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Emmanuelle Bardin, Céline Dietrich, Messaouda Attaïlia, Agnès Ferroni, Anne Jamet, et al.. Restored Cytokine-Producing Capacities of Mucosal-associated Invariant T Cells in Pediatric Cystic Fibrosis Patients Treated with Elexacaftor/Tezacaftor/Ivacaftor. *American Journal of Respiratory and Critical Care Medicine*, 2024, 210 (2), pp.243-245. 10.1164/rccm.202401-0201LE . hal-04660891

HAL Id: hal-04660891

<https://hal.science/hal-04660891>

Submitted on 24 Jul 2024

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Restored Cytokine-Producing Capacities of Mucosal-Associated Invariant T Cells in Pediatric Cystic Fibrosis Patients Treated with Elexacaftor/Tezacaftor/Ivacaftor

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Keywords: ETI treatment, cystic fibrosis, children, MAIT cells, inflammation.

Author contributions:

ISG, MLM contributed to conception and design of the research; EB, CD, MA performed experiments and data acquisition; EB, AF, AJ, GL, ISG contributed to data collection; EB, ISG, MLM drafted the manuscript.

To the Editor:

Cystic fibrosis (CF) is an autosomal recessive inherited disorder that leads to chronic airway disease accompanied by long-lasting airway inflammation and bacterial infections, such as those caused by *Pseudomonas aeruginosa* and *Staphylococcus aureus* (1,2). Airway epithelial biopsies show a marked T-lymphocyte infiltrate, suggesting an important role for adaptive immune responses in the orchestration of sustained inflammation (3). The lung contains distinct innate-like T lymphocytes, including mucosal-associated invariant T cells (MAIT). MAIT are the most abundant T-cell population that recognizes bacterial metabolic compounds, enabling them to play an important role in antimicrobial immunity (4). MAIT can accumulate in the bronchoalveolar lavage of people with CF (6), suggesting their possible involvement in the control of lung infection in these patients by their ability to produce IFN γ , TNF α , GrzB and IL-17A (4,5). Previous studies showed that *S. aureus* antigens induce robust MAIT-cell activation, rapidly followed by a state of anergy/exhaustion that may compromise their antimicrobial role (7,8). Such hyperactivation is characterized by the high production of IFN γ and TNF α , whereas anergic/exhausted MAIT highly express the checkpoint markers CD279(PD-1), CD223(LAG-3), and anti-CD366(TIM-3) (8). In patients with active tuberculosis, MAIT highly expressed CD279/PD-1 marker, and its blockade resulted in significantly higher IFN γ production (9). These cytokines and markers could be useful for the analysis of the functional properties of MAIT.

The advent of the CFTR modulators elexacaftor, tezacaftor, and ivacaftor (ETI) dramatically improves lung function and respiratory symptoms of patients with CF (1,10). Here, we aimed to determine the activation state and cytokine-producing abilities of MAIT in children with CF (cwCF) and the impact of ETI treatment on these cells.

Twenty-four cwCF (16 girls, 8 boys; 9 [7-11] years of age) with at least one F508del mutation (13 homozygous, 11 heterozygous) and 24 age-matched control children (14 girls, 10 boys; 9.5 [7-12.7] years of age) were enrolled between September 2021 and July 2023 at the Necker Enfants Malades Hospital. A second cohort of cwCF (5 [3-6] years of age) was enrolled between November 2023 and Mars 2024. The study was approved (NCT04301856, NCT03776474) and all parents gave informed consent. Patients were evaluated at baseline (M0), one month (M1), and three months (M3) after ETI initiation.

Circulating MAIT profiling was performed by flow cytometry, as described (5). Peripheral blood mononuclear cells (PBMCs) were stained with anti-CD3, anti-CD161, anti-CD25, anti-CD279/PD-1, anti-CD223/LAG-3, anti-CD366/TIM-3 and MR1-5-OP-RU tetramers (National Institutes of Health Tetramer Core Facility, Atlanta, GA). MAIT were identified as CD3⁺CD161⁺MR1-5-OP-RUtetramer⁺. For intracellular cytokine analysis to allow the identification of GrzB⁻, TNF α ⁻, IFN γ ⁻, and IL-17A-producing MAIT, PBMCs were cultured for 5h with the non-specific activators 25ng/mL phorbol 12-myristate 13-acetate and 1 μ M ionomycin plus 10ng/mL brefeldin A. Antibodies and molecules were purchased from BioLegend (San Diego, CA) and Sigma-Aldrich (Saint-Quentin Fallavier, France), respectively. Events were acquired on a LSR Fortessa cell analyzer and processed using FlowJo software v10.8.1 (Becton Dickson, Ashland).

Data are reported as medians (quartiles). Comparisons versus controls were made using two-tailed non-parametric unpaired Mann-Whitney tests; comparisons between M0, M1, and M3 groups used paired Friedman tests corrected for multiple comparisons by controlling the FDR. Statistical analyses were performed using Prism 10.1.1 (GraphPad Software, Boston).

CwCF patients had mild CF lung disease, as assessed by the percentage predicted forced expiratory volume in 1s (FEV_{1,pp}) at baseline (**Table 1**). As expected, FEV_{1,pp} increased significantly from M1, and sweat chloride concentration significantly decreased (**Table 1**). Sputum bacterial colonization improved, showing a significant decrease in *S. aureus* inoculum (**Table 1**).

At baseline, the number of circulating MAIT was significantly lower in cwCF than in controls (**Figure 1A**). Likewise, the frequency of CD25+ (marker of activation), TNF α +, GrzB+ and IL-17A+ cells among gated MAIT was lower in cwCF (**Figures 1C, 1D, 1E and 1G**).

The number of total MAIT significantly increased as early as one month after ETI initiation reaching levels similar to those of the controls at M3 (**Figures 1A**). This result was replicated in the second cohort (**Figure 1B**). Treatment similarly restored the frequency of CD25+, TNF α +, GrzB+ and IL-17A+ cells among MAIT and enhanced their ability to produce IFN γ (**Figures 1C to 1G**).

Given that MAIT can promote anti-infectious responses, we divided cwCF into two groups according to MAIT numbers. The threshold of 15,000/mL was defined according to the median level of MAIT in controls (**Figure 1A**). CwCF in the group with >15,000 MAIT had significantly less *S. aureus* in their sputum (1×10^4 ($0-1 \times 10^5$) versus 0 ($0-1 \times 10^4$) CFU/mL, $p=0.048$). Further analysis failed to show a significant linear regression ($R^2=0.02$, $p=0.234$) or nonparametric Spearman correlation ($r=-0.224$; $p=0.06$) between the number of MAIT and *S. aureus* CFU/mL.

MAIT from cwCF expressed a high frequency of the co-inhibitory molecules CD279/PD-1, CD223/LAG-3, and CD366/TIM-3 (**Figures 1H to 1J**), suggesting that these cells could be in an anergic/exhausted state, as described (8,9). ETI treatment resulted in a progressive loss of

expression of these molecules, their frequency returning to a level similar to that of the controls at M3 (**Figures 1H to 1J**).

Our results show that cwCF with mild lung disease have a reduced number of circulating MAIT. The MAIT were less activated and showed an anergic/exhausted phenotype, reflecting their reduced cytokine-producing capacity. ETI treatment restored the number and functional properties of MAIT to levels similar to those of controls.

Activated MAIT can provide protection against pulmonary bacterial infections (4,9). The observation that cwCF with a higher number of MAIT had a significantly lower *S. aureus* load suggests that these cells may contribute to infection control in these patients. The main mechanism by which MAIT play their anti-infectious role is cytokine production. MAIT respond rapidly to *S. aureus* stimulation and can induce the apoptosis of *S. aureus*-infected cells by producing GrzB (7). Therefore, the enhanced cytokine-producing capacity of MAIT observed after ETI treatment may represent a novel mechanism that could contribute to infection control. These changes in MAIT cell number and activation state could be patient-specific and should be further investigated.

Our study had several limitations. For ethical reasons, we were unable to profile MAIT in the BAL of cwCF after ETI treatment. The number of T cells in cwCF sputum is very low, limiting the analysis of MAIT. Consequently, we cannot assert that the differences observed in blood reflect those in BAL or sputum. Moreover, given the small size of our cohort, the results need to be confirmed in larger series.

Our results suggest that ETI treatment could be associated with functional restoration of MAIT and could help boost their antimicrobial role in cwCF.

FIGURE LEGEND

Figure 1. Longitudinal analysis of circulating MAIT cells following ETI initiation. Total numbers of MAIT (Mucosal-Associated Invariant T cells) in cohort 1 (9.5 [7-12.7] years of age) and in cohort 2 (5 [3-6] years of age) are represented in **(A)** and **(B)**, respectively. Percentages of activated cluster of differentiation (CD)25+ **(C)**, tumor necrosis factor (TNF) α + **(D)**, Granzyme B (GrzB)+ **(E)**, interferon (IFN γ)⁺ **(F)**, interleukin (IL)-17A+ **(G)**, CD279 (PD-1 or programmed cell death 1)+ **(H)**, CD223 (LAG-3 or lymphocyte-activation gene 3)+ **(I)** and CD366 (TIM-3 or T-cell immunoglobulin and mucin domain 3)+ **(J)** among gated MAIT from control or cwCF at M0 (initiation) and treated with ETI at M1 (1 month) and M3 (3 months). MAIT were identified as CD3+CD161+MR1-5-OP-RU [MHC related protein 1-5-(2-oxopropylideneamino)-6-D-ribitylamino] tetramers+. Statistical differences were determined using Kruskal-Wallis (CONT versus M0 and CONT versus M3) and paired Friedman tests (between M0, M1, and M3). False discovery rate (FDR) corrections were applied. Significant *p* values (*p* < 0.05) are indicated.

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Table 1. Clinical characteristics of cwCF treated with elexacaftor, tezacaftor, and ivacaftor (ETI)

	<i>p</i> value				
	M0	M1	M3	M0 x M1	M0 x M3
Clinical and microbiological endpoints					
Weight, kg	26.5 (22.2-34.8)	26.9 (22.8-35.1)	27.9 (25.2-36.2)	0.0065	0.0002
Sweat chloride concentration, mEq/L	89 (73-103)	35 (20.5-46.5)	nd	< 0.0001	nd
FEV₁, pp	97 (80.6-103)	106 (93.4-111)	115 (106-142)	< 0.0001	< 0.0001
FVC, pp	102 (93.5-111)	106 (101-113)	106 (101-115)	0.0019	0.0237
FEF₂₅₋₇₅, pp	59.1 (46.6-92.5)	78 (63.3-110)	86.6 (62.1-121)	0.0006	0.0296
Bronchial colonization					
<i>Staphylococcus aureus</i> (CFU/mL)	100x10 ³ (0-7.7x10 ⁶)	1x10 ³ (0-1x10 ⁵)	1x10 ³ (0-1x10 ⁵)	0.027	0.042
<i>Pseudomonas aeruginosa, Achromobacter xylosoxidans or Stenotrophomonas maltophilia</i> (nb positive/nb tested)	4/24	2/23	3/22	0.825	0.825

Data from 24 cwCF (16 girls, 8 boys; 9 [7-11] years of age) with at least one F508del mutation (13 homozygous, 11 heterozygous) and 24 age-matched control children (14 girls, 10 boys; 9.5 [7-12.7] years of age) were enrolled between September 2021 and July 2023 at the Necker Enfants Malades Hospital. Definition of abbreviations: FEV₁= Forced expiratory volume in the first second; FVC= forced vital capacity; FEF₂₅₋₇₅=forced mid-expiratory flow; M0=month 0; M1=month 1; M3= month 3; pp =percentage predicted; nb: number; nd : not done. Data are given as median (interquartile range). Bronchial colonization refers to the chronic identification of bacteria in sputum. p< 0.05 considered as significant and indicated in bold (Friedman test, two-tailed, for paired samples).

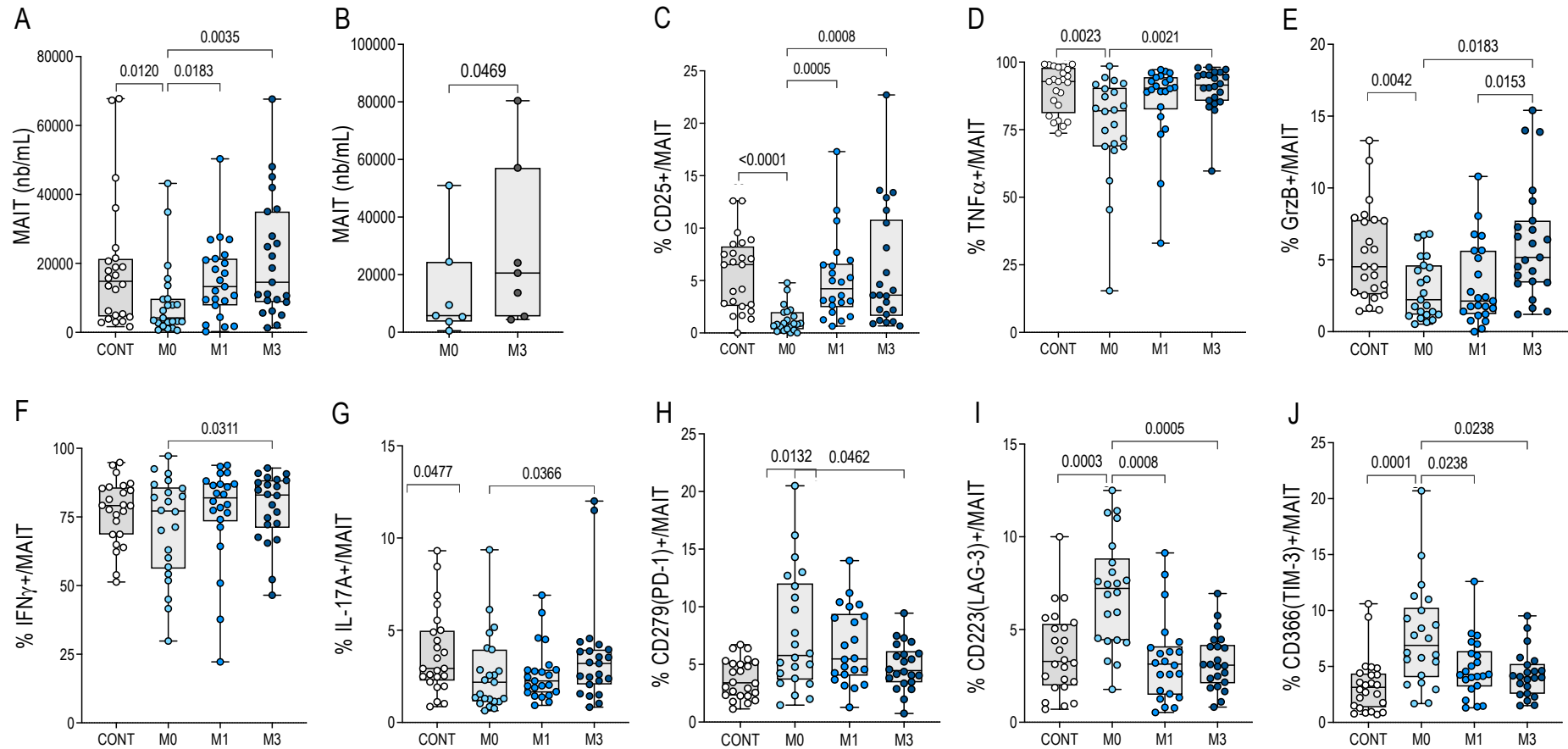


Figure 1. Longitudinal analysis of circulating MAIT cells following ETI treatment. Data represent the number of MAIT (Mucosal-Associated Invariant T cells) (A and B) and the percentage of activated cluster of differentiation (CD)25+ (C), tumor necrosis factor (TNF) α + (D), Granzyme B (GrzB)+ (E), interferon (IFN) γ + (F), interleukin (IL)-17+ (G), CD279 (PD-1 or programmed cell death 1)+ (H), CD223 (LAG-3 or lymphocyte-activation gene 3)+ (I) and CD366 (TIM-3 or T-cell immunoglobulin and mucin domain 3)+ (J) among gated MAIT from control or cwCF patients treated with ETI at M0 (initiation), M1 (1 month) and M3 (3 months). MAIT were identified as CD3+CD161+ MR1-5-OP-RU[MHC related protein 1-5-(2-oxopropylideneamino)-6-D-ribitylaminouracil] tetramers+. Data from the cohort 1 (9.5 [7-12.7] years of age) are represented at Figures A, C, D, E, F, J, H, I, J and data from the cohort 2 (5 [3-6] years of age) are represented at Figure B. Each point within the box plot represents one individual. Statistical differences were determined by Mann-Whitney (CONT versus M0 and CONT versus M3) and paired Friedman test (for M0, M1 and M3). FDR (false discovery rate) corrections were used. Significant p values ($p < 0.05$) are indicated.