Glutaminolysis and lipoproteins key factors in late immune recovery in successfully treated HIV-infected patients

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Background

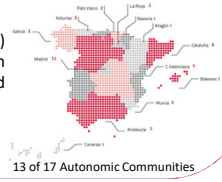
Up to one fourth of HIV-infected subjects who start combined antiretroviral therapy (cART) when they are severely immunosuppressed do not experience sufficient CD4 T-cell increases after two years of successful viral suppression. They are so-called "immunological non-responders (INR)" or "poor responders" and are associated with worse long-term prognosis, including a higher risk of progression towards AIDS and non-AIDS-defining clinical events and death. The immunological, biochemical and molecular mechanisms associated with poor immune recovery are far from known, and metabolomics profiling offers an additional value to traditional soluble markers. Here, we search for predictive markers of late immune response and disease progression in a cohort of HIV-subjects with increased CD4 T-cell turnover and inflammation preceding their poor immune recovery.

Keywords - CD4⁺ T-cell turnover, Inflammation, NMR-based lipoprotein profile, MS-based metabolomics, Poor immune recovery

Methods

- We executed a nuclear magnetic resonance (NMR) and mass spectrometry (MS)-based circulating metabolomics approach in 41 cART-naive HIV-infected patients aged ≥18 years recruited from the cohort of the Spanish AIDS Research Network (CoRIS)*. The study subjects included patients who started cART with counts of <200 CD4 cells/µl but did not achieve more than 250 CD4 cells/ µl after 96 weeks of suppressive treatment (INR subjects, n = 18) and a control group of patients who had also started cART with <200 CD4 cells/µl but achieved more than 250 CD4 cells/ µl under the same conditions (IR subjects, n = 23). Available follow-up samples (INR subjects, n = 9 and IR subjects, n = 8) after 96 weeks under suppressive cART were also analyzed. Additionally, a group of healthy HIV-uninfected (n=9) volunteers, matched by gender (all male) and age (43 [40-48] years), was also analyzed for reference values.
- Integrated data from NMR-based lipoprotein profiles, MS-based metabolomics and soluble plasma biomarkers help to build prognostic and immunological progression tools that enable the differentiation of HIV-infected subjects based on their immune recovery status after 96 weeks of suppressive cART.

Statistical analyses – Partial least squares discriminant analysis (PLS-DA) and Random forest (RF) were performed to identify the variables that best partitioned the overall study population according to immune recovery predisposition. Network of metabolite-protein interaction and functional enrichment analyses were generated to identify the metabolomics pathways affected.



13 of 17 Autonomic Communities

*CoRIS is an open, prospectively and multicentric Spanish cohort (44 centres from 13 Autonomic Communities)

Conclusions

Metabolomics improve the value of soluble parameters and shows novel and relevant data that may contribute to a better understanding of molecular mechanisms preceding discordant response and immunological progression under suppressive stable cART. The metabolomics signature of ART-naïve HIV subjects with late immune recovery is the expression of pro-inflammatory molecules and glutaminolysis, which is probably related to their higher T-cell turnover.

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Results

The metabolite PLS-DA score plot, including lipidomic markers, was different between INR subjects and IR subjects before cART onset (**Figure 1A**). In fact, univariate analysis revealed an elevated concentration of L-tyrosine (P = 0.04) and a decreased concentration of L-glutamate (P = 0.05) and phosphatidylcholine PC (16:1) (P = 0.01) at baseline associated with later poor immune recovery. Additionally, higher IL-6 levels and a greater number of subjects having hsCRP ≥ 5 mg/L where found in INR subjects compared to IR subjects. RF provided additional evidence indicating that hsCRP ≥ 5 mg/L, palmitoylcarnitine, IL-6 and PC (16:1) were among the most discriminatory parameters between INR subjects and R subjects before cART onset (**Figure 1B**). We also evaluated for which metabolic pathways were affected by the interaction of these selected metabolites and proteins (**Figure 1C**). The enriched KEGG pathway revealed a strong relationship between pre-cART immune recovery not only with immune processes (hematopoietic cell lineage, Jak-STAT signaling pathway, T-cell receptor) but also with key regulatory factors of T-cell glycolysis (HIF-1 signaling pathways), inflammatory disorders (IBD, NAFLD), and alanine, aspartate and glutamate metabolism.

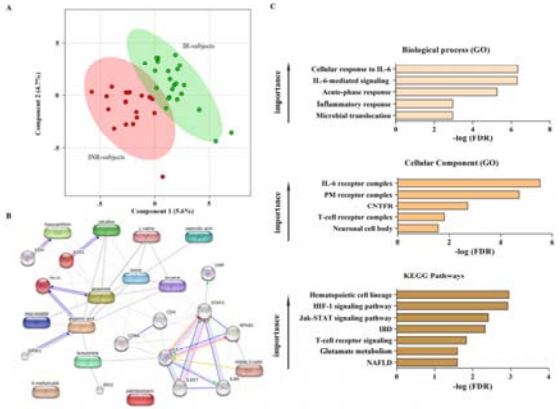


Figure 1. Baseline integrated data associated with poor immune recovery. (**A**) Two-dimensional PLS-DA score plot of metabolomics and lipidomics analyses distinguishes INR subjects (red dots) from IR subjects (green dots). (**B**) Network of metabolite-protein interaction generated with the set of 35 parameters that constitute the best predictors of immune recovery. The names of the integrated data (soluble protein markers and metabolites) were used as input in the Search Tool for Interactions of Chemicals (STITCH) database. (**C**) The STITCH database identified different functional enrichments associated with our network using the false discovery rate (FDR). The strong biological process and KEGG pathways included the expected IL-6-mediated signaling pathways but also microbial translocation, inflammatory bowel disease (IBD) and glutamate metabolism, among others. CNTFR, ciliary neurotrophic factor receptor; NAFLD, non-alcoholic fatty liver disease. Glutamate metabolism refers to alanine, aspartate and glutamate metabolism

Next, we examined lipoprotein alterations and analyzed soluble parameter changes after suppressive cART therapy in a group of 17 HIV-infected subjects with available follow-up samples after 96 weeks on stable cART. INR subjects (n = 9) had increased HDL cholesterol (P = 0.05) and increased HDL particle sizes (P = 0.04), mainly due to increased medium HDL-P, compared to IR subjects (n = 8). Regarding the soluble plasma markers, INR subjects had reduced concentrations of transforming growth factor beta (TGF-β) after suppressive cART (P = 0.03). In addition, longitudinal evaluation of lipoprotein metabolism indicated that LDL particle subclasses were completely recovered in IR subjects , achieving similar values of healthy subjects (H-subjects). The number of HDL particles increased in HIV-subjects but remained lower in the subset of IR subject compared to H-subjects.

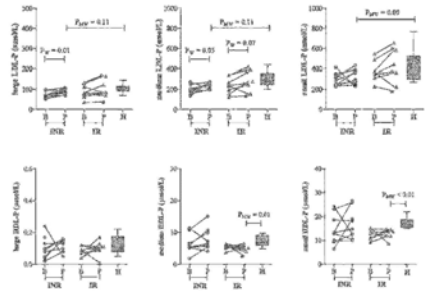


Figure 2. Longitudinal evolution of the lipoprotein profile in a follow-up subgroup of 8 INR and 9 IR subjects in comparison with a group of healthy volunteers.

Finally, hierarchical clustering clearly distinguished INR subjects from IR and healthy subjects, indicating an anomalous immune response after suppressive cART. Notably, after cART, IR subjects had similar metabolomic signatures as non-HIV-infected healthy volunteers (**Figure 3**).

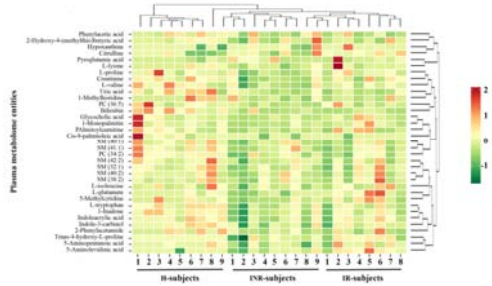


Figure 3. Hierarchal clustering of metabolites altered during cART. Hierarchical clustering of a set of 35 metabolites that were different throughout the follow-up in both groups of HIV patients and different between healthy volunteers and HIV-infected patients at 96 weeks of cART.

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