VARIABILITY IN IMMUNOMETABOLIC SIGNATURES AMONG HIV PATIENTS DEFINES IMMUNE OUTCOMES AND PROGRESSION

360

Rachelle Saleh[1], **Simon Gressens[1]**, Mohamed Joma[1], Lara Tabet[1], Julien Pansiot[1], Cédric Caradeuc[2], Cassiopée Adam[1], Katia Bourdic[3], Gildas Bertho[2], Guislaine Carcelain[1], Nicolas Noel[3], Olivier Lambotte[3] and Mireille Laforge[4]

1:NeuroDiderot, INSERM, Université Paris Cité, Paris, France. 2:Université Paris Cité, CNRS, Laboratoire de Chimie et de Biochimie Pharmacologiques et Toxicologiques, Paris, France; 3: Université Paris-Saclay, CEA, INSERM, UMR1184, Immunology of Viral, Auto-immune, Hematological and Bacterial diseases (IMVA-HB/IDMIT Department), Fontenay-aux-Roses/Le Kremlin-Bicêtre, France.

BACKGROUND

HIV infection leads to significant loss of CD4 T lymphocytes (CD4TL), compromising immune function despite antiretroviral therapy (ART). People living with HIV (PLHIV) can be categorized as immune responders (IR) or immune non-responders (INR) based on their ability to achieve immune reconstitution. IR restore CD4TL counts >500 cells/mm³, while INR remain below this threshold despite ART. CD4TL subpopulations have different energy needs and signatures based on their functions. Recent research has shown that CD4TL from PLHIV exhibit bioenergetic defects, particularly in INR, linked to issues in their mitochondria. This study explores how IR manage to recover despite these issues, while INR do not, aiming to find ways to help INR improve their immune function.

METHODS

We included 30 PLHIV based on age, ART length, undetectable virus, CD4TL count and CD4/CD8 ratio. We performed a multiapproach immuno-metabolic profiling of CD4TL isolated from blood sample of INR and IR patients and healthy donors using flow cytometry, Seahorse analysis, biochemistry for bioenergetics studies combined with mitokines analysis on plasma.

Mitochondrial Issues: INR have more stressed, and bioenergetic default. INR showed less mitochondrial biomass and default on mito/autophagy mechanism.

Activation Without Fuel: INR show high activation markers when stimulated overnight with anti-CD3/anti-CD28 but fail to boost glucose metabolism showing non-classical metabolic signature upon stimulation.

Compensation mechanisms: CD4T cells of IR patients also have mitochondrial bioenergetic defects but they compensate for mitochondrial dysfunction by stimulating mito/autophagy mechanism to eliminate damage mitochondria and boost mitochondrial biogenesis.

RESULTS

- **INR (Immune Non-Responders) showed:**
  - Reduced mitochondrial biomass.
  - Higher levels of cell activation markers (e.g., CD25, CD69, HLA-DR, CD95, PD-1).
  - Fewer naive CD4 T cells and more exhausted T cells.
  - Inability to upregulate Glut1 upon activation, despite high activation markers.
  - Ineffective mito/autophagy in INR
  - Significantly higher levels of GDF15 in plasma, indicating mitochondrial stress.
- **IR (Immune Responders) showed:**
  - Default on mitochondrial bioenergetic
  - High level of mitokines but less then the INR
  - Efficient mitochondrial biogenesis
  - Efficient mito/autophagy

CONCLUSIONS

- **IR compensate for mitochondrial defects, preserving immunity; INR fail to recover.**
- **Enhancing mitochondrial repair could help INR improve immune function.**

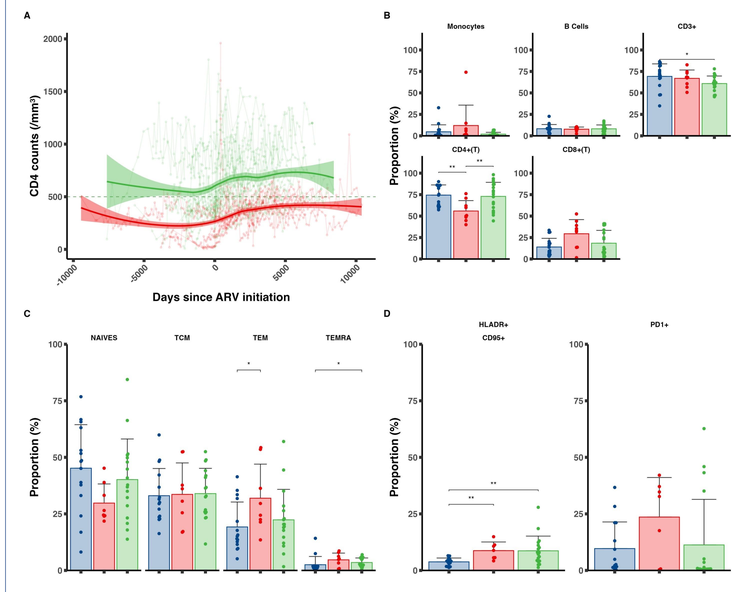


Figure 1. A. Study design, classification and phenotype of patients into Immune Responders (IR) and Immune Non-Responders (INR). *1(A) Evolution of CD4 counts over time following ARV initiation (Day 0). Representative curves (local polynomial regression fitting) illustrate trends for each treatment response group: Immune Responders (IR, green) and Immune Non-Responders (INR, red). Individual patient trajectories are shown as fainter curves. (B) Distribution of PBMC (peripheral blood mononuclear cells) using Flow Cytometry in IR and INR. (C) Distribution of CD4 T cell subsets (naïve, central memory, TEM, TEMRA) using Flow Cytometry in IR and INR groups. INR exhibit a significant reduction in naïve CD4 T cells and an increase in TEM cells, reflecting an altered immune profile. (D) Expression of late activation markers (HLADR, CD95 and PD1) on CD4 T cells using Flow Cytometry from IR and INR patients. PLWHIV show markedly higher activation marker levels.*

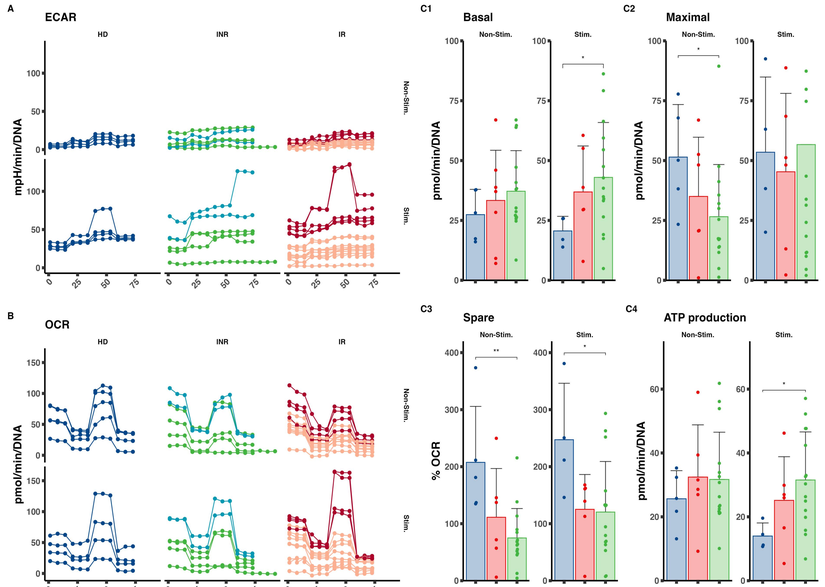


Figure 2: Ex-vivo Seahorse Analysis of CD4 T Cell isolated from healthy donors, IR and INR. *2(A) Extracellular acidification rate (ECAR) in CD4 T cells, reflecting anaerobic glycolytic activity. 2(B) Oxygen consumption rate (OCR) in CD4 T cells, reflecting mitochondrial respiration. 2(C) Analysis was done +/- the addition of anti-CD3/anti-CD28. 2(C1) Basal respiration in CD4 T cells. 2(C2) Maximal respiration in CD4 T cells. 2(C3) Spare respiratory capacity in CD4 T cells. 2(C4) ATP production in CD4 T cells.*

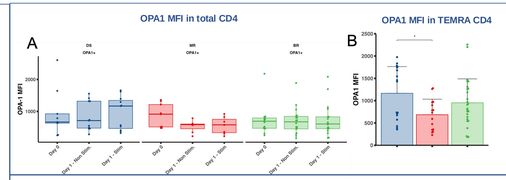


Figure 3: OPA1 Expression in CD4 T Cells *3(A) Mean fluorescence intensity (MFI) of OPA1 protein expression in total CD4 T cells at baseline (J0) and +/- the addition of anti-CD3/anti-CD28 (J1). 3(B) MFI of OPA1 protein expression in TEMRA (terminally differentiated effector memory CD4 T cells). INR exhibit significantly lower OPA1 protein expression in TEMRA cells compared to healthy donors, suggesting mitochondrial dysfunction in this T cell subset.*

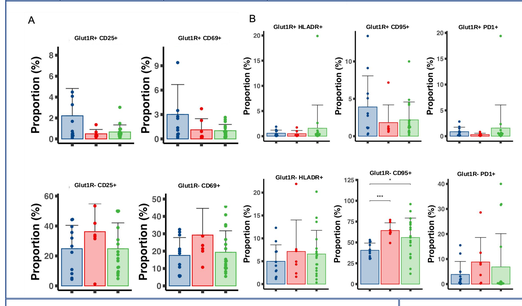
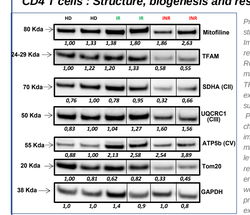


Figure 4: Association of Glut1 expression with Activation and Exhaustion Markers *4(A) Correlation between Glut1 surface expression and early activation markers (CD25 and CD69) in CD4 T cells. Immune Non-Responders (INR, red) show a weaker association between Glut1 and CD25/CD69 compared to Immune Responders (IR, green), indicating impaired metabolic activation. 4(B) Correlation between Glut1 surface expression and late activation/exhaustion markers (HLA-DR and PD-1) in CD4 T cells. INR exhibit a stronger association between Glut1 and HLA-DR/PD-1 compared to IR, suggesting a link between metabolic dysfunction and T cell exhaustion.*

Figure 5: Western Blot analysis for mitochondrial proteins in CD4 T cells : Structure, biogenesis and respiratory complexes



Protein expression of mitofilin, a mitochondrial structure protein.
Immune Non-Responders (INR, red) show reduced mitofilin levels compared to Immune Responders (IR, green), indicating impaired mitochondrial structure. Protein expression of TFAM (mitochondrial transcription factor A), INR exhibit lower TFAM levels compared to IR, suggesting reduced mitochondrial biogenesis.
Protein expression of mitochondrial respiratory chain complexes (Complex II, III, and V) implicated in oxidative phosphorylation and mitochondrial respiration. INR show decreased levels of these complexes compared to IR, reflecting impaired mitochondrial function and energy production. All mitochondrial proteins were normalized to TOM20, a mitochondrial protein. To calculate mitochondrial biomass, the expression of TOM20 was normalized to GAPDH, a cytosolic protein for loading control. TOM20 levels are reduced in INR compared to IR showing less mitochondria biomass.

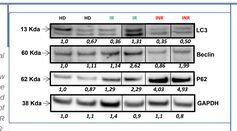


Figure 6: Western Blot Analysis of Autophagy Markers in CD4 T cells *Expression levels of autophagy-related proteins (LC3, Beclin, and P62) were analyzed by Western blot and normalized to GAPDH. Immune Non-Responders (INR) show lower levels of LC3 and Beclin, along with higher levels of P62, compared to Immune Responders (IR), indicating impaired autophagy activity in CD4 T cells.*

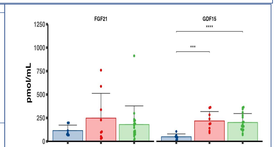


Figure 7: ELISA Analysis of Mitokines (FGF21 and GDF15) in Plasma *Plasma levels of mitokines (FGF21 and GDF15) were measured by ELISA. GDF15 levels are elevated in both Immune Responders (IR) and Immune Non-Responders (INR). FGF21 levels were also analyzed in plasma to assess mitochondrial stress.*