Abstract 3298

Propagation Competence of a Self-Antigen-Targeting Arenavirus Vector Based Cancer Therapy Determines Antitumor Efficacy in Mouse Melanoma

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ABSTRACT

Breaking self-tolerance and inducing a strong tumor-specific immune response is one of the main goals of a self-antigen-based therapeutic vaccine. Here we compared two molecularly similar recombinant lymphocytic choriomeningitis virus (LCMV)-based vectors carrying the melanoma-associated antigen tyrosinase-related protein 2 (TRP2). Both the propagating artLCMV-TRP2 and the non-propagating rLCMV-TRP2 induced strong immune responses to foreign antigens. However, only artLCMV induced detectable CD8+ T-cell responses to the self-antigen TRP2 and could control B16F10 melanoma growth in a TRP2-specific manner. artLCMV-TRP2 transduced significantly more antigen-presenting cells and induced a stronger type I interferon (IFN-I) response. Moreover, concurrently with the peak of TRP2-specific CD8+ T cells, the frequency of regulatory T cells (T_{regs}) was reduced in artLCMV-TRP2-vaccinated mice, resulting in an improved effector-to-T_{reg} ratio, also in the tumor. In conclusion, propagation competence is essential for the success of a self-antigen-targeting cancer vaccine.

RESULTS

Non-propagating rLCMV Vector Induces Strong Immune Responses Against Foreign but Not Self-antigens

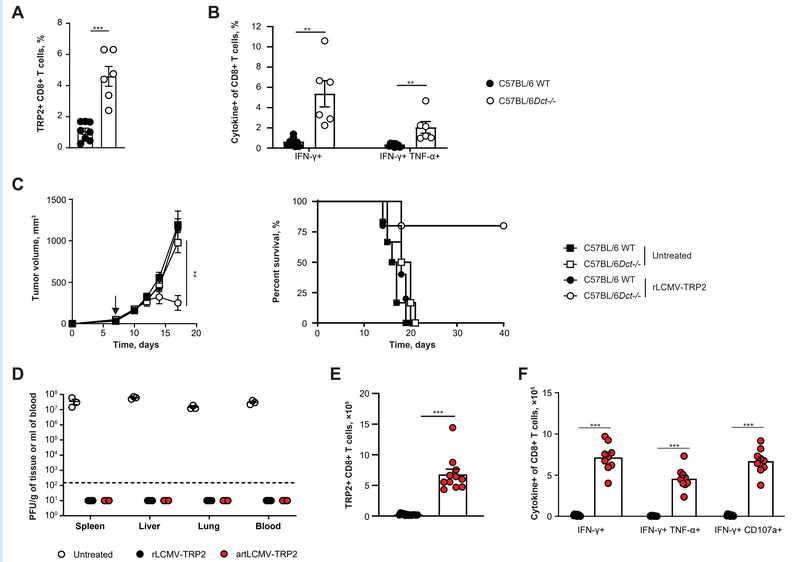

Figure 1. The propagating artLCMV vector overcomes tolerance to self-antigens. **(A)** WT and TRP2-deficient *Dct-/-* mice were immunized with rLCMV-TRP2. Frequency of TRP2+ CD8+ T cells in spleen. **(B)** Cytokine production of splenocytes from immunized mice after stimulation with TRP2 peptides. **(C)** Tumor growth in and survival of immunized and unimmunized mice. **(D)** WT mice previously vaccinated with rLCMV-TRP2, artLCMV-TRP2 or untreated were challenged with LCMV clone 13. Plaque assay of indicated organs. **(E)** Frequency of TRP2+ CD8+ T cells in spleen of immunized WT mice. **(F)** Cytokine production of splenocytes from immunized WT mice after stimulation with TRP2 peptides. *Dct*, dopachrome tautomerase; IFN, interferon; LCMV, lymphocytic choriomeningitis virus; TNF, tumor necrosis factor; WT, wild-type.

Propagation Competence Is Important for APC Transduction and Activation of Innate Immunity

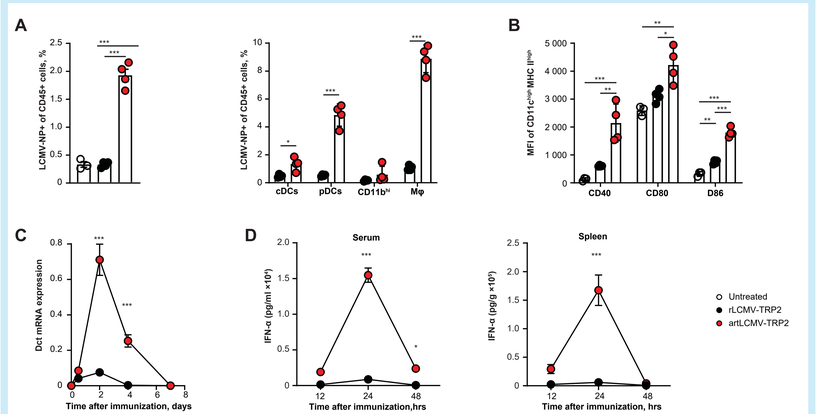

Figure 2. Antigen expression and activation of APCs by recombinant LCMV vectors. WT mice were vaccinated with rLCMV-TRP2 or artLCMV-TRP2. **(A)** Infection of CD45+ cells and transduction of various APCs. **(B)** Surface expression of costimulatory molecules on cDCs. **(C)** Expression of *Dct* mRNA in spleens of vaccinated mice. **(D)** IFN-α level in serum and spleen of immunized mice. APC, antigen presenting cell; DC, dentritic cell.

A Propagation-Competent Vaccine Vector Ensures an Optimal Effector-to-T_{reg} Ratio

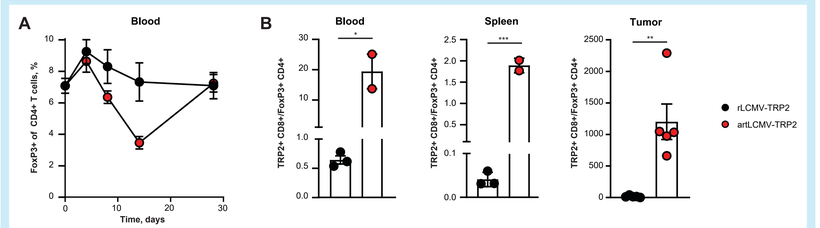

Figure 3. artLCMV vector increases the ratio of CD8+ effector to regulatory T cells. **(A)** Frequency of T_{regs} in blood of immunized mice. **(B)** Ratio of TRP2+ CD8+ T cells to T_{regs} in blood, spleen and tumor of immunized mice.

Propagation-Competent artLCMV Vector Controls Tumor Growth in an IFN-I-dependent Manner

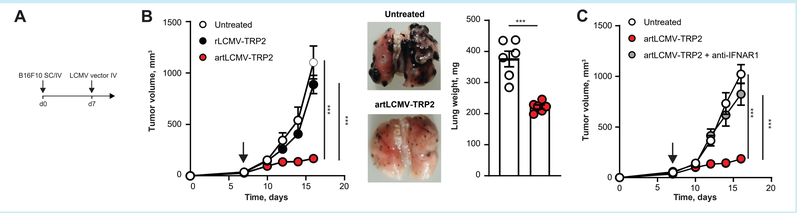

Figure 4. Treatment with a propagation-competent vector confers tumor control. **(A)** Experimental setup of tumor growth experiments. WT mice were subcutaneously inoculated with B16F10 melanoma cells and intravenously immunized with rLCMV-TRP2 or artLCMV-TRP2 at day 7 of tumor growth. Arrow indicates timepoint of tumor cell injection or immunization. In lung metastasis experiments, tumor cells were injected intravenously. **(B)** Growth of subcutaneous tumors and lung metastases in immunized and unimmunized mice. **(C)** Tumor growth in unimmunized mice, artLCMV-TRP2-immunized mice and artLCMV-TRP2-immunized mice treated with IFNAR1-blocking antibodies. IV, intravenous; SC, subcutaneously.

Only artLCMV Can Activate Tumor-Specific CD8+ T Cells Sufficiently for Complete Tumor Rejection

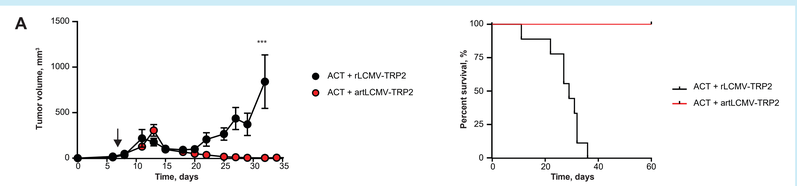

Figure 5. Adoptive transfer of naive TRP2+ CD8+ T cells from TRP2 TCR transgenic mice. The cells were injected intravenously on the day of immunization. **(A)** Tumor growth and survival in immunized mice receiving ACT. ACT, adoptive cellular therapy.

Treatment With a Propagation-Competent Vector Induces More Immune Infiltration of Tumors

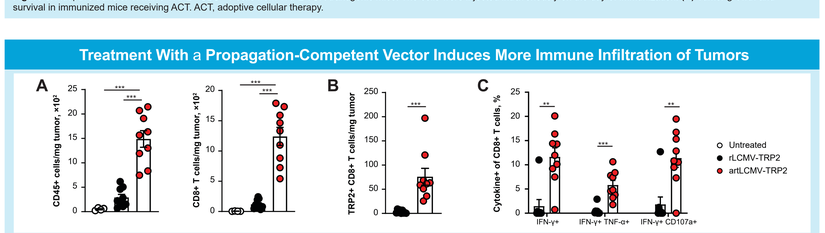

Figure 6. Treatment with a propagation-competent vector confers tumor control. **(A)** Number of tumor-infiltrating CD45+ cells and CD8+ T cells in immunized and unimmunized mice. **(B)** Number of tumor-infiltrating TRP2+ CD8+ T cells in immunized mice. **(C)** Cytokine production of tumor-infiltrating TRP2+ CD8+ T cells from immunized mice after stimulation with TRP2 peptides.

CONCLUSION

- Recombinant LCMV vectors have great potential in cancer therapy. Unlike the propagation-deficient rLCMV, replicating artLCMV can overcome tolerance to self and induce a strong IFN-I response, which leads to a potent antitumor response by self-antigen-specific cytotoxic T cells infiltrating the tumor. In sum, this study uncovers the necessity of using propagation-competent vaccine vectors for active immunization for targeting tumor self-antigens

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