MAIT cell activation augments adenovirus vector vaccine immunogenicity

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Mucosal-associated invariant T (MAIT) cells are innate sensors of viruses and can augment early immune responses and contribute to protection. We hypothesized that MAIT cells may have inherent adjuvant activity in vaccine platforms that use replication-incompetent adenovirus vectors. In mice and humans, ChAdOx1 (chimpanzee adenovirus OX1) immunization robustly activated MAIT cells. Activation required plasmaid dendritic cell (pDC)–derived interferon (IFN)–α and monocyte-derived interleukin-18. IFN-α–induced, monocyte-derived tumor necrosis factor was also identified as a key secondary signal. All three cytokines were required in vitro and in vivo. Activation of MAIT cells positively correlated with vaccine-induced T cell responses in human volunteers and MAIT cell–deficient mice displayed impaired CD8+ T cell responses to multiple vaccine-encoded antigens. Thus, MAIT cells contribute to the immunogenicity of adenovirus vectors, with implications for vaccine design.

Mucosal-associated invariant T (MAIT) cells are unconventional T cells that recognize microbe-derived metabolites of vitamin B2 biosynthesis such as 5-(2-oxopropylideneamino)-6-O-ribitylaminouracil (5-OP-RU) (7). However, MAIT cells can also be activated by cytokines and thereby respond to viruses, which do not synthesize vitamin B2. In vivo, MAIT cells respond to influenza virus to amplify early local immune responses and protect against lethal infection (2–4). We hypothesized that the ability of MAIT cells to augment early immune responses may play a key role in viral vector vaccine immunogenicity. Replication-incompetent adenovirus (Ad) vectors are highly potent vaccine platforms for many human diseases (5). They have recently been licensed for use against the Ebola virus (6) and show promise for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (7, 8). We sought to determine whether such vectors activate MAIT cells and whether this affects vaccine immunogenicity.

To determine whether MAIT cells respond to Ad vectors, we stimulated human peripheral blood mononuclear cells (PBMCs) with Ad5 and chimpanzee adenovirus OX1 (ChAdOx1), which are leading SARS-CoV-2 candidate vaccines (7, 8). ChAdOx1 induced dose-dependent up-regulation of CD69, granzyme B, and interferon (IFN)–γ by MAIT cells (Fig. 1, A to C, and fig. S1, A to D), whereas Ad5 only weakly activated MAIT cells. This activation was confirmed using the MRI-5–OP-RU tetramer to identify MAIT cells (fig. S1E).

Species C–derived Ad vectors have been shown to poorly stimulate innate immune responses as compared with non–species C vectors (9–11). We tested the relative ability of three species C vectors (Ad5, Ad6, and ChAdN19) and five non–species C vectors (Ad24, Ad35, ChAd63, ChAd68, and ChAdOx1) (fig. S1F) to activate MAIT cells. After stimulation, we observed greater average activation by non–species C vectors as compared with species C vectors (Fig. 1, D and E).

We next tested the ability of Ad vectors to activate MAIT cells in vivo. Intramuscular (i.m.) ChAdOx1 immunization of C57BL/6J mice strongly induced up-regulation of CD69 and granzyme B by MAIT cells, whereas Ad5 induced significantly weaker activation (Fig. 1, F and G, and fig. S2, A to C). We also observed significant up-regulation of CD69 on MAIT cells 1 day after immunization of human volunteers with a candidate ChAdOx1 vaccine (Fig. 1H and fig. S3, A to C). Plasma IFN-γ levels markedly increased after activation (Fig. S3D), as seen in nonhuman primates (10). This increase correlated with levels of MAIT cell activation (Fig. 1I).

To investigate the pathways involved, RNA sequencing (RNA-seq) of MAIT cells was performed. Eighty-four genes were significantly up-regulated in human MAIT cells after vaccination (Fig. 2A and data S1). Gene set enrichment analysis (GSEA) (12) identified the strong induction of type I IFN, interleukin (IL)–1 family, IL-12 family, and IL-2 family signaling pathways (Fig. 2B). Changes in post-vaccination plasma IFN-α or CCL2, an IFN-regulated chemokine (13), strongly correlated with MAIT cell activation (Fig. 2C and fig. S3, D and E). Comparison of genes up-regulated in MAIT cells after human vaccination, vaccination of mice, or in vitro stimulation showed a high degree of overlap. Ninety-eight percent of vaccine–up-regulated genes in humans were up-regulated in at least one of the other two conditions, and 63% were up-regulated in both (Fig. 2D; fig. S4, A and B; and data S2 to S4). GSEA on murine MAIT cells and in vitro-stimulated human MAIT cells identified similar enrichment of these cytokine signaling pathways (fig. S4, C and D).

In vitro inhibition of type I IFN signaling reduced MAIT cell IFN-γ production by >50% (Fig. 2E). Blockade of IL-18 (an IL-1 family member) or IL-12 also reduced MAIT cell activation. By contrast, blockade of IL-15 (an IL-2 family member) had no effect (Fig. 2F and fig. S5A). MAIT cell activation by Ad vectors was independent of T cell receptor signaling (fig. S5B) (2, 3).

To understand the cellular origins of these critical cytokines, we examined the cell populations transduced by Ad5 and ChAdOx1. Monocytes or conventional dendritic cells were the major transduced population by both vectors (>80% of green fluorescent protein–expressing (GFP+) cells) (fig. S5, C to F). ChAdOx1 also efficiently transduced CD123+ plasmacytoid dendritic cells (pDCs), whereas Ad5 did not (fig. S5F) (11). Notably, depletion of CD123+ pDCs resulted in a significant (67%) reduction in IFN-γ production by MAIT cells (Fig. 2G) and reduced IFN-α levels by >99% after ChAdOx1 stimulation (Fig. 2H).

Depletion of CD44+ monocytes significantly reduced MAIT cell activation after ChAdOx1 stimulation (Fig. 2I and fig. S5G) and abrogated the secretion of IL-18 (Fig. 2J). The cathespin B–NLRP3 inflammasome pathway (14) was the source of IL-18 in response to ChAdOx1 (fig. S6). Thus, pDC-derived IFN-α and monocyte-derived IL-18 play critical roles in activating MAIT cells in response to Ad vectors. Ad5 induced negligible amounts of IFN-α (fig. S7, A and B) (10, 11). Despite transducing monocytes, Ad5 did not induce IL-18 or IL-12p70 (fig. S7, C and D). By contrast, ChAdOx1 induced robust production of IFN-α and IL-18.

Although IFN-α/β and IL-18 together induced production of IFN-γ by MAIT cells in PBMC culture, this was not seen using isolated CD8+ T cells (~75% of human MAIT cells express CD8+ (15)) (Fig. 3A), despite the induction of CD69 (fig. S8A). Depletion of monocytes reduced MAIT cell IFN-γ production after stimulation with IFN-α and IL-18 (fig.

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Fig. 1. Activation of human and murine MAIT cells by adenovirus vectors. (A to C) Human PBMCs (n = 9 donors; four experiments) were stimulated with Ad5-GFP or ChAdOx1-GFP (multiplicity of infection (MOI) = 0 to 10^4 vp (viral particles)). MAIT cell CD69 (A), granzyme B (GzmB) (B), and IFN-γ (C) expression was measured after 24 hours. (D and E) Human PBMCs (n = 5 donors; two experiments) were stimulated with the indicated vectors (species in parentheses). MAIT cell GzmB (D) and IFN-γ (E) expression was measured after 24 hours. C, species C; non-C, non-change in plasma IFN-γ. (F) MAIT cell CD69 expression 1 day before and 1 day after immunization. (G) Pearson correlation of vectors (species in parentheses). MAIT cell GzmB (D) or IFN-γ (E) expression was measured after 24 hours. (H) Inguinal lymph node (iLN) and liver MAIT cell CD69 (F) and GzmB (G) expression was measured after 24 hours. (I) Healthy human volunteers (n = 14) were immunized with a 5 × 10^{10} vp dose of ChAdOx1 MenB1. (H) MAIT cell CD69 expression 1 day before and 1 day after immunization. (I) Pearson correlation of change in plasma IFN-γ levels after vaccination with the change in MAIT cell CD69 expression. *P < 0.05; **P < 0.01; ***P < 0.001. Unpaired t test [(A) to (C)], two-way analysis of variance (ANOVA) [(D) and (E)], one-way ANOVA with Sidak correction for multiple comparisons [(F) and (G)], or Wilcoxon rank-sum test (H). Symbols indicate average response [(A) to (C)] or individual mice or volunteers [(D) to (I)]. Mean ± SEM is shown.
which lack MAIT cells (16), were used (fig. S11, A to C). After vaccination with ChAdOx1 expressing an optimized hepatitis C virus (HCV) antigen (17), Mr1−/− mice had significantly reduced frequencies of HCV-specific CD8+ T cells compared with WT mice (Fig. 4C and fig. S11D). No significant defect in HCV-specific CD4+ T cells was observed (fig. S11E). We also observed defects in the CD8+ T cell responses of Mr1−/− mice vaccinated with the candidate SARS-CoV-2 vaccine, ChAdOx1-nCoV-19 (Fig. 4D and fig. S11F) (7). WT and Mr1−/− mice were then given a homologous ChAd63-ovalbumin (OVA) prime-boost immunization (fig. S11G) (18). Mr1−/− mice displayed reduced OVA-specific CD8+ T cell responses after both priming and boosting (Fig. 4, E and F). Differences in the microbiome (19) or general immunodeficiency of Mr1−/− mice did not explain these differences in immunogenicity (fig. S12).

MAIT cells can sense the diversity of the Ad vector–induced innate immune activation landscape (e.g., IFN-α, TNF, IL-18), integrating these signals to augment vaccine-induced CD8+ T cell immunity. The blend of signals required to maximally trigger MAIT cells described here includes a critical pathway via type I IFN–dependent TNF release, relying on cross-talk between two distinct populations of transduced cells, and varies between

**Fig. 2. Activation of MAIT cells by adenovirus vectors requires pDC-derived IFN-α and monocyte-derived IL-18.** (A and B) Gene expression analysis of MAIT cells isolated from the PBMCs of human volunteers 1 day before and 1 day after vaccination with ChAdOx1 MenB1 (n = 14 vaccinees). (A) Volcano plot of differentially expressed genes [log2 fold change (FC) > 1, adjusted P < 0.05]. The top 10 up-regulated genes are annotated. (B) Selected cytokine signaling pathways from the Reactome database enriched by GSEA. NES, normalized enrichment score. (C) Pearson correlation of change in plasma IFN-α level after vaccination with the change in MAIT cell CD69 expression. (D) Overlap of genes up-regulated in MAIT cells from ChAdOx1-vaccinated volunteers (“human in vivo”), from human PBMCs stimulated with ChAdOx1 (“human in vitro”), and from the draining inguinal LNs of ChAdOx1-vaccinated mice (“mouse in vivo”). (E and F) Human PBMCs were stimulated with ChAdOx1-GFP, and the following inhibitors were used: vaccinia virus–derived type I IFN antagonist B18R (1 or 10 μg/ml; n = 7 donors; three experiments) or anti-IFNAR2 antibody (10 or 25 μg/ml; n = 5 or 3 donors; two or one experiments, respectively) (E); or anti-IL-12, anti-IL-15, or anti-IL-18 antibodies (10 μg/ml; n = 5 donors; two experiments) (F). MAIT cell IFN-γ expression was measured after 24 hours. PBS, phosphate-buffered saline. (G and H) PBMCs were depleted of CD123+ pDCs or left untreated and stimulated with ChAdOx1-GFP. MAIT cell IFN-γ expression (n = 8 donors; three experiments) (G) or levels of IFN-α in the cell culture supernatant (n = 4 donors; one experiment) (H) were measured after 24 hours. (I and J) PBMCs were depleted of CD14+ monocytes or left untreated and stimulated with ChAdOx1-GFP. MAIT cell IFN-γ expression (n = 4 donors; two experiments) (I) or IL-18 levels in the supernatant (n = 4 donors; three experiments) (J) were measured after 24 hours. *P < 0.05; **P < 0.01; ***P < 0.001. Repeated-measures one-way ANOVA with Dunnett correction [(E) and (F)] or unpaired t test [(G) to (J)]. Symbols indicate individual donors. Mean ± SEM is shown.
adenovirus serotypes. Our data, coupled with studies in the lung (4, 20, 21), support a model that places MAIT cells in a critical bridging position between innate and adaptive immunity. The mechanism by which MAIT cell activation promotes antigen-specific CD8+ T cell responses remains to be defined. However, local production of chemokine CXCL10 represents a promising candidate as it can promote CD8+ T cell priming (22).

It is notable that the activation of MAIT cells is tightly linked to the immunogenicity of antigens. MAIT cell activation is enhanced by pro-inflammatory cytokines, particularly IFN-α, which is produced by infected cells in response to viral infection (4, 20, 21). Our studies in the lung (4, 20, 21) and in vivo models (22) support this model. The mechanism by which MAIT cell activation promotes antigen-specific CD8+ T cell responses remains to be defined. However, local production of chemokine CXCL10 represents a promising candidate as it can promote CD8+ T cell priming (22).

Fig. 3. IFN-α acts directly and indirectly through the induction of TNF to activate MAIT cells. (A) Human PBMCs or purified CD8+ T cells (n = 3 donors; one experiment) were stimulated with the indicated cytokines (50 ng/ml). MAIT cell IFN-γ expression was measured after 24 hours. (B) Purified CD8+ T cells with or without CD14+ monocytes (n = 4 donors; one experiment) were stimulated with IFN-α and IL-18 (50 ng/ml). MAIT cell IFN-γ expression was measured after 24 hours. (C) Purified monocytes (n = 3 donors; one experiment) were stimulated with IFN-α (50 ng/ml) or left untreated. After 24 hours, supernatants were transferred with or without IL-18 (50 ng/ml) to autologous purified CD8+ T cells. MAIT cell IFN-γ expression was measured after 24 hours. (D) TNF production by IFN-α–treated CD14-purified monocytes was measured after 24 hours (n = 3 donors; one experiment). (E) Purified CD8+ T cells (n = 10 donors; four experiments) were stimulated with IFN-α and IL-18 with or without TNF (50 ng/ml) or anti-TNF2R2 agon antibody (2.5 μg/ml). MAIT cell IFN-γ expression was measured after 24 hours. (F) PBMCs were stimulated with ChAdOx1, and the following inhibitors were added: vedolizumab (anti-α4β7 integrin antibody, n = 8 donors; two experiments), adalimumab (anti-TNF antibody, n = 11 donors; three experiments), or etanercept (TNFR2-Fc fusion protein, n = 8 donors; two experiments). MAIT cell IFN-γ expression was measured after 24 hours. (G) PBMCs with or without CD14 depletion were stimulated with ChAdOx1. Concentration of TNF in the supernatant was measured after 24 hours (n = 4 donors; one experiment). (H) Purified monocytes (n = 4 donors; one experiment), or Ifnar−/− mice were immunized i.m. with 10^5 IU of ChAdOx1–GFP. Naïve C57BL/6J mice (n = 4) were used as a control. After 24 hours, MAIT cells were isolated from the iLNs and sorted for RNA-seq (one experiment). (H) Principal components analysis. (I) Heatmap of the up-regulated differentially expressed genes (log2 FC > 1, adjusted P < 0.05) between MAIT cells from ChAdOx1-immunized and naïve C57BL/6J mice, with all other groups shown for comparison. (J) Overlap of the genes up-regulated (log2 FC > 1, adjusted P < 0.05) in MAIT cells from ChAdOx1-immunized and naïve C57BL/6J mice, and the genes up-regulated in MAIT cells from ChAdOx1-immunized C57BL/6J mice as compared to each of the ChAdOx1-immunized knockout strains. *P < 0.05; **P < 0.01. Unpaired t test [(B), (C), and (G)], repeated-measures one-way ANOVA with Dunnett correction [(E) and (F)]. Symbols indicate individual donors. Mean ± SEM is shown.
of adenovirus vectors. This technology has emerged as a potent platform for T cell immunogenicity in clinical trials for HIV (23) and as vaccines for emerging viruses such as Ebola (6) and SARS-CoV-2 (7, 8). This knowledge can be harnessed to improve the design of these vaccines against major pathogens and cancers.

**REFERENCES AND NOTES**

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SUPPLEMENTARY MATERIALS

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Materials and Methods

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Vaccines get a help-MAIT

Mucosal-associated invariant T (MAIT) cells are a T cell subset important for mucosal homeostasis. These cells recognize derivatives of microbiota-derived vitamin B2 precursors but can also be activated by certain cytokines in the context of viral infections. Provine et al. report that a leading adenoviral vector vaccine, ChAdOx1, activated MAIT cells in immunized mice (see the Perspective by Juno and O'Connor). This activation required interferon-γ produced by plasmacytoid dendritic cells as well as monocyte-derived interleukin-18 and tumor necrosis factor. MAIT cell activation positively correlated with vaccine-mediated T cell responses in human subjects, and mice deficient in MAIT cells showed impaired CD8+ T cell immunity to target antigens after vaccination. This work suggests an additional pathway that could be exploited to enhance the efficacy of vaccines.

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