁺ T cells in blood. Male immunocompetent C57BL/6J mice or B-cell deficient μ MT mice (n=6/group) were preexposed as described in (A) on day 0, and blood was harvested on day 7 for flow cytometry analysis. Data represent mean \pm SEM. Significance levels: ***p<0.001 and ****p<0.0001.

CONCLUSION

Combined with the data described in **Chapter 5**, these results show that Reo-specific NAbs responses are required to prevent Reo-induced pathology, but they also directly hamper the antitumor efficacy of Reo monotherapy. Depletion of CD4⁺ T cells can abrogate NAbs production and enhance the antitumor efficacy of Reo therapy, without concomitant Reo-induced viremia.

REFERENCES

1. Qiao J, Wang H, Kottke T, White C, Twigger K, Diaz RM, *et al.* Cyclophosphamide Facilitates Antitumor Efficacy against Subcutaneous Tumors following Intravenous Delivery of Reovirus. *Clinical Cancer Research* **2008**;14:259-69
2. Swain SL, McKinstry KK, Strutt TM. Expanding roles for CD4+ T cells in immunity to viruses. *Nature Reviews Immunology* **2012**;12:136-48
3. Chen J, Lau YF, Lamirande EW, Paddock CD, Bartlett JH, Zaki SR, *et al.* Cellular immune responses to severe acute respiratory syndrome coronavirus (SARS-CoV) infection in senescent BALB/c mice: CD4+ T cells are important in control of SARS-CoV infection. *J Virol* **2010**;84:1289-301
4. Sette A, Moutaftsi M, Moyron-Quiroz J, McCausland MM, Davies DH, Johnston RJ, *et al.* Selective CD4+ T cell help for antibody responses to a large viral pathogen: deterministic linkage of specificities. *Immunity* **2008**;28:847-58
5. Thomsen AR, Nansen A, Andersen C, Johansen J, Marker O, Christensen JP. Cooperation of B cells and T cells is required for survival of mice infected with vesicular stomatitis virus. *International immunology* **1997**;9:1757-66
6. Provine NM, Badamchi-Zadeh A, Bricault CA, Penalzoza-MacMaster P, Larocca RA, Borducchi EN, *et al.* Transient CD4+ T Cell Depletion Results in Delayed Development of Functional Vaccine-Elicited Antibody Responses. *J Virol* **2016**;90:4278-88
7. Crotty S. T follicular helper cell differentiation, function, and roles in disease. *Immunity* **2014**;41:529-42
8. Pearce AC, Bamford MJ, Barber R, Bridges A, Convery MA, Demetriou C, *et al.* GSK137, a potent small-molecule BCL6 inhibitor with *in vivo* activity, suppresses antibody responses in mice. *Journal of Biological Chemistry* **2021**;297
9. León B, Bradley JE, Lund FE, Randall TD, Ballesteros-Tato A. FoxP3+ regulatory T cells promote influenza-specific Tfh responses by controlling IL-2 availability. *Nat Commun* **2014**;5:3495
10. Shao H-Y, Huang J-Y, Lin Y-W, Yu S-L, Chitra E, Chang C-K, *et al.* Depletion of regulatory T-cells leads to moderate B-cell antigenicity in respiratory syncytial virus infection. *International Journal of Infectious Diseases* **2015**;41:56-64
11. Dietze KK, Zelinskyy G, Gibbert K, Schimmer S, Francois S, Myers L, *et al.* Transient depletion of regulatory T cells in transgenic mice reactivates virus-specific CD8+ T cells and reduces chronic retroviral set points. *Proc Natl Acad Sci U S A* **2011**;108:2420-5

