Fellowship in "Biotechnology and Biosciences", University of Parma. Third year Report

PhD in "Biotechnologies and Biosciences", University of Parma - XXXII Cycle
Research Project by Dr. Maria Nicastro *Tutor: Dr. Gabriella Moroni*

PROJECT TITLE: Cellular and molecular approaches to the study of Erdheim-Chester Disease and Chronic Periaortitis

My research activities during my three-year PhD in *Biotechnologies and Biosciences* concerned some chronic renal diseases in the domain of fibroinflammatory disorders. In particular, I was heavily involved in a project on Erdheim-Chester Disease (ECD) and on Chronic Perioaortitis (CP). However, I also worked as a secondary investigator in additional investigations. This annual report contains a fairly exhaustive description on my activity in ECD and CP projects as well as an outline of the above-mentioned additional studies.

Procollagen I expression by ECD foamy histiocytes

ECD is a rare form of non-Langerhans cell histiocytosis. It presents clinically with a broad spectrum of manifestations, ranging from focal/isolated to diffuse/infiltrative forms which often involve multiple organ systems and, frequently, carry a high mortality rate. From a histopathological standpoint, ECD is characterized by skeletal and extra-skeletal areas where the normal tissue architecture is replaced by foci of $CD68^{+}/CD1a^{-}$ foamy histiocytes which are organized into small nests and/or cellular groups with, in between, a noteworthy amount of collagen type I extracellular matrix. Interestingly, the fibroblastic component in ECD foci is, very often, quantitatively minor, even in cases with substantial amounts of extracellular collagen fibre matrix. Hence, the question arises whether additional cells – other than fibroblasts – might be capable of producing collagen type I in these ECD locations. The purpose of this investigation was to explore whether ECD histiocytes are also capable of producing collagen fibres.

During my **third PhD year**, I performed RNA In Situ Hybridization in 10 ECD cases in which laser microdissection could not be performed since I had both suitable and unsuitable cases for microdissection.

RNA In Situ Hybridization confirmed the previous finding that the histiocytes expressed these pro-collagen genes. The presence of reddish clusters in ECD cases – compared to the small dots in positive controls – was indicative of a significant gene expression in ECD

Subsequently, by using immunohistochemistry, I demonstrated that PPAR-γ was expressed in foamy histiocytes from ECD biopsies.

Data from the literature demonstrate that, in vitro, the presence of serum lipoproteins induces monocyte differentiation into CD14low CD1a- cells, which is the typical phenotype of foamy histiocytes. In contrast, removal of these lipoproteins skews their differentiation into CD1a+ cells. Of note is the fact that development of CD1a- monocytes is associated with expression of peroxisome proliferator-activated receptor–gamma (PPARG) gene, a major regulator of adipogenesis as well as a potent modulator of systemic lipid metabolism. Interestingly, it has been shown that PPAR-γ also increases expression of ARG I, thereby accelerating foam-cell formation and collagen synthesis. Arg1catalyses the conversion of L-arginine into urea and L-ornithine, which is further converted into polyamines and L-proline, essential substrate for collagen synthesis. Unpublished data obtained from ECD patients show significant dyslipidaemic traits in terms of higher levels of cholesterol and/or triglycerides. All these data support our initial hypothesis that histiocytes produce collagen I.

Future developments:

- Evaluating COL1A1 and COL1A2 genes by RT-PCR in monocytes and in foamy histiocytes obtained after culturing pan-monocytes (isolated from buffy coat, and subsequently, by indirect magnetic labelling system by depletion of non-monocytes) from patients and controls
- Exploring Ornithine expression in ECD biopsies
- Exploring Ornithine and PPARγ in the culturing of foamy histiocytes

If this hypothesis is proven, a next step could be to inhibit Ornithine and PPARγ to evaluate whether pro-collagen type I synthesis is reduced in ECD histiocytes

Fibrocytes in Chronic Periaortitis

In this study, we explored the role of fibrocytes in Chronic Periaortitis (CP). CP is a rare disease characterized by a newly-formed periaortic fibro-inflammatory tissue which extends eccentrically into the retroperitoneum. Fibrocytes are peripheral blood cells that originate from bone marrow mesenchymal progenitor cells. Several studies have demonstrated that fibrocytes participate in different fibrosing diseases such as idiopathic pulmonary fibrosis. However, their role in the pathogenesis of CP has not been investigated thus far.

During my **third year,** I performed a flow cytometric analysis to measure fibrocyte percentages after one month of prednisone therapy in 8 of the 21 patients included in the flow cytometry study and in only one case with relapse.

We observed a post-treatment reduction in circulating fibrocyte concentrations, albeit only close to statistical significance (P=0.09). In only one case with relapse, we could see a post-treatment reduction in circulating fibrocyte percentages and an increase during recurrence. Furthermore, I performed a quantitative sandwich ELISA to determine the circulating levels of CXCL12/SDF-1 (ligand for CXCR4) after one month of prednisone therapy in 8 patients of the 16 patients included in ELISA test. We observed a post-treatment reduction in SDF-1 plasma levels, to statistical significance (P=0.02)

Some of these results have been published as a manuscript in *Arthritis & Rheumatology*.

Nicastro M, Vescovini R, Maritati F, Palmisano A, Urban ML, Incerti M, Fenaroli P, Peyronel F, Benigno GD, Mangieri D, Volpi R, Becchi G, Romagnani P, Corradi D, Vaglio A. **(IF: 6.918).**

Other studies:

Clinical and Prognostic Significance of Serum IgG4 in Chronic Periaortitis. An Analysis of 113 Patients.

Chronic periaortitis (CP) is a rare fibro-inflammatory syndrome included in the spectrum of IgG4-related diseases. Since CP patients rarely undergo diagnostic biopsies, serum IgG4 levels are often used to classify CP as IgG4-related. However, the clinical and prognostic significance of serum IgG4 in CP is unknown.

In this study I performed immunohistochemical analyses of cases with biopsies, in order to assess IgG4+ plasma cell infiltration. Unstained retroperitoneal biopsy slides were analysed with IgG and IgG4 antibodies. The number of IgG4+ and IgG+ plasma cells was assessed in 10 microscopic fields for each of the tested slides at a magnification of 400x. The IgG4+/IgG+ plasma cell ratio was calculated by dividing the total number of IgG4+ plasma cells by the total number of IgG+ plasma cells and multiplying this value by 100. Furthermore, we measured serum IgG4 in active CP patients, healthy and disease controls. The findings demonstrated that there is not always a correlation between tissue IgG4+/IgG+ plasma cell ratio and serum IgG4 levels. In addition, our data appear to confirm that serum IgG4 levels cannot be considered a good biomarker of IgG4-Related Disease.

This manuscript has now been accepted for publication in *Frontiers in Immunology* (IF: 6.429).

Maritati F, Rocco R, Accorsi Buttini E, Marvisi C, **Nicastro M**, Urban ML, Fenaroli P, Peyronel F, Benigno GD, Palumbo AA, Corradi D, Emmi G, Pipitone N, Palmisano A, Vaglio A.

Identification of molecular markers in patients with Giant Cell Arteritis.

I performed this study in collaboration with the Laboratory of Allergology, Autoimmunity and Innovative Biotechnology at Reggio Emilia Hospital.

Giant Cell Arteritis (GCA) is a large-vessel and medium-vessel vasculitis that affects individuals older than 50 years of age. The aetiology is still unknown but various studies have suspected an infectious cause and a genetic predisposition. The histologic picture of GCA is characterized by panarteritis including lymphomononuclear inflammatory cell infiltrate, with or without giant cells. To date, no specific biomarkers have been identified and the gold standard to diagnose GCA remains a biopsy of the temporal artery.

The aim of this study was to identify any potential gene changes that would be able to predict the efficacy of steroid treatment in relapsed patients vs. patients without a relapse.

The inclusion criteria in our study were:

Patients with biopsy-proven GCA and grading +2, +3 of the inflammatory infiltrate

Patients without steroid treatment at the time of biopsy

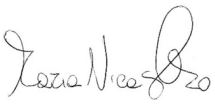
Patients with available 3-year follow-up data

This patient cohort was subdivided into patients with no relapses and patients with one relapse

With my colleagues, we performed a NanoString nCounter Gene Expression Assay in 10 relapsed patients and 10 patients with no relapses and then normalized the data obtained with a large number of housekeeper gene using nSOLVER Software. We identified a down and up regulated mRNA in patients with relapsed disease vs. no relapse. In this study I worked to validate the gene changes obtained from NanoString Assay. After extracting RNA from frozen biopsies, I performed an RT-PCR analysis in a new GCA cohort of 16 relapsed patients and 15 patients with no relapses. To date, I'm analysing the data obtained by PCR with those of the NanoString nCounter Gene Expression Assay. This study is still ongoing.

Parma, 05.10.2019

In witness whereof,



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