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Notes and Insights

Adenovirus vectors activate Vδ2$^+$ γδT cells in a type I interferon-, TNF-, and IL-18-dependent manner

Unlike conventional αβT cells, γδT cells, ~4% of circulating human T cells, are restricted by diverse non-MHC elements [1]. Up to 75% of circulating γδT cells express a paired Vδ2/Vγ9 T-cell receptor (TCR) (hereafter Vδ2$^+$ γδT cells) [2], which are restricted by butyrophilin 2A and 3A (BTN2A/BTN3A) family proteins, enabling recognition of self- and microbe-derived phosphoantigens [3]. This association of semi-invariant TCR (Vδ2/Vγ9) with monomorphic restricting element (BTN2A/BTN3A) is akin to two other unconventional αβT cell populations: mucosal-associated invariant T (MAIT) cells (Vα7.2-Jα33 TCR binding MR1) and invariant natural killer T (iNKT) cells (Vα24-Jα18/Vβ11 binding CD1d) [4].

Cytokines can activate MAIT and iNKT cells without TCR triggering [4]. Adenovirus (Ad) vaccine vectors, including ChAdOx1, can activate MAIT cells by the cytokines IL-18, type I interferon (IFN), and TNF [5], which activate Vδ2$^+$ γδT cells [6–8]. Thus, we sought to determine if Ad vector-induced cytokines activate Vδ2$^+$ γδT cells.

To test this, human peripheral blood mononuclear cells (PBMCs) were stimulated with an increasing multiplicity of infection (MOI) of two Ad vectors: ChAdOx1 or Ad5, and γδT cell activation was measured. After 24 h, ChAdOx1 induced expression of CD69 and IFN-γ by Vδ2$^+$ γδT cells, which were confirmed to co-express Vγ9 TCR (Fig. 1A–C; Supporting Information Fig. S1A and B). An MOI of 10^3 viral particles (vp) induced maximal activation (72% CD69$^+$ and 26% IFN-γ$^+$), which declined at a higher dose. In contrast, Ad5 induced minimal

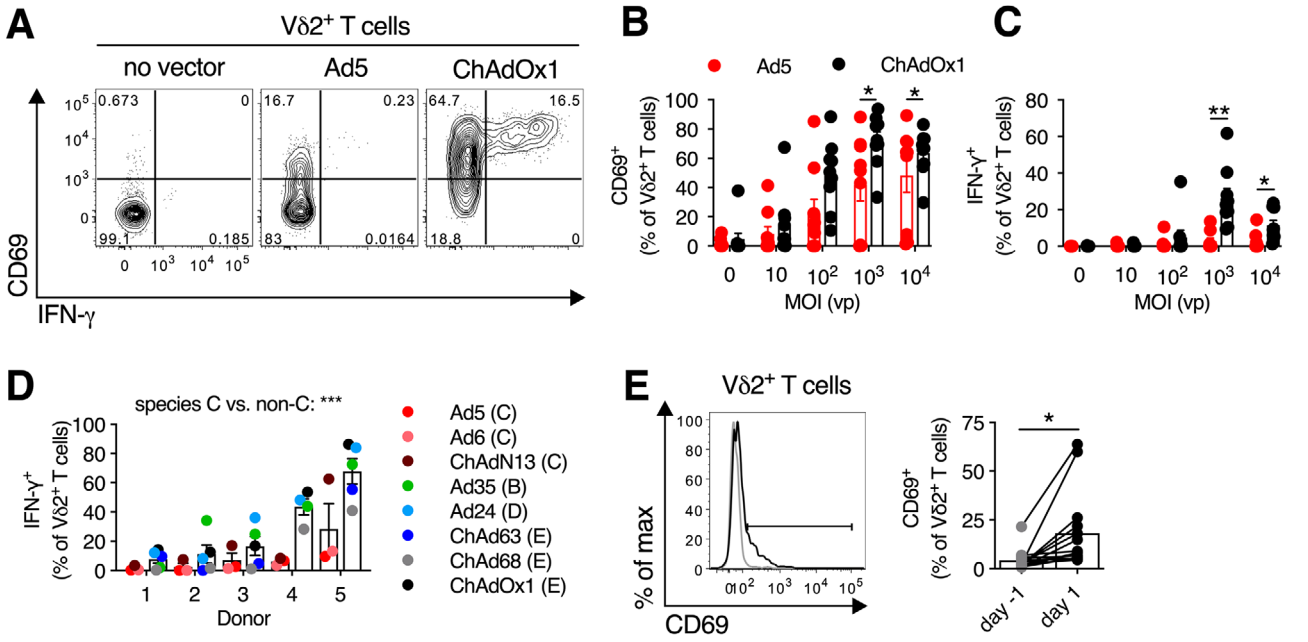

Figure 1. Adenovirus vectors activate Vδ2$^+$ γδT cells in vitro and in vivo. (A–C) Human PBMCs were stimulated with an increasing MOI of Ad5-GFP or ChAdOx1-GFP for 24 h. (A) Representative Vδ2$^+$ γδT cell CD69 and IFN-γ expression [MOI = 10^3 vp (viral particles)]. (B,C) Summary (n = 9 donors, three experiments) of Vδ2$^+$ γδT cell CD69 (B) and IFN-γ (C) expression. (D) Human PBMCs were stimulated with the indicated vectors (viral species in parentheses; n = 4 donors for ChAd63 and n = 5 donors for all others, two experiments). Vδ2$^+$ γδT cell IFN-γ expression was measured after 24 h. (E) PBMCs from human volunteers (n = 14 volunteers, two experiments) were collected 1 day before and 1 day after vaccination with ChAdOx1 MenB.1 (5×10^{10} vp dose) and Vδ2$^+$ γδT cell CD69 expression was measured. Mean ± SEM are shown. Dots represent individual donors/volunteers. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. (B,C) Unpaired t test; (D) two-way ANOVA; (E) paired Wilcoxon rank-sum test.

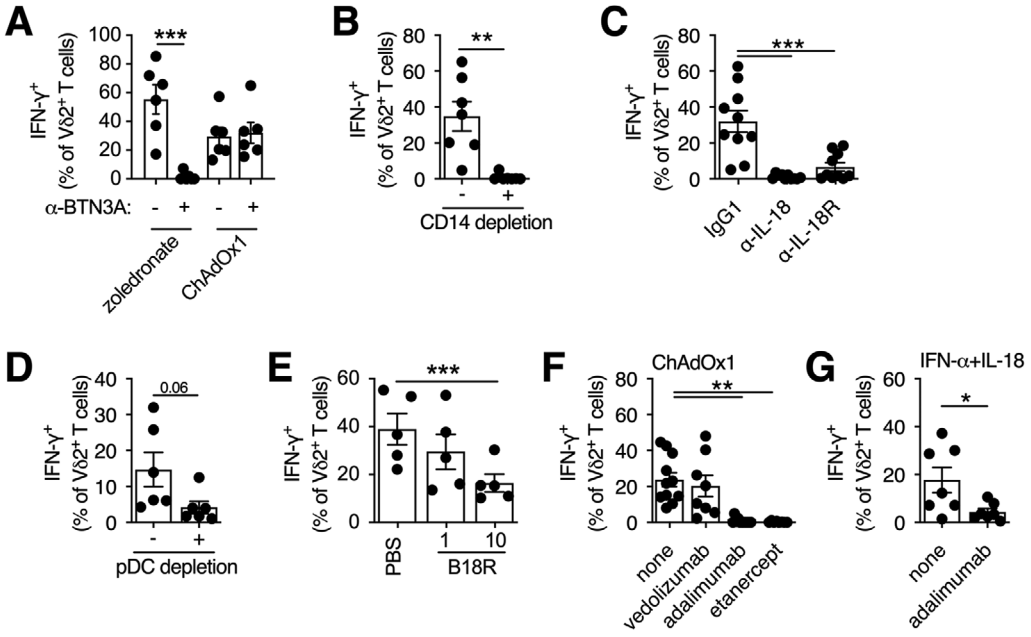

Figure 2. Activation of Vδ2$^+$ γδT cells by ChAdOx1 requires IL-18, type I interferon, and TNF. In all experiments, Vδ2$^+$ γδT cell IFN-γ production was measured after 24 h. (A) PBMCs ($n = 6$ donors, two experiments) were stimulated with zoledronate or ChAdOx1-GFP plus anti-BTN3A blocking or control antibody. (B) PBMCs or CD14-depleted PBMCs ($n = 7$ donors, two experiments) were stimulated with ChAdOx1-GFP. (C) PBMCs ($n = 10$ donors, three experiments) were treated with anti-IL-18 or anti-IL-18Rα antibodies (10 μg/ml) and stimulated with ChAdOx1-GFP. (D) PBMCs or CD123-depleted PBMCs ($n = 6$ donors, two experiments) were stimulated with ChAdOx1-GFP. (E) PBMCs ($n = 5$ donors, two experiments) were treated with B18R (type I interferon antagonist; 1 or 10 μg/ml) and stimulated with ChAdOx1-GFP. (F) PBMCs (three experiments) were treated with vedolizumab (anti-α4β7 integrin antibody; $n = 8$ donors), adalimumab (anti-TNF antibody; $n = 11$ donors), or etanercept (TNFR2-Fc fusion protein; $n = 8$ donors), or untreated ($n = 11$ donors), and stimulated with ChAdOx1-GFP. (G) PBMCs ($n = 7$ donors, two experiments) were treated with adalimumab (10 μg/ml) and stimulated with recombinant IFN-α and IL-18. Mean ± SEM are shown. Dots represent individual donors. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. (A,B,D,G) Unpaired *t* test; (C) Repeated-measure one-way ANOVA with Holm-Sidak's multiple comparison test; (E) repeated-measure one-way ANOVA with test for linear trend; (F) mixed-effects analysis with Dunnett's multiple comparison test.

CD69 or IFN-γ expression. Both vectors modestly induced Granzyme B, while negligible TNF was induced (Supporting Information Fig. S1C and D). Activation was biased to the Vδ2$^+$ γδT cell subset, as only CD69 was induced on non-Vδ2 γδT cells (Supporting Information Fig. S1E).

Species C Ad vectors induce distinct, weaker antiviral cytokine responses than non-species C vectors [9]. We examined three species C vectors (Ad5, Ad6, and ChAdN13) and five non-species C vectors (Ad24, Ad35, ChAd63, ChAd68, and ChAdOx1), and found that on average, the non-species C vectors induced significantly more Vδ2$^+$ γδT cell IFN-γ production (Fig. 1D). Thus, Ad vectors can activate Vδ2$^+$ γδT cells in vitro, and viral species differ in their stimulatory capacity.

To test if Ad vectors can activate Vδ2$^+$ γδT cells in vivo, we collected PBMCs from human volunteers 1 day pre- and 1 day post-immunization with a ChAdOx1 MenB.1 construct. Vaccination significantly increased CD69 expression on circulating Vδ2$^+$ γδT cells (Fig. 1E). Thus, Ad vectors activate human Vδ2$^+$ γδT cells in vivo.

We next determined how Vδ2$^+$ γδT cells were activated. Ad vector-driven activation of MAIT cells involves two processes: (1) production of IL-18 by monocytes and (2) production of type I interferon by plasmacytoid dendritic cells (pDCs), which acts directly but also by triggering TNF production by monocytes [5]. We tested Vδ2$^+$ γδT cell activation by these pathways.

Activation of Vδ2$^+$ γδT cells by ChAdOx1 was TCR-independent, as blocking BTN3A [10] had no effect (Fig. 2A). Monocyte depletion (Fig. 2B) and IL-18 signaling blockade (Fig. 2C) significantly inhibited Vδ2$^+$ γδT cell IFN-γ production. pDC depletion reduced Vδ2$^+$ γδT cell IFN-γ production by 71% ($p = 0.06$; Fig. 2D). Type I interferon signaling blockade using the vaccinia virus-derived B18R protein significantly reduced Vδ2$^+$ γδT cell IFN-γ production (Fig. 2E). TNF signaling blockade using adalimumab (anti-TNF antibody) or etanercept (TNFR2-Fc fusion protein) significantly reduced Vδ2$^+$ γδT cell IFN-γ production (Fig. 2F), while a control anti-α4β7 integrin antibody (vedolizumab) had no impact. TNF blockade of PBMCs stimulated with recombinant IFN-α and IL-18 significantly reduced Vδ2$^+$ γδT cell IFN-γ production (Fig. 2G), confirming that TNF was an intermediate of type I interferon-induced activation.

We found that Ad vectors activate human Vδ2$^+$ γδT cells in vitro and in vivo, and vectors derived from different adenovirus species are differentially stimulatory. Activation involves monocytes and pDCs, and signaling via IL-18, type I interferon, and TNF. This is concordant with the mechanism of Ad vector-induced MAIT cell activation [5], and supports the growing literature of shared innate-like

functionality of Vδ2$^+$ γδT, MAIT, and iNKT cells [6–8].

Our data raise a major question: what contribution do Vδ2$^+$ γδT cells make to the immune response induced against the Ad vector-encoded transgene? In mice, which lack an equivalent butyrophilin-restricted γδT cell population [1], the absence of MAIT cells dampens immune responses against the encoded transgene antigen [5]. From the highly overlapping biology of MAIT cells and Vδ2$^+$ γδT cells in humans, we hypothesize that these populations have possibly redundant roles in modulating vaccine immunogenicity. Further studies will be required to disentangle the shared functionality of these populations.

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Conflict of interest: C.D., C.S.R., and A.J.P. are named inventors on a patent application in the field of meningococcal vaccines. A.J.P. waives these patent rights. P.K. is a named inventor on a patent application in the field of cancer vaccines. The other authors declare no commercial or financial conflict of interest.

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Nicholas M. Provine[#1], **Ali Amini**[#1], **Lucy C. Garner**[1], **Michael E.B. FitzPatrick**[1], **Christina Dold**[2,3], **Laura Silva Reyes**[2,3], **Senthil Chinnakannan**[4], **Blanche Oguti**[2,3], **Meriel Raymond**[2,3], **Fulvia Troise**[5,6], **Stefania Capone**[7], **Antonella Folgori**[7], **Eleanor Barnes**[1,4], **Christine S. Rollier**[†2,3], **Andrew J. Pollard**[2,3] **and Paul Klenerman**[1,4]

[1] Translational Gastroenterology Unit, Nuffield Department of Medicine, University of Oxford, Oxford, UK
[2] Oxford Vaccine Group, Department of Paediatrics, University of Oxford, Oxford, UK
[3] NIHR Oxford Biomedical Research Centre, Oxford, UK
[4] Peter Medawar Building for Pathogen Research, University of Oxford, Oxford, UK
[5] Nouscom, SRL, Rome, Italy
[6] Ceinge Biotechnologie Avanzate, Naples, Italy
[7] ReiThera, SRL, Rome, Italy

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Full correspondence: Dr. Nicholas M. Provine and Paul Klenerman, Translational Gastroenterology Unit, Nuffield Department of Medicine, University of Oxford, Oxford, UK.
E-mail: nicholas.provine@ndm.ox.ac.uk; paul.klenerman@ndm.ox.ac.uk

[#]Authors Nicholas M. Provine and Ali Amini contributed equally to this work.
[†]Present address: Christine S. Rollier, School of Biosciences and Medicine, University of Surrey, Guildford, UK.

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