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 μMT 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 μMT 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.

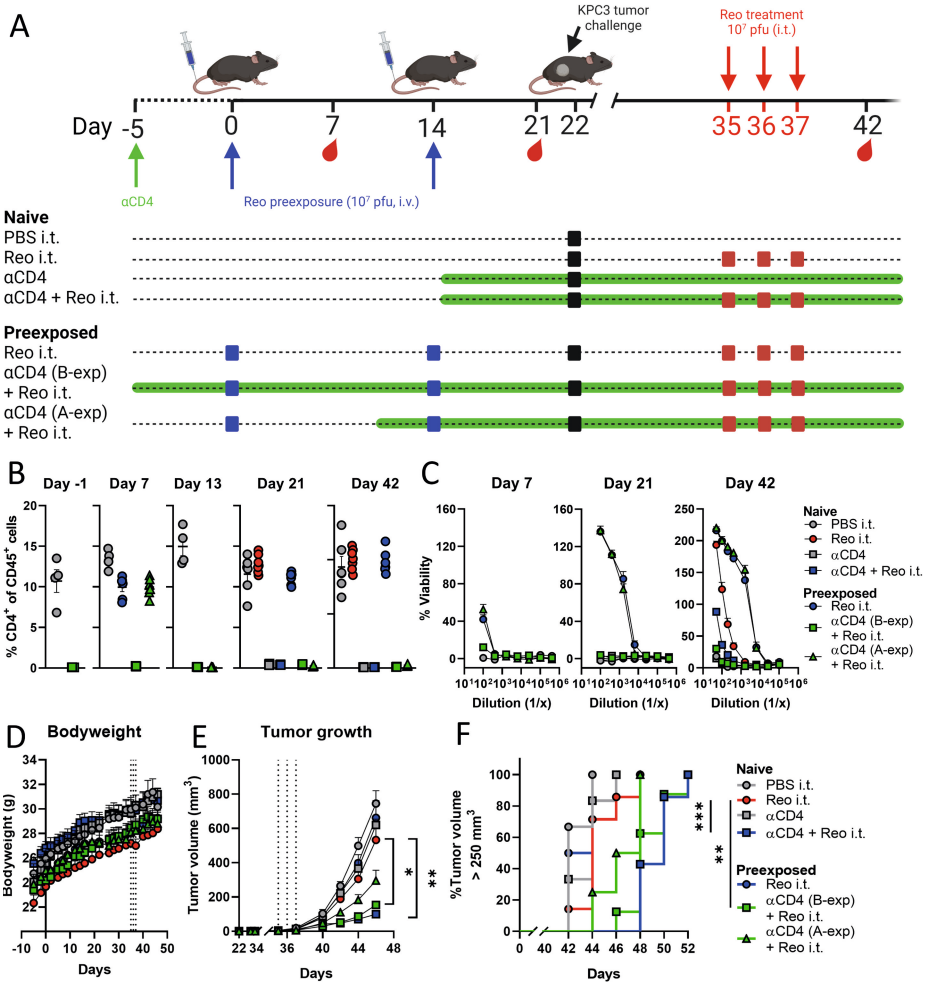

Figure 1. Abrogated production of Reo-specific neutralizing antibodies by CD4⁺ T-cell depletion improves Reo monotherapy efficacy without inducing weight loss. (**A**) Overview of experiment described in (B-F). Male C57BL/6J mice (n=6-8/group) were preexposed by intravenous injection of Reo (10^7 plaque-forming 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 ($1x10^5$/mouse) and received intratumoral Reo injections (10^7 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 mm^3. Data represent mean±SEM. IC_{50} 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.

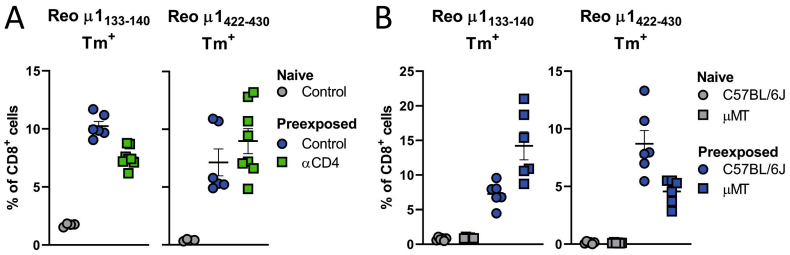

Figure 2. Reo-specific CD8⁺ T-cell responses are present after CD4⁺ T-cell depletion or in B-cell deficient μMT mice. (**A**) Frequency of Reo-specific CD8⁺ T cells in blood. Male immunocompetent C57BL/6J mice (n=6-8/group) were preexposed by intravenous (i.v.) injection of Reo (10^7 plaque-forming units (pfu)/injection) on day 0. Depletion of CD4⁺ T cells (αCD4, 100 μg/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 μMT 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±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 NAb 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 NAb production and enhance the antitumor efficacy of Reo therapy, without concomitant Reo-induced viremia.

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