Biochemical Society Summer Vacation Studentship Report 2023

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Introduction

Juvenile Idiopathic Arthritis (JIA) is the most prevalent chronic inflammatory condition of childhood (Martini et al., 2022). JIA encompasses the following groups of childhood arthritides, systemic arthritis, enthesis-related arthritis (ERA), rheumatoid factor positive (RF+), rheumatoid factor negative (RF-) oligo/polyarticular arthritis (OA/PO) and undifferentiated arthritis, according to the International League Against Rheumatism classification (Martini et al., 2022). Each of these JIA subtypes have differences associated with their clinical characterisation but all share similarities including pain, warmth and swelling in at least one joint. Pathogenic changes to the gut-site, such as dysbiosis of the gut-microbiota, are central to the pathogenic process in both adult-onset rheumatoid arthritis (RA) and JIA. However, the cellular and molecular mechanisms underpinning the associations between the gut and joint inflammation remain unknown. Previously it has been shown that serum levels of intestinal permeability biomarkers, including lipopolysaccharide binding protein (LBP) and intestinal-fatty acid binding protein (I-FABP) are higher in RA patients compared to healthy controls (HCs) (Matei et al., 2021). A paper that focussed on paediatric JIA patients that were systemic treatment-naïve, found that patients with PO JIA, OA JIA and spondyloarthropathies had significantly greater circulating LBP concentrations than HCs (Fotis et al., 2017).

Aims and Objectives

In this studentship, I set out to investigate whether the biomarkers of intestinal permeability, namely LBP and I-FABP, are increased in the serum of our cohort of adolescent JIA patients (n=61) compared to age-matched HCs (n=29). The age range of our cohort was from 13 to 23.

Summary of work undertaken

I first curated a collection of serum samples from a bioresource of peripheral blood samples taken from JIA patients. The different JIA subtypes that we chose to investigate were ERA, RF+ OA/PO and RF- OA/PO. These groups can be subdivided into whether their disease was active (at least one joint inflamed) or inactive (no current joint inflammation). Importantly, a similar number of samples for each subtype within each JIA group was selected.

After ELISA practice, I ran three ELISA plates for LBP and one for I-FABP following the protocols given by the manufacturer that came with the kits ordered; DuoSet ELISA LBP and Millipore I-FABP. Consistency across different plates and dates was achieved by using four samples as references across the three LBP plates. To make sure that all groups were represented on each plate to allow integration of the data, there was at least one of each JIA subtype (ERA, RF+ OA/PO, RF- OA/PO) as well as a HC. The given concentration of the standard was plotted against the optical density to generate a standard curve which was used to interpolate the unknown

concentrations of LBP and I-FABP in the serum samples. To standardise the concentration of LBP across plates I divided each concentration by the average of the two chosen references on the plate and multiplied it by the average of the average of the three pairs of references. The standard error of the mean and an unpaired t-test or one-way ANOVA were completed to calculate the error bars and p-value, respectively.

Description of results and forward directions

First, I compared LBP concentrations in the HCs to the whole cohort of JIA patients (see Figure 1A). I found that the LBP levels in JIA patients on average were not significantly increased compared to HCs. However, when splitting our JIA cohort into different JIA subtypes - ERA, RF+ and RF- OA/PO subtypes - there was a statistically significant differences in the level of serum LBP between the HC and ERA, as well as between ERA and RF- OA/PO (see Figure 1B). To investigate whether this was associated with disease activity or treatment, ERA was split further, but neither disease activity nor biologics showed any significant differences in LBP concentration (see Figure 1C and D). The increased LBP concentration in ERA indicates that patients within the ERA subtype may experience heightened intestinal permeability. Of interest, it is well-known that ERA has a strong association with subclinical gut inflammation and patients often have swelling in joints concurrently with symptoms of inflammatory bowel disease (Aggarwal et al., 2016).

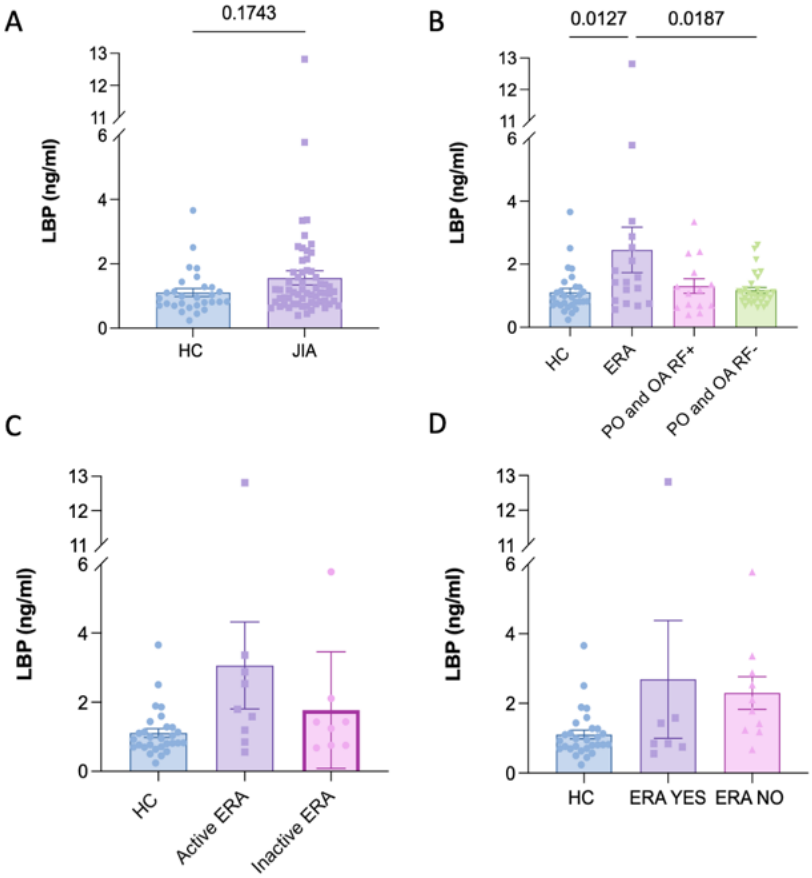

Figure 1. There is increased LBP in the serum of JIA patients with ERA. Standardised concentrations of LBP (ng/mL). A) LBP concentrations (ng/mL) in HC compared to JIA. B) LBP concentrations in HC compared to JIA subtypes. C) LBP concentrations in HC compared to ERA active and ERA inactive. D) LBP concentrations in HC compared to ERA biologics (ERA YES) or ERA no biologics (ERA NO). Error bars were calculated using the standard error of the mean. The lack of p-values indicates non-significance.

Due to time limitations, it was not possible to perform the I-FABP ELISA on enough samples. It would be necessary to complete at least one more ELISA plate. A future direction is to perform these necessary analyses and to investigate the levels of other biomarkers of 'gut-health' such as serotonin, D-Lactate, zonulin and retinoic acid in JIA patients compared to controls. The Rosser group is now planning to build on the work from my summer studentship and carry out these proposed experiments.

Impact

The research I carried out contributes to the increased understanding of the role of gut inflammation in the pathogenesis of JIA subtypes. With continued research in this field, LBP or other intestinal permeability markers could be used as a diagnostic tool for JIA patients, particularly ERA patients, to allow the application of personalised drug strategies such as those that target gut permeability. This is important since early treatment of JIA is associated with better long-term disease outcomes and some patients still do not respond to current treatment regimens (Garner et al., 2021). The Biochemical Society has supported me, an early career molecular bioscientist, which is in line with their strategy. I may have helped build the Biochemical Society's international reputation by doing weekly updates tagging BiochemSoc via Twitter.

Contribution to future goals and transferrable skills

I enjoyed how multifaceted working as a researcher is, from the manual aspects, data analysis, giving presentations, reading papers to writing. I have also confirmed my passion for Immunology and thus learned that I would like to work in Immunology research. Learning how to perform ELISAs is therefore a valuable skill. Moreover, I had such a great time that I requested to carry out my BSc laboratory project in the Rosser lab. Lastly, I have greatly improved my teamwork and communication skills. More specifically, I recognised the importance of attending weekly meetings to ensure everyone works well as a team and is well integrated.

Figure 2. The Rosser group. I am on the left, Persephone Jenkins fourth from the left, Elizabeth Rosser fifth from the left and Diana Matei seventh from the left. It was great working with the whole team!

References

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