# Progress Report

**Description of activities:**

**Project "Crosstalk between MAIT cells and gut microbiota in the development of type 1 diabetes in children"**
**Supervision : Prof. Dr Jacques Beltrand, Dr Agnès Lehuen**
**Hopital Necker/ Inserm**

**Introduction:**

Mucosal Associated Invariant T (MAIT) cells are innate-like T cells recognizing bacterial metabolites, derived from the synthesis of riboflavin, presented by the non-polymorphic class I like molecule MR1.They could represent a key player linking microbiota and gut mucosa homeostasis to the autoimmune destruction of pancreatic islets. Recent results in T1D patients and in NOD mice indicate an abnormal MAIT cell frequency and activation in this pathology, which can occurs at diagnosis and probably before disease onset in at risk subjects[17].

No concomitant data are available on changes in MAIT cells and gut microbiota (including CVB) during the course of the disease, especially in the years before the appearance of hyperglycemia and during the first months of diagnosis when patients will still have a residual beta cell function. The overall objective of the project is to bring new knowledge on the physiopathology of T1D to open new avenues for innovative therapeutic strategies based on microbiota mediated MAIT cell triggering for the prevention of T1D development.

**Aims:** To investigate in a prospective way changes in MAIT cells frequency, phenotype and function in link with the gut microbiota, gut integrity and the presence of CVB in three cohorts of pediatric patients: at risk of T1D individuals (AR) and patients at onset of T1D (RO) by comparison with control subjects (C).
Tasks:

1. To measure blood MAIT cells frequency, phenotype and function in the three cohorts
2. To analyze gut microbiota and the presence of Coxsackie B enterovirus and their impact on MAIT cell function
3. To evaluate gut integrity and analyze the gut mucosa
4. To integrate all the data obtained with T1D development and evolution

**Research steps during this period**

1. Protocol for Ethics Committee approval- The project was elaborated and sent for Inserm promotion by Dr Passone, under Dr Beltrand supervision.

2. Learning of specific techniques for lab research development:

- Preparation of cells for cytometer analysis: surface, intracellular and exhaustion protocols of lymphocytes, specially MAIT cells
- Formation in flowcytometry- Fortessa and Aria cytometers
- Preparation of stools samples for MAIT cell ligands by bio-assay
- Formation in FLOW JO software analysis

3. Protocol follow-up:

During this period, we analyzed two groups: recent onset type 1 diabetes (RO) and control group. Thirty patients were evaluated, including 17 recent onset type 1 diabetic patients. Their characteristics are in table 1.

The inclusion criteria for RO cohort were: newly diagnosed T1D defined as hyperglycemia with ketonemia, low C-peptide at diagnosis (<0.7 ng/ml) and at least one auto-antibody or at-risk HLA. The control group was restricted to patients older than 12 months, without any auto-immune/ inflammatory disease, without corticosteroid and antibiotic use in the last month.

These patients were selected according study protocol, and Dr Passone analyzed their blood. The stool samples were also collected and frozen.

For FACS analysis, MAIT cells were identified as CD3+ CD4- CD161high Vα7.2+ T cells. Surface markers were analyzed to determine their activation status (CD25, CD69), their exhaustion (PD1,TIM3), their migration capacity (CCR6), and their proliferation and survival will be analyzed by Ki67 and BCL2 expression. Cytokine production were assessed after PMA-ionomycin activation, followed by intracytoplasmic staining with antibodies against IL-2, IFN-γ TNF-α, IL-2, IL-4, IL-10, IL-17 and granzyme B. To determine the capacity of MAIT cells to response to TCR stimulation (exhaustion), in vitro stimulation was performed in the presence of specific bacterial ligands. Activation marker expression were analyzed by FACS and cytokines released in the supernatant by Cytometry based assay.

For surface cells analysis and cell sorting Aria cytometer was required. For all others parameters Fortessa cytometer was used.

Our preliminary results showed a lower frequency of MAIT cells in recent onset diabetic cohort comparing to control group. MAIT cells frequency are correlated with age as previous studies.

Regarding intracellular panel, MAIT cells expressed higher levels of IL-4, IL-17 and lower levels of IFN γ. These results collaborate with our previous cohort.

No differences were found until this moment for other parameters.

From a subgroup of patients, we performed single cells analysis to identified differences in gene expression.

*Preliminary results:*

| | Type | age | sex | Stature | BMI | Hbglic | Anti-IA2 | anti-GAD | ZnT8 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | DT1 | 13.4 | M | 156 | 32.25 | 12,10% | + | + | + |
| 2 | Control | 6.4 | F | 106 | 14.10 | | | | |
| 3 | DT1 | 9.5 | F | 141 | 17.10 | 10.2% | + | + | neg |
| 4 | Control | 11.8 | M | 133 | 16.95 | | | | |
| 5 | DT1 | 12.9 | F | 163 | 15.80 | 15.6% | + | + | neg |
| 6 | DT1 | 9.2 | F | 127 | 14.88 | 12.7% | + | neg | neg |
| 7 | Control | 8.1 | F | 138 | 17.06 | | | | |
| 8 | DT1 | 13.4 | M | 171 | 21.20 | 10.6% | + | neg | neg |
| 9 | Control | 6.8 | F | 107 | 13.62 | | | | |
| 10 | Control | 11.5 | F | 137 | 15.98 | | | | |
| 11 | DT1 | 12.9 | F | 150 | 14.22 | 11.3% | + | + | neg |
| 12 | Control | 9.3 | M | 121 | 14.82 | | | | |
| 13 | Control | 12.1 | M | 149 | 18.10 | | | | |
| 14 | Control | 12 | F | 137 | 14.81 | | | | |
| 15 | DT1 | 5.5 | F | 116 | 13.37 | 10.9% | neg | + | neg |
| 16 | DT1 | 11.6 | F | 158 | 23.23 | 12.1% | neg | + | neg |
| 17 | Control | 10.7 | M | 132 | 13.77 | | | | |
| 18 | DT1 | 9.2 | F | 131 | 13.63 | 8.8% | + | + | neg |
| 19 | DT1 | 12.6 | M | 159 | 14.63 | 13.7% | + | + | |
| 20 | Control | 10.7 | F | 131 | 15.26 | | | | |
| 21 | DT1 | 7.7 | F | 129 | 12.91 | 17.4% | + | + | + |
| 22 | DT1 | 13 | F | 140 | 20.10 | 8.6% | neg | + | neg |
| 23 | DT1 | 1.6 | M | 80 | 17.18 | 10.5% | + | + | neg |
| 24 | DT1 | 1.6 | F | 78 | 19.72 | 11.1% | neg | + | neg |
| 25 | Control | 5.7 | F | 100 | 14.00 | | | | |
| 26 | DT1 | 14.9 | F | 170 | 17.30 | 11.5% | + | neg | neg |
| 27 | Control | 8.2 | F | 139 | 16.87 | | | | |
| 28 | DT1 | 1.9 | F | 80 | 15.62 | 10.5% | + | + | neg |
| 29 | Control | 2.2 | M | 87 | 17.17 | | | | |
| 30 | DT1 | 12 | F | 148 | 16.89 | 8.7% | + | + | neg |

Table 1: Characteristics of included patients

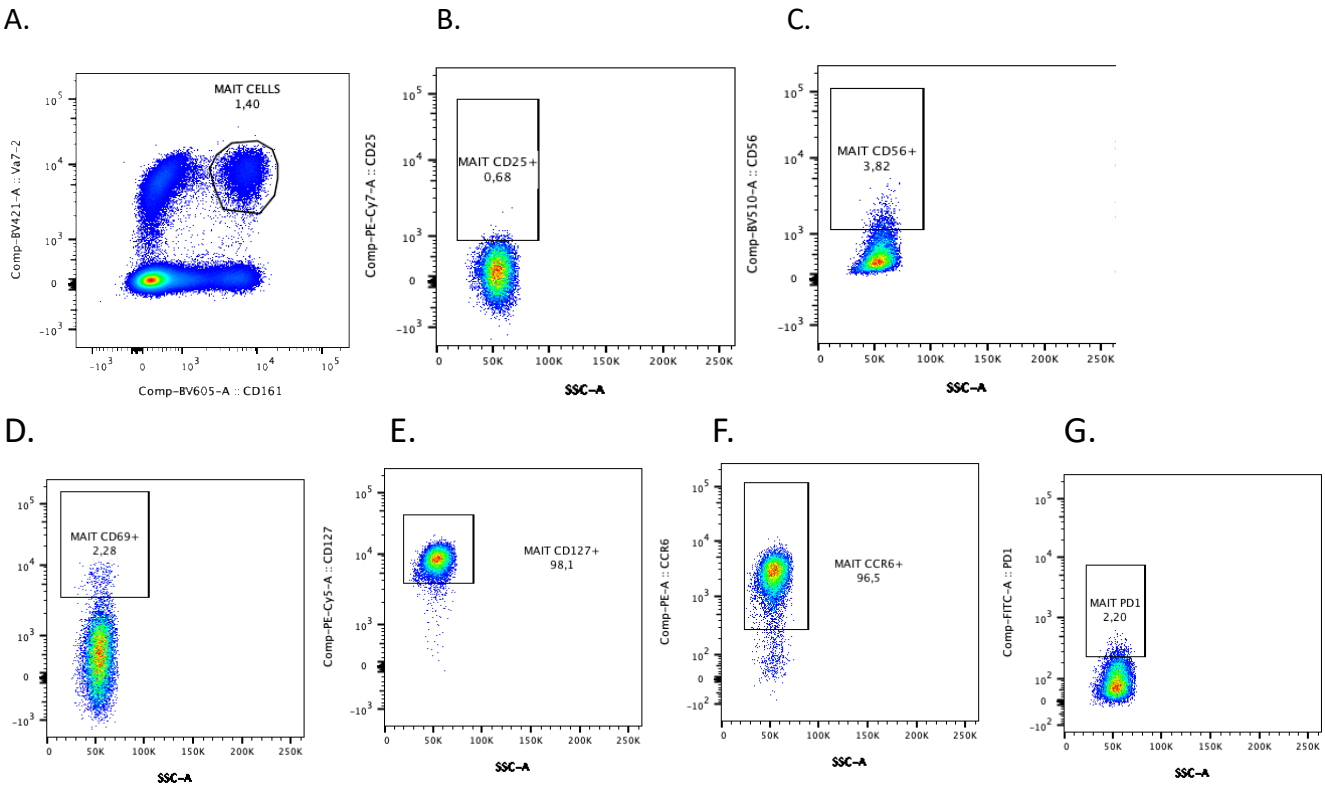


Figure 2: Example of surface analyses of MAIT cells. A: MAIT cells gate B: MAIT CD25+ C :CD56+, D : MAIT CD69+. E : MAIT CD127+ F : MAIT CCR6+ G : MAIT PD1

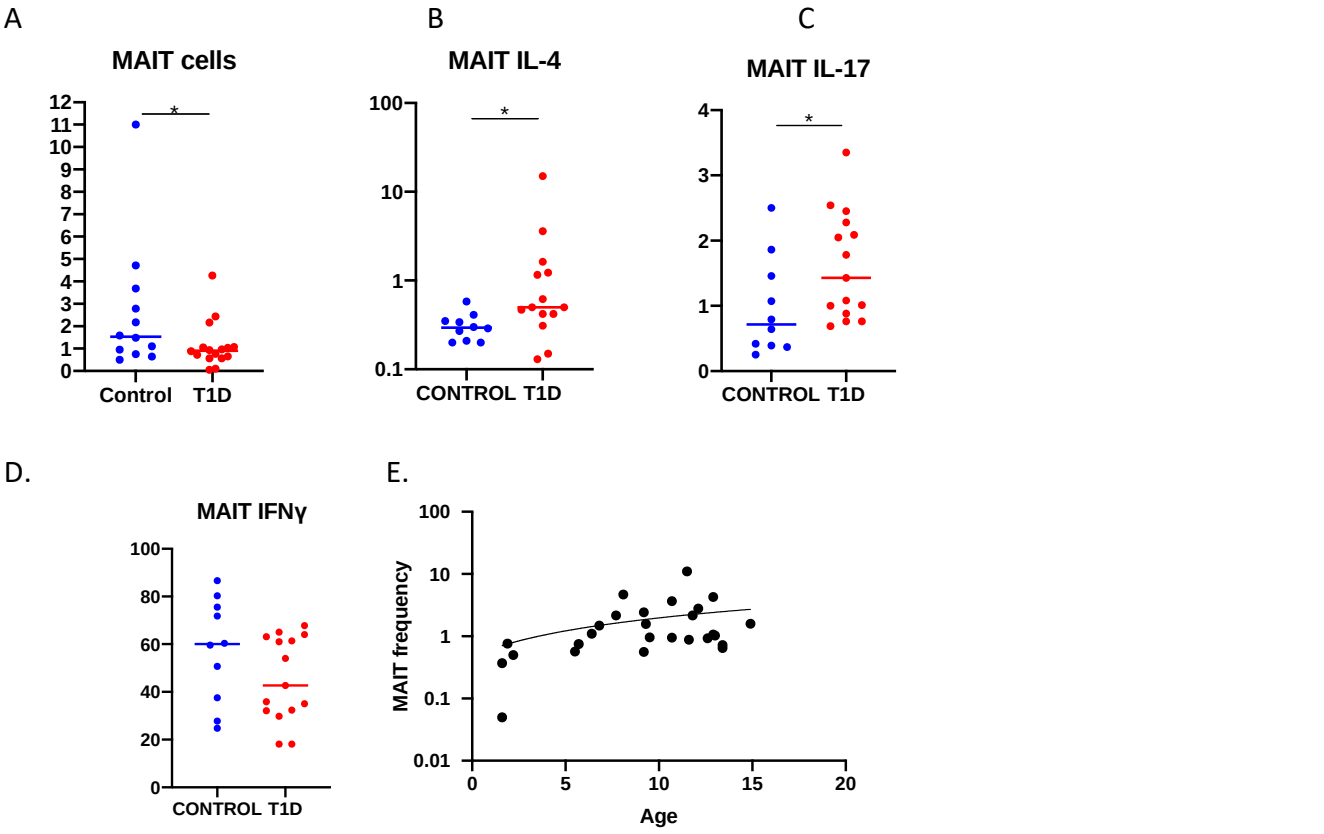


Figure 3: A) MAIT frequency among T1D versus control patients B) IL-4 MAIT cells expression C) Levels of IL-17 expressed by MAIT cells D) Levels of IFN expressed by MAIT cells E) Frequency of MAIT related to age in all patients. *p<0.05.

**Publication plan: We will include more 25 type 1 diabetic patients, 20 controls and 60 patients at risk of type 1. The results will be submitted next year.**
**For the second year of this project Dr Passone received a grant from the diabetes french association ( AJD)**

- Other projects developed during this period:

**DIABELOOP – Closed Loop system**- Dr Passone participated in the first DIABELOOP clinical trial for children and now she is organizing a new clinical trial for adolescents.
**Hopital Necker**
**Supervision: Dr Jacques Beltrand**

**Content:** Diabeloop is a close-loop system/ algorithm created in France. The first studies were performed in an adult cohort. This year the first experience in children was started. The algorithms could be reviewed for specific age demands during this follow-up period. A new trial will start in adolescents at the end of this year.

**Case report:** Poster presentation at ISPAD meeting: **Neonatal diabetes caused by RFX6 mutations: barriers to follow-up management.**
Content: We report two cases of rare cases of neonatal diabetes and their follow-up management. One of them received a liver and b-cell transplant that was not reported before in literature for this disease. An article will be produced after the conference.