THU0509

MONOCYTES PROTEOMIC PROFILE OF PATIENTS WITH DIFFERENT AUTOINFLAMMATORY DISEASES: A NEW APPROACH TO CHARACTERIZE THESE DISEASES

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Background: Autoinflammatory diseases are a group of inherited diseases characterized by early onset and systemic inflammation. These pathologies are caused by mutations in genes involved in the regulation of innate immune response with a consequent inflammatory phenotype. The most common genetically defined periodic fevers are Familial Mediterranean Fever (FMF), Cryopyrin-associated periodic syndromes (CAPS), TNF receptor-associated periodic syndrome (TRAPS) and mevalonate kinase deficiency (MKD/HIDS).

Some patients show clinical features similar to autoinflammatory diseases but no genetic mutation has been found. Our aim is to evaluate the differences in the expression of proteins or pathway in monocytes, and plasma metabolites in patients with autoinflammatory diseases compared with healthy subjects to clusterize and better understand the mechanisms underlying different genetically defined disorders and try to characterize the genetically undefined pathologies.

Methods: Monocytes, purified from peripheral blood and incubated with or without LPS, were collected from patients and healthy donors; samples have been processed by iST protocol. Each digested sample was analyzed by high-resolution liquid chromatography and tandem mass spectrometry (LC-MS/MS) based on Orbitrap technology. The quantification strategy is a label-free approach (LFQ) available in MaxQuant suite. PCA analysis and Person’s correlation show good reproducibility of data and a good separation between the different groups. The data were then submitted to an appropriate statistic. T-Tests highlighted differentially expressed proteins and through Cytoscape with the ClueGo app we obtained the differently regulated pathways in the different conditions. It has also been constructed, starting from significative proteins, a network related to disease using the information of String Disease database. We observed that the expression of proteins is differently enriched according to the different conditions. For each autoinflammatory disease, a list of significantly modulated proteins was obtained: some of which are already known to be related to these disorders, while others have not yet been described. In FMF, MEFV, RhoA and some related proteins were significantly up-regulated together with genes linked to the interferon pathway. In TRAPS relevant proteins turn up related to the maintenance of Golgi and cellular trafficking. The bioinformatics analysis allows us to better understand the functional interaction between these monocytes proteins and which are involved in the disease.

Conclusion: Here, we addressed how a high-resolution proteomics approach could be used to better understand the biology of autoinflammatory diseases. The characterization of a broad spectrum of proteins and their interaction network will allow us to identify new biomarkers for the different pathologies and better comprehend and recognize the genetically undefined disorders.

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5.49; p<0.001 respectively. ∆MFI and SI on DHR between patients and healthy controls were comparable. There was no difference in oxidative burst activity between active and inactive SLE patients. Two patients showed a double peak in DHR flow cytometry consistent with mosaic pattern associated with a probable carrier state for X-linked granulomatous disease. Both oxidative burst activity and phagocytic activity did not show significant correlation with the dose of prednisolone.

Conclusion: This study suggests that there is impaired neutrophil function in childhood SLE and also there may be a correlation between neutrophil dysfunction and disease activity. The E.coli based phagocytic functions of neutrophils were significantly reduced in pediatric SLE patients compared to healthy controls. Phagocytic activity of neutrophil was significantly lower in patients with disease activity and coexistent infection in patients with pediatric SLE. Oxidative burst activity was reduced in patients with pediatric SLE compared to healthy controls. However, there was no significant correlation of oxidative burst activity to the age, disease activity and coexistent infections.

REFERENCES:

Abstract THU0510 – Figure 1. Phagocytic functions in patients with active SLE versus inactive disease

Abstract THU0510 – Figure 2. Correlation of SLEDAI and phagocytic activity

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THU0511

COMPARISON OF PAXGENE AND TEMPUS WHOLE BLOOD RNA COLLECTION AND ISOLATION SYSTEMS FOR THE QUANTIFICATION OF TYPE I INTERFERON-STIMULATED GENE EXPRESSION

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Background: Type I interferons (IFN) have important roles in many pediatric and adult rheumatic diseases and are a new therapeutic target for which several “anti-interferon (anti-IFN)” treatments are currently in use or in development. Since the direct detection of these proteins in biological samples has proved challenging, indirect methods are often used to infer the presence of type I IFN. IFN-stimulated genes (ISGs) and the relative expression of interferon-stimulated genes (ISGs) that are used to calculate an interferon score (IS) (1). This score has been used for example to assess type I IFN activity in pediatric patients with type I interferonopathies, systemic lupus erythematosus, dermatomyositis and systemic juvenile idiopathic arthritis (2). Both qPCR and Nanostring technology have similar sensitivity and reproducibility for IS determination (3). The use of different whole blood RNA collection systems on the IS have not been evaluated however despite evidence of method-dependent changes in gene expression (4).

Objectives: The aim of the study was to compare expression of six common ISGs (IFI27, IFI44L, IFIT1, ISG15, RSAD2, SIGLEC1) and the corresponding IS in RNA derived from two commonly used whole blood RNA collection systems (PAXgene and Tempus).

Methods: Whole blood was collected from ten healthy individuals (median age 25.5 years) in sodium heparin tubes and incubated without or with recombinant human interferon alpha 2b (rhIFNα, 2 IU/ml, 4 hrs, 37°C), 5% CO₂. Next, samples were divided between PAXgene (PreAnalytiX, Becton Dickinson) and Tempus (Applied Biosystems) tubes and RNA was isolated according to the manufacturer’s protocols. cDNA was synthesized (500ng input RNA; qScript cDNA synthesis kit) and ISG expression measured on a QuantStudio 6 Real-Time PCR instrument using a TaqMan Fast Advanced Assay. For each ISG, expression was normalized against the geometric mean of two housekeeping genes (18S rRNA and HPRT1) and calculated using the formula 2^-ΔΔCt. Relative gene expression is reported as the normalized expression of each ISG divided by the median of normalized expression of the same ISG in unstimulated samples. The median relative expression of all six ISGs was used to calculate the IFN score for each sample.

Results: There was not a statistically significant difference in the normalized expression of any of the six ISGs in either the rhIFNα-stimulated or unstimulated samples derived from PAXgene or Tempus tubes. The greatest difference in mean normalized expression in both unstimulated and stimulated samples was observed for ISG15 (difference in mean normalized expression was 0.0034 and 0.11, respectively). Overall there was a strong correlation of the IFN score between PAXgene and Tempus tubes for both the unstimulated (R² = 0.9117, p<0.0001) and rhIFNα-stimulated samples (R² = 0.8529, p<0.0001).

Conclusion: Despite reported differences in gene expression patterns associated with samples collected in PAXgene versus Tempus tubes, our results demonstrate that 6-gene interferon scores do not differ significantly between RNA samples obtained with these two systems. These results suggest that health care and research centres can use either tubes for IFN score determination using these 6 ISGs and results can be directly compared irrelevant of the RNA collection system employed.

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