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THE ROLE OF MAIT CELL IN PROTECTION AGAINST INFECTION *IN-VIVO*

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ABSTRACT:
MAIT cells are also activated during human viral infections, yet it remains unknown whether MAIT cells play a significant protective or even detrimental role during viral infections *in vivo*. Using murine experimental challenge with two strains of influenza A virus, we show that MAIT cells accumulated and were activated early in infection, with upregulation of CD25, CD69 and Granzyme B, peaking at 5 days post infection. Activation was modulated via cytokines independently of MR1. MAIT cell-deficient MR1-/- mice showed enhanced weight loss and mortality to severe (H1N1) influenza. This was ameliorated by prior adoptive transfer of pulmonary MAIT cells in both immunocompetent and immunodeficient RAG2-/-gC-/- mice. Thus, MAIT cells contribute to protection during respiratory viral infections, and constitute a potential target for therapeutic manipulation.
Key words: Mucosal associated invariant T cell, T cell, infection, influenza virus.

1. INTRODUCTION:

The *innate* immune system comprises anatomical, physiological, and inflammatory barriers, and uses innate immune cells to recognize structural motifs shared by many pathogens – the pathogen-associated molecular patterns (PAMPs. In this way, rapid responses against a broad range of pathogens can be mounted in an unspecific manner. In contrast, the *adaptive* immune system relies on specialized cells that recognize unique pathogenic motifs, the *antigens* [1]

1.1. Unconventional T Cells:

Unconventional T cells recognize antigens presented by antigen-presenting molecules encoded by genes that display a low degree of polymorphism. [2,3]

1.2. Towards the discovery of MAIT cells:

The semi-invariant TCR rearrangement characteristic of MAIT cells in humans, Vα7.2-Jα33, was identified for the first time in 1993 when Porcelli *et al.* examined the TCRα chains of peripheral blood CD8-CD4- (double-negative, DN) T cells from healthy individuals. Later in 1999, Tilloy *et al.* reported that this TCR rearrangement defines a new cell population of DN and CD8αα T cells in humans with an effect or memory phenotype.

1.3 Evolutionary conservation of MR1 and MAIT cells: The *MR1* gene, discovered in 1995 is believed to have been established 160 to 220 million years ago in a common ancestor of placental and marsupial mammals MR1 and MAIT cells are present and highly conserved across mammals, and are found not just in humans and mice but also in non-human primates cattle, sheep, bats, elephants,Tasmanian devils, and opossums.

1.4 Identification of MAIT cells:

In 2009, Martin *et al.* developed the monoclonal antibody (clone 3C10) that recognizes the human TCR Vα7.2 segment and showed that the Vα7.2-Jα33 gene rearrangement characteristic of MAIT cells was only found in Vα7.2+ cells expressing high levels of CD161**.** Later in 2011, Le Bourhis *et al.* reported that CD161 and interleukin (IL)-18 receptor α (IL-18Rα) were co-expressed on MAIT cells Thus, co-expression of Vα7.2, and IL-18Rα or high levels of CD161 within the T cell compartment was adopted in the field to identify MAIT cells by flow cytometry.

1.5 MAIT cell development and phenotype:

Like NKT cells, MAIT cells develop in the thymus, where they are selected by MR1-expressing CD8+CD4+ (double-positive, DP) thymocytes. Two studies initially alluded to this through the detection of high levels of endogenous MR1 in mouse and human DP thymocytes.

1.6 MAIT cell tissue localization:

Adult peripheral blood MAIT cells express a distinct combination of chemokine receptor that mediate their trafficking to peripheral tissues. This includes the expression of CCR6 and CXCR6, liver-homing chemokine receptors, as well as α4β7 and intermediate levels of CCR9, receptors involved in lymphocyte migration to the gut.

1.7 Antigen presentation to MAIT cells:

MAIT cell TCR: Most MAIT cells express the TCRα chain defined by the Vα7.2-Jα33 rearrangement, whereas a minority expresses either Vα7.2-Jα12 or Vα7.2-Jα20.

1.8 MAIT cell effector functions:

MAIT cells respond to riboflavin-producing microbes through the upregulation of the activation markers CD69 and CD25 (or IL-2Rα chain) and the secretion of cytokines.

1.9 MAIT cell antimicrobial role *in vivo:*

The high evolutionary conservation of the MAIT cell-MR1 axis among mammals and the ability of MAIT cells to recognize intermediates of the riboflavin biosynthesis pathway, which is conserved among many different species of bacteria and fungi, suggest that MAIT cells play an essential role in host protection against microbes.

1.9.1 *Fungal infections:*

Several species of fungi, including *Candida albicans* and *Saccharomyces cerevisiae*, possess the riboflavin biosynthesis pathway and can activate MAIT cells *in vitro.* However, to date, the role of MAIT cells in fungal infections in either humans or in animal models has not been investigated.

1.9.2 *Parasitic infections:*

So far only one study has investigated MAIT cells in parasitic infections. Mpina *et al.* reported that following intradermal administration of a high dose of *Plasmodium falciparum* sporozoites to Tanzanian volunteers, peripheral blood MAIT cell levels decreased during early blood-stage parasitemia (11 to 18 days post-infection). Surprisingly, after treatment, MAIT cells rebounded and were maintained in levels higher than those initially measured up to several months post-infection.

2. MATERIAL AND METHODS:

The assays performed throughout this thesis can be broadly divided into *phenotypic* and *functional* experiments.

2.1 Experimental approaches

Functional experiments were conducted to study the activation, proliferation, and cytotoxicity of MAIT cells. They required either PBMC mixtures as the source of MAIT cells and APCs, or magnetically purified Vα7.2+ cells as the MAIT

cell source, and autologous monocytes or the 293T cell line stably transfected with human MR1 (293T-hMR1 cells) as APCs. In some functional experiments, pure MAIT cell populations obtained by fluorescence-activated cell sorting (FACS) were directly cultured with monocytes. Microbes, either *E. coli* or *C. albicans*, were used as the source of MAIT cell antigens. In selected experiments, PBMCs were cultured with the cytokines IL-12 and IL-18 to assess MAIT cell activation in response to an innate cytokine stimulus. The readouts of these assays (broadly presented in Table 1) were ultimately determined by flow cytometry.

2.2 Selection of experimental approach

Specific experimental approaches were selected based on the type of assay to be performed and the type of biological material available. PBMC mixtures allowed for MAIT cell functions to be assessed in the presence of other cell types, and both direct and bystander activation of MAIT cells occurred in this system.

2.3 Selection of MAIT cell stimulus

The MAIT cell activating antigens identified to date are intermediate metabolites of the riboflavin pathway. This pathway is present in *E. coli,* and this bacterium was used as the natural source of MAIT cell agonists to establish Methodologies.

2.4 Activation assay

In order to establish the activation assay, several technical parameters were optimized using CD69 upregulation concomitant with IFNγ production (CD69+IFNγ+) as functional readout for MAIT cell activation. The parameters optimized included microbial dose (*i.e.*, the *E. coli* colony forming units (cfu):monocyte ratio), Vα7.2+ cell:monocyte ratio, requirement of anti- CD28 as a co-stimulatory signal, and duration of the culture.

2.5 Proliferation assay

The proliferation assay was established using dilution of cell trace violet (CTV), a fluorescent proliferation-tracing reagent, in MAIT cells as functional readout. We optimized both the duration of the culture and the microbial dose, and found the 5-day assay to result in clear MAIT cell proliferation patterns that were predominantly MR1-dependent. The detection of discernible CTV dilution peaks at the end of the assay can be further used to selectively study MAIT cells with different proliferation capacities.

2.6 Cytotoxicity assay

The cytotoxicity assay was established in order to be able to evaluate the capacity of MAIT cells to degranulate and kill target cells. To this end, we used 293T-hMR1 cells as APCs (and target cells) because they are relatively resistant to *E. coli*-induced cell death.

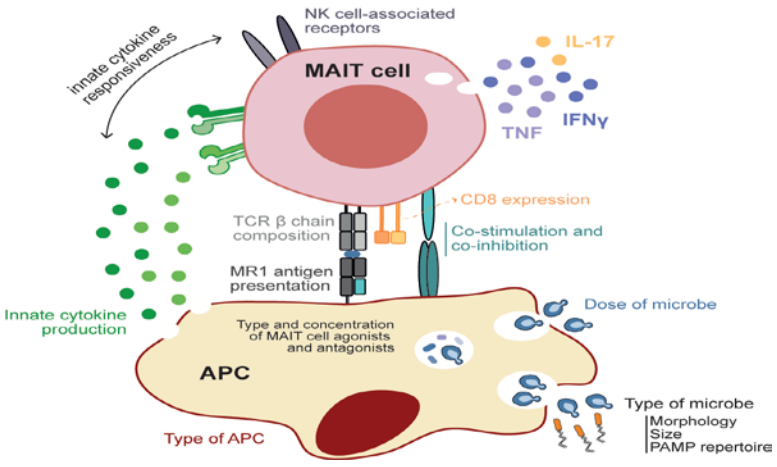

Figure 1: Influence of APC characteristics on MAIT cell antimicrobial responses

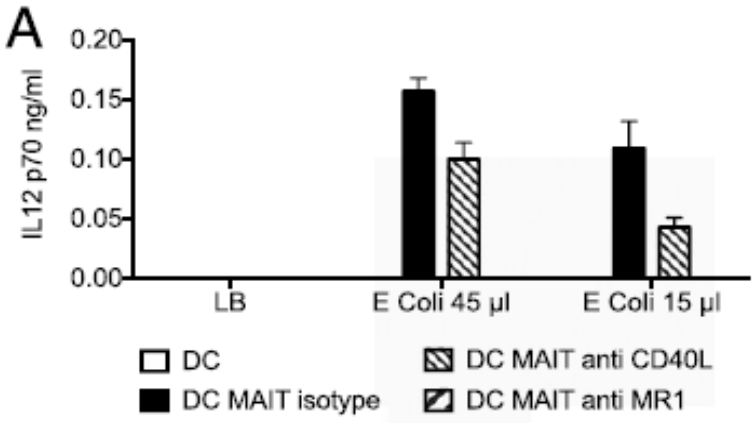

Figure 2: cytokine stimulus

Table: 1 MAIT cell functional readouts. Readouts assessed in MAIT cell analyses at the end of the activation, proliferation, and cytotoxicity assays. In some cytotoxicity assays, target cell death was also evaluated

Assays	Surface readouts	Intracellular readouts Activation
Activation	CD69 CD25 CD38 HLA-DR PD-1 TIM-3	IFNγ TNIL-17
Proliferation		CTV dilution
Cytotoxicity	CD107a	GrzA Grz Brf Gnly

3. RESULT AND DISCUSSION:

3.1 Characteristics of MAIT cells influencing their antimicrobial responses

TCR β chain composition

In an attempt to further understand factors that influence MAIT cells in their antimicrobial responses, we focused on their Vβ segment expression, which, although more restricted than that of other T cells. In summary, we show that MAIT cell TCR-mediated responses may be influenced by two factors intrinsic to the MAIT cells themselves: the TCR β chain composition and CD8 expression. Vβ-defined MAIT cell subpopulations are associated with different degrees of responsiveness to microbial stimulation, and CD8+ MAIT cells display higher functional capacity than DN MAIT cells both to microbial and mitogen stimulations.

3.2 Influence of APC characteristics on MAIT cell antimicrobial responses

Repertoire of co-signaling receptors

The MAIT cell effect or functions against *E. coli* and *C. albicans* were studied using monocytes as APCs (**paper I**, Fig. 1). In our experimental system, we found that the addition of anti-CD28 in the monocyte-Vα7.2+ cell co-cultures stimulated with *E. coli* boosted MAIT cell IFNγ production.

3.3 Microbe-mediated activation-induced MAIT cell death

MAIT cell loss by engagement in antimicrobial responses

It has been hypothesized that the loss of MAIT cells from peripheral blood of HIV-1-infected patients is due to continuous engagement of these cells in antimicrobial responses as a consequence of microbial translocation.

4. CONCLUSION:

We established MAIT cell-based experimental platforms that can be used to study several functions of these cells and adapted to answer a wide variety of research questions. We showed that MAIT cell responses to distinct riboflavin biosynthesis-competent microbes depend on the microbial dose and differ in the type and amount of cytokines produced. We demonstrated functional compartmentalization of the MAIT cell population, as the TCR β chain composition and CD8 expression, as well as the expression of NK cell associated receptors, influence their capacity to respond to TCR and innate cytokine stimulation, respectively. In an attempt to understand the relationship between CD8+ and DN MAIT cells, we showed that CD8+ MAIT cells are functionally superior to DN MAIT cells and that the latter may derive from the former *in vivo*.

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