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## **Pregnant type 1 diabetes women with rises in C-peptide display higher levels of regulatory T cells: A pilot study**

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**Full Title:** Pregnant Type 1 diabetes women with C-peptide rise display higher level of regulatory T cells, a pilot study.

**Running Title :** Immune markers during Type 1 diabetes pregnancy

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Elevation of C peptide concentration have been described during Type 1 diabetes (T1D) pregnancy (1). Number of regulatory T cells (Tregs), which increase during physiological pregnancy (2), play a central role in T1D disease by limiting the pathogenicity of T effectors cells responsible for pancreatic beta cell destruction (3). Indeed, a defect of Tregs as of interleukin-2 (IL-2) (major cytokine controlling Tregs survival/function) is described in this disease (4). Pathological immune adaptation to pregnancy is observed during T1D (5). We questioned in this pilot monocentric, prospective, observational study if the immune status could be associated with an eventual beta cell adaptation to pregnancy. We included 13 pregnant T1D women without other pathology between 2015 and 2017.

We assessed, on non-fasted blood samples, immunological parameters (Tregs (CD4+CD25hiCD127-/lo Foxp3+) by flow cytometry (as described (6)), interleukin 2 (IL2) by Milliplex kits (Merck Millipore) and diabetes parameters (HbA1C by high performance liquid chromatography, glycaemia, C peptide by chemiluminescence considered interpretable if contemporary glucose level was over 5 mmol/l). Diabetes history was collected at first visit. Clinical (weight, exogenous insulin doses per day) and biological parameters were collected on first, second and third trimester (1<sup>st</sup>T : 6 to 14; 2<sup>nd</sup>T: 20 to 26; 3<sup>rd</sup>T: 30 to 37 weeks of gestation) and post-partum (PP, within 6 months). All participants gave their oral consent after oral and written information. This study was in accordance with ethical French rules.

Immunological parameters were compared with those of a healthy pregnant cohort (Qute trial, clinical trial NCT02931695).

At inclusion mean age was 29.2±3.1 years old, mean weight 67.7±12 kg and mean duration of diabetes 16.6±8 years. Diabetes was complicated with retinopathy in 10 (77%) patients.

Seven women had a positive beta cell response to pregnancy (Group 1) defined by a C peptide rise between 2<sup>nd</sup>T and 3<sup>rd</sup>T (4/7 women), and (1/7 women) /or (3/7 women) a decrease of insulin

dose-adjusted A1C (IDAA1C) index along pregnancy (individual exogenous insulin need estimation) (Fig1 A, B).

In 6 women (Group 2), we did not observed beta cell function improvement during pregnancy, C peptide level never raised, IDAA1C index increased (5/6 women) or was stable (1/6 women) (Fig1 A, C). The 2 groups had comparable clinical characteristics at baseline (not shown).

In comparison with the healthy cohort, pregnancy of T1D is a situation of Tregs insufficiency (Fig1 D). However, among T1D women, we objective a significant higher level of Tregs and IL2 in Group 1 compared with Group 2 during pregnancy, and at PP for Tregs (Fig1 E, F). However, on the opposite of physiological observations (2), no significant rise of immune parameters was objective during pregnancy (Fig 1 E, F), possibly consequence of the small number of patients.

To our knowledge association between beta cell functionality and immunological parameters during the pregnancy of T1D women have never been investigated. Our results suggest that during T1D pregnancy 1) Tregs insufficiency reported in T1D (4) persists ; 2) higher levels of Tregs and IL2 seem to allow a significant beta cell adaptation to pregnancy.

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## **Disclosure of interest**

No potential conflicts of interest relevant to this article were reported.

**Author Contributions.** C.A. ; M.H. ; M.P. ; S.J. ; C.C. ; O.B. ; F.A. ; J.E.S. ; A.H. ; M.R. collected data. C.A. ; A.H. and M.R. analyzed data. E.T. contributed to data analysis. C.A. wrote the manuscript. D.K. ; J.E.S. ; A.H. and M.R. reviewed the manuscript.

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### Figure legend

Figure 1 : A Non fasted C peptide measurement at 2<sup>nd</sup> and 3<sup>rd</sup> trimester of pregnancy in Group 1 (empty rounds represent C peptide measurement at serum glucose <5mmol/l) and Group 2 ; B and C: IDAA1C index (HbA1C (percent) + [4 x insulin dose (units per kilogram per 24 h)]) in Group 1 (triangle shapes represent the 3 patients with a decrease of IDAAC index without C peptide rise during pregnancy), and Group 2 ; D: Tregs values at 3<sup>rd</sup> trimester of pregnancy and post-partum state of Type 1 diabetes women compared with Healthy women (\* represent  $p < 0,05$  and \*\* $p < 0,01$  by Kruskal Wallis test). E: represent Tregs levels in Group 1 and 2 all along pregnancy, (\* represent  $p < 0,05$  by Mann and Whitney test). F: represent IL2 levels in Group 1 and 2 all along pregnancy, (\* represent  $p < 0,05$  by Mann and Whitney test).

Data are expressed as mean  $\pm$  standard deviation (SD), Statistical analysis were performed by Graph Pad Software, Prism 7