Th2-skewing of human circulating iNKT cells in the context of obesity

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1 Introduction

In addition to induce low-grade inflammation contributing to type 2 diabetes, obesity also causes immune dysregulation by negatively impacting several immune cell populations. This results in a loss of immunosurveillance which has been proposed to contribute to increased susceptibility of obese patients to develop some cancers. Peripheral invariant Natural Killer T (iNKT) cells are unconventional T cells that express semi-invariant TCR. iNKT cells recognize through their TCR lipid antigens presented by CD1d on antigen presenting cells, causing the rapid release of Th1 and/or Th2 and/or Th17 cytokines. iNKT cells play an important role in tissue homeostasis, defense against infection and tumor immunosurveillance.

We previously demonstrated a global phospholipidome alteration of PBMCs in obese patients in comparison to lean individuals which is correlated to fasting insulin levels [1]. Based on these results and because the frequency of iNKT cells is downmodulated in obesity, we hypothesized that a chronic and/or excessive stimulation of iNKT cells could disrupt their activity and contribute to the loss of immunosurveillance.

[1] Wilkin C et al. New Insights on the PBMCs Phospholipidome in Obesity Demonstrate Modulations Associated with Insulin Resistance and Glycemic Status. Nutrients. 2021;13(10):3461. doi:10.3390/nu13103461

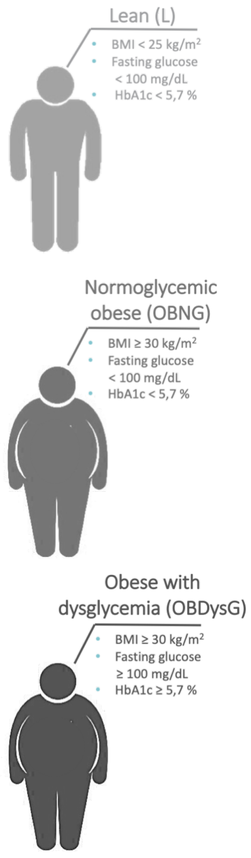

Figure 1. Patient recruitment criteria

2 Materials and Methods

A total of 54 individuals (aged between 18-65 years) were recruited on a voluntary basis. The study protocol was approved by the ethics Committee of the Liège University Hospital, and all patients provided written informed consent. Subjects with inflammatory or malignant diseases were excluded. The participants were categorized in three groups based on the BMI and glycemic status (Figure 1 and Table 1): (i) lean with normoglycemia (Lean; n=20), (ii) obese with normoglycemia (OBNG; n=20) and (iii) obese with dysglycemia (OBDysG; n=14).

	LEAN	OBNG	OBDysG
n (f/m)	20 (14/6)	20 (14/6)	14 (8/6)
Age (years)	42.1 ± 7.9	41.4 ± 11.4	48.7 ± 10.5
Body Weight (kg)	63.9 ± 8.0	109.3 ± 16.1 ***	105.4 ± 21.4 ***
BMI (kg/m2)	21.3 ± 1.3	37.9 ± 5.2 ***	37.3 ± 5.0 ***
Waist (cm)	76.2 ± 5.8	110.8 ± 11.3 ***	118.9 ± 12.0 ***;†
Fasting glucose (mg/dL)	89.2 ± 6.5	88.2 ± 6.1	128.2 ± 31.9 ***;†††
Fasting insulin (mU/L)	5.2 ± 2.2	11.8 ± 4.5 **	15.3 ± 5.1 ***;††
HOMA-IR	1.17 ± 0.51	2.57 ± 1.00 ***	4.86 ± 1.68 ***;†
HbA1c (%)	5.22 ± 0.23	5.35 ± 0.29	6.50 ± 0.91 ***;†††
Triglycerides	71.3 ± 23.8	125.9 ± 70.2 **	166.3 ± 62.3 ***
HDL/total cholesterol	0.35 ± 0.07	0.26 ± 0.09 **	0.24 ± 0.06 **
Type 2 diabetes	0/20	0/20	4/14

Table 1. Anthropometric, clinical and biological characteristics of the participants. Data are mean ± SD. Kruskal–Wallis followed by Dunn post hoc test was performed on data. Normoglycemic obese (OBNG) or obese with dysglycemia (OBDysG) vs. Lean * p<0.05; ** p<0.01; *** p<0.001. OBDysG vs. OBNG † p<0.05; †† p<0.01; ††† p<0.001.

3 Results

1. Activation and Th2 skewing of iNKT cells in obese patients

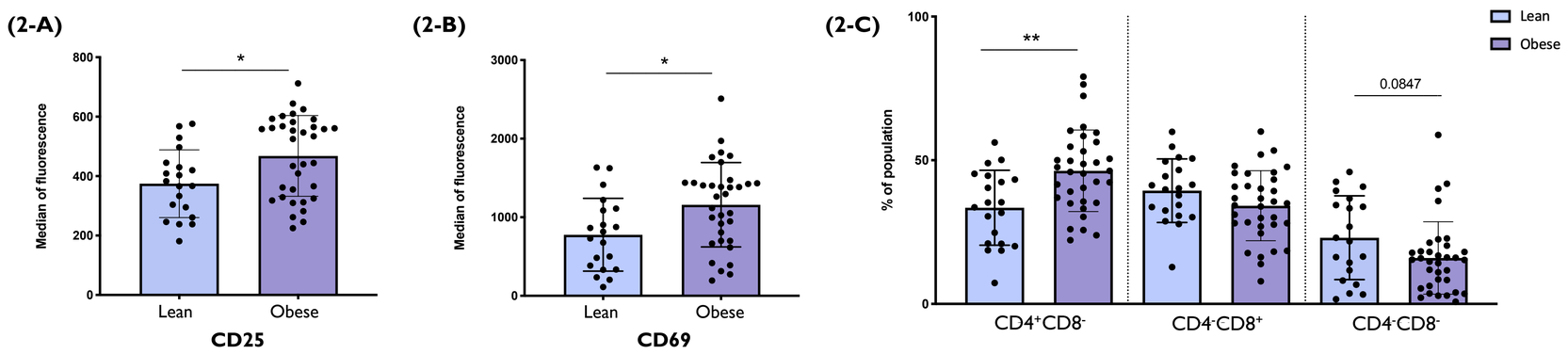

Figure 2. Median of fluorescence of (A) CD25, (B) CD69 at iNKT cells surface. (C) iNKT cells subpopulation expressed as the percentage of total iNKT cells population. Data are mean ± SD. * p<0.05; ** p<0.01.

The expression of activation markers (CD25 and CD69) is upregulated with obesity. Moreover, the anti-inflammatory CD4⁺ subpopulation is increased to the detriment of pro-inflammatory double negative subpopulation in obese patients compared to lean individuals.

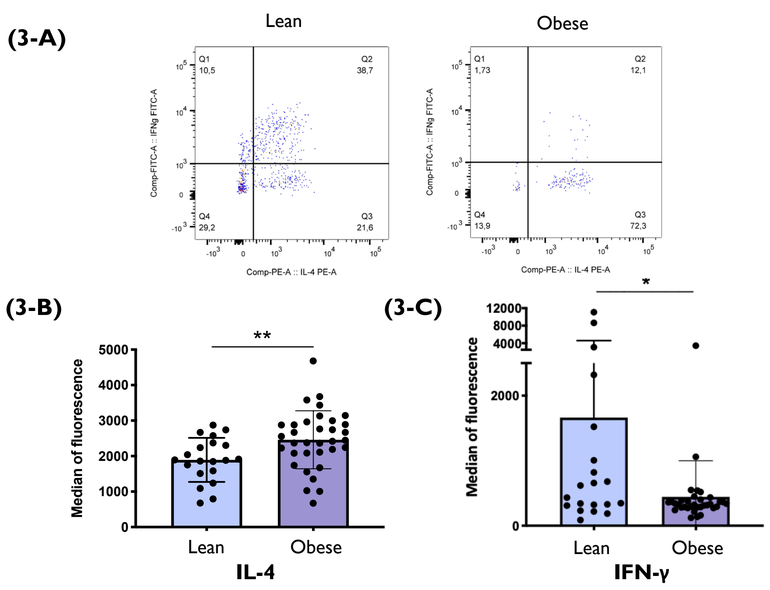

Figure 3. (A) Dot plot of intracellular IL-4 (x-axis) and IFN-γ (y-axis) production by iNKT cells following PMA-ionomycin stimulation in Lean and Obese patients. Median of fluorescence of intracellular (B) IL-4 and (C) IFN-γ produced by iNKT cells following PMA-ionomycin stimulation. Data are mean ± SD. * p<0.05; ** p<0.01.

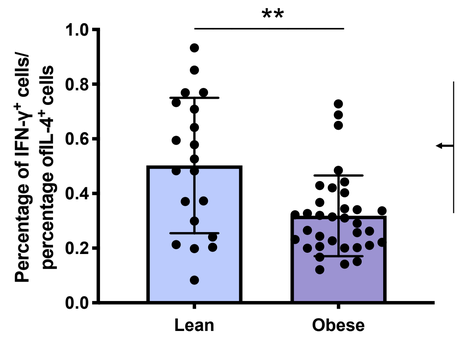

Figure 4. Percentage of IFN-γ⁺ cells/percentage of IL-4⁺ cells following PMA-ionomycin stimulation. Data are mean ± SD. ** p<0.01.

IL-4 production by iNKT cells increases while IFN-γ production decreases following PMA-ionomycin stimulation in obese patients compared to lean individuals. Moreover, the ratio (% of IFN-γ⁺ cells/% of IL-4⁺ cells) decreases with obesity indicating a shift towards anti-inflammatory Th2 phenotype.

2. Dysglycemia does not seem to have a significant impact on iNKT cells

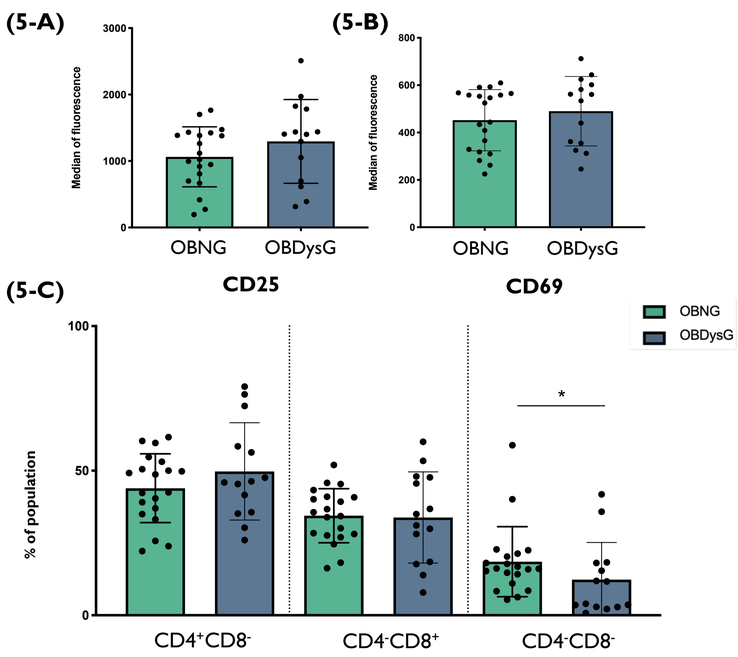

Figure 5. Median of fluorescence of (A) CD25, (B) CD69 at iNKT cells surface. (C) iNKT cells subpopulation expressed as the percentage of total iNKT cells population. Data are mean ± SD. * p<0.05.

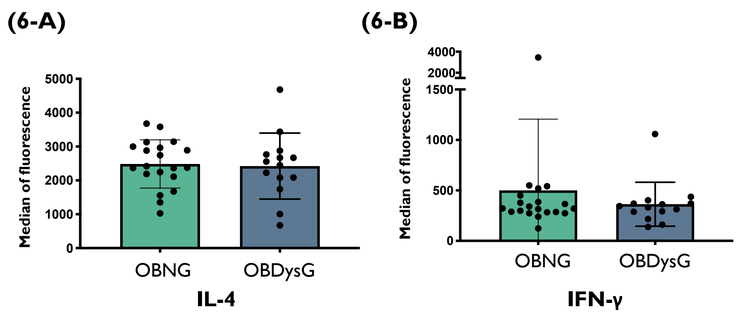

Figure 6. Median of fluorescence of intracellular (A) IL-4 and (B) IFN-γ produced by iNKT cells following PMA-ionomycin stimulation.

3. iNKT cells phenotype and activity are correlated to plasma lipid content and fasting insulin levels

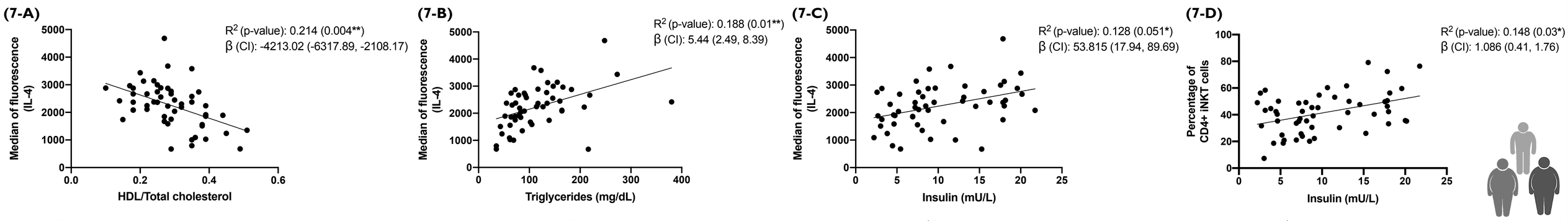

Figure 7. Linear regression of median of fluorescence of intracellular IL-4 produced by iNKT cells following PMA-ionomycin stimulation and (A) HDL/Total cholesterol ratio, (B) plasma triglycerides levels (mg/dL) or (C) fasting insulin plasma levels (mU/L). (D) Linear regression of the percentage of CD4⁺ iNKT cells and fasting insulin plasma levels (mU/L). Data presented as β-coefficients and corresponding 95% confidence intervals (CI). All p-values represent significance of associations after correcting for multiple comparisons using the Benjamini-Hochberg method. * p<0.05; ** p<0.01.

4 Discussion

We have observed a disruption of peripheral circulating iNKT cells in obese patients compared to lean individuals. Correspondingly, we found that iNKT cells are more activated in obese patients. Moreover, iNKT cells shift towards Th2-phenotype with obesity as demonstrated by an increased percentage of CD4⁺ iNKT cells, a decreased ratio of IFN-g/IL-4 producing cells and a higher IL-4 production by iNKT cells in response to PMA-ionomycin stimulation in obese patients. This strong alteration of iNKT cells activity seems to be correlated to plasma lipid content and fasting insulin levels. Thus, iNKT cells of obese patients could be less efficient to respond to microbial and tumor aggression.