SCIENTIFIC HIGHLIGHTS

Induction of antitumor responses by antigen targeting *in vivo*

Antigen Delivery to CD11c⁺CD8⁻ Dendritic Cells Induces Protective Immune Responses against Experimental Melanoma in Mice In Vivo

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... antigen delivery to CD11c⁺CD8⁻ DCs induced a much less efficient CD8⁺ cytotoxic T cell response as opposed to CD11c⁺CD8⁺ DCs...

... unexpectedly, in the melanoma model, both CD11c⁺CD8⁺ and CD11c⁺CD8⁻ DCs induced protective as well as therapeutic immune responses ...

Dendritic cells (DCs) are key players for the induction of immune responses but also for the maintenance of peripheral tolerance. Therefore, DCs might be ideal target cells for immunotherapeutic approaches to improve (tumor) or dampen immune responses (autoimmune diseases). In the past, we have generated antibodies, which efficiently bind to uniquely expressed endocytic receptors on murine DC subpopulations (DEC205, DCIR2). By genetic modifications, these antibodies carry an antigen of choice and cannot bind to Fc receptors. Upon injection and binding to their endocytic receptors, the antigen targeting antibodies are internalized, and the antigens are processed and presented as peptide MHC complexes, thereby allowing an efficient activation of antigen specific T cells.

In a recent study, we delivered antigens to CD11c⁺CD8⁻ or CD11c⁺CD8⁺ DCs in the presence or absence of an immune stimulus in naïve animals. We found that antigen delivery to CD11c⁺CD8⁺ DCs induced a pronounced CD8+ cytotoxic response as well as CD4⁺ T cell responses. In contrast, the delivery of antigens to CD11c⁺CD8⁻ DCs was less efficient in inducing CD8⁺ cytotoxic T cell responses, but showed similar CD4⁺ helper T cell responses. The measured antibody titers against the engulfed antigen suggested that a mixed TH1/TH2 immune response was induced, which was independent of the DC subset that originally presented the antigen.

Next, we analyzed whether the induced immune responses would be protective for the outgrowth of melanoma cells. To this end, we used the B16F10 melanoma model, in which ovalbumin (OVA) was a surrogate antigen. We found that it did not matter, which of the DC subsets presented the antigen, as both CD11c⁺CD8⁺ and CD11c⁺CD8⁻ DCs induced protective as well as therapeutic immune responses. This finding was unexpected as CD11c⁺CD8⁻ DCs were less efficient inducers of cytotoxic CD8⁺ Tcells which usually are critical for the fighting against tumor cells .

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K. Neubert · C.H. Lehmann · L. Heger · A. Baranska · A.M. Staedtler · V.R. Buchholz · S.Yamazaki · G.F. Heidkamp · N. Eissing · H. Zebroski · M.C. Nussenzweig · F. Nimmerjahn · D. Dudziak. 2014. Antigen delivery to CD11c⁺CD8⁻ dendritic cells induces protective immune responses against experimental melanoma in mice in vivo. *The Journal of Immunology* 192:5830-5838.

FIGURE 1

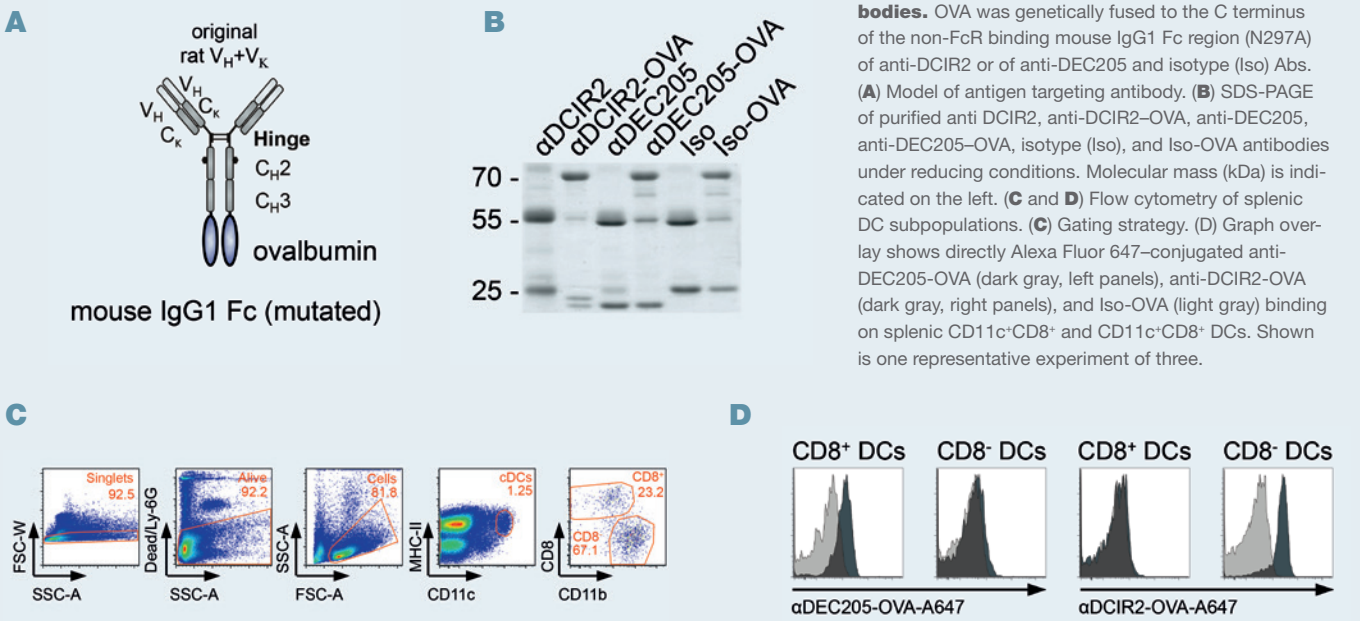

Figure 1: Production of antigen targeting antibodies. OVA was genetically fused to the C terminus of the non-FcR binding mouse IgG1 Fc region (N297A) of anti-DCIR2 or of anti-DEC205 and isotype (Iso) Abs. (**A**) Model of antigen targeting antibody. (**B**) SDS-PAGE of purified anti DCIR2, anti-DCIR2–OVA, anti-DEC205, anti-DEC205–OVA, isotype (Iso), and Iso-OVA antibodies under reducing conditions. Molecular mass (kDa) is indicated on the left. (**C** and **D**) Flow cytometry of splenic DC subpopulations. (**C**) Gating strategy. (D) Graph overlay shows directly Alexa Fluor 647–conjugated anti-DEC205-OVA (dark gray, left panels), anti-DCIR2-OVA (dark gray, right panels), and Iso-OVA (light gray) binding on splenic $CD11c^+CD8^+$ and $CD11c^+CD8^+$ DCs. Shown is one representative experiment of three.

FIGURE 2

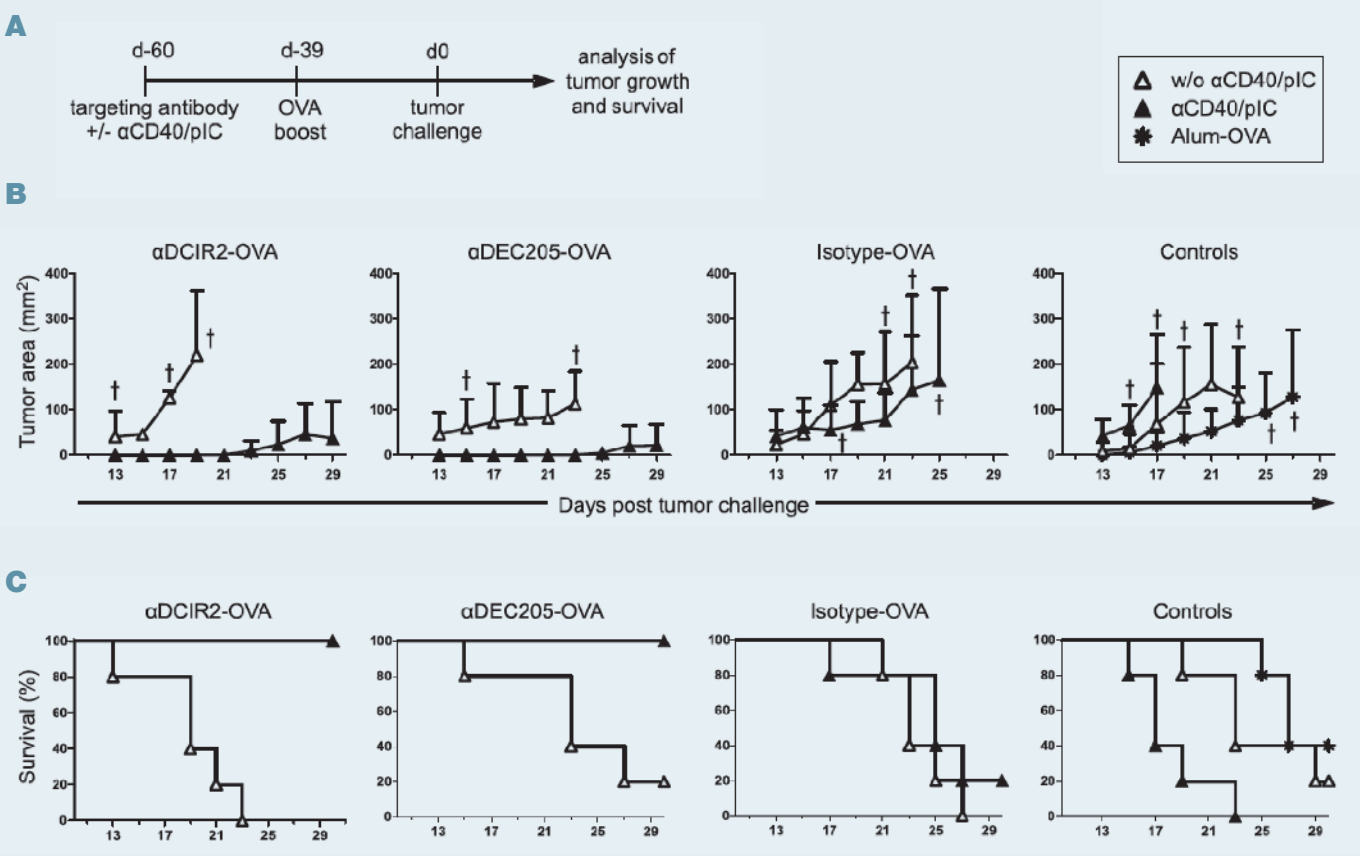

Figure 2: Preventive antigen targeting to $CD11c^+CD8^+$ and $CD11c^+CD8^-$ DCs reduces tumor cell growth and prolongs survival. Sixty days prior to tumor cell application, five C57BL/6 mice each were injected i.p. with 1 µg of anti-DCIR2-OVA, anti-DEC205-OVA, Iso-OVA, or PBS in the presence (filled) or absence (open) of the maturation stimuli 50 µg anti-CD40 Ab and 50 µg poly(I:C) (anti-CD40/pIC) or Alum-OVA (*). On day -39 (21 d after immunization), mice were boosted with 10 µg endotoxin-free soluble OVA protein and challenged with 2×10^5 B16F10-OVA cells on day 0. (**A**) Experimental setup. (**B**) Mean tumor sizes. Lines were discontinued when >50% of the mice needed to be sacrificed because of high tumor burden or died (†). Data are mean + SD. (**C**) Survival shown as Kaplan–Meier plots for five mice/group.