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PRL-R ISOFORMS LONG / SHORT RATIO IS INCREASED IN SYSTEMIC LUPUS ERYTHEMATOSUS BLOOD MONONUCLEAR CELLS

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ABSTRACT

Prolactin (PRL) displays several functions in the whole body by binding its receptor (PRL-R). Two main PRL-R isoforms are reported that differ in their capacity to trigger signaling pathways, PRL-R long isoform is the activation receptor, and the short isoform is the inhibitory one. Although many autoimmune diseases display hyperprolactinemia, the role of each PRL-R isoform expression in autoimmunity remains unknown. This work aimed to correlate PRL-R isoforms expression in human peripheral blood mononuclear cells (PBMCs) of female Systemic Lupus Erythematosus patients and healthy controls. To this end, PBMCs from lupus patients (n=9) and healthy controls (n=5) were enriched by the ficoll-hypaque method. Then, RNA extraction, cDNA synthesis, and real-time PCR were performed to determine mRNA expression. We found that PRL-R long and short isoforms expression from lupus patients display similar expression to healthy controls. However, the PRL-RL/PRL-RS ratio is higher than healthy controls (P-value two-tailed <0,05). Additionally, PRL-RL isoform expression correlates with the short isoform in lupus patients (Spearman r 0,8945; 95% confidence interval 0,6834 to 0,9676; P-value two-tailed <0,0001). However, the expression of neither long and short isoforms correlates with active disease nor disease duration. Similarly, lupus patients display similar PRL-R isoforms expression than healthy controls. Although much work must be done, our data indicate that similar mechanisms may regulate the expression of both PRL-R isoforms in immune cells, and “their expression goes hand by hand”.

Keywords: *autoimmunity, prolactin, expression, lupus, receptor.*

1. INTRODUCTION

For many years, numerous advances have been reported concerning the pathogenic mechanisms of autoimmune diseases. However, specific and effective therapies for systemic autoimmune disorders such as Systemic Lupus Erythematosus (SLE) or Rheumatoid Arthritis (RA) have not yet been developed (Suurmond, Zou, Kim, and Diamond, 2015). The great heterogeneity surrounding all autoimmune diseases, such as their prognosis, diagnosis, treatment, and etiology, hinders the development of new therapies (Li *et al.*, 2013). Despite numerous efforts focused on searching for more effective therapies for diseases systemic autoimmune systems, most of the current therapies are based on the administration of immunosuppressive drugs leading to a generalized suppression with severe

complications (Houssiau and Ginzler, 2008). Due to this, it is crucial to identify pathogenic mechanisms in the development of autoimmunity in an individualized way, thus allowing the design of personalized immunosuppressive therapies. What to protect the competence of the immune system.

In recent years, much progress has been made in understanding the modulatory role of PRL on the immune system (Ben-Jonathan, LaPensee, and LaPensee, 2008). Studies indicate that in the thymus PRL would have a role in the differentiation of T lymphocytes promoting the survival of CD4-CD8- thymocytes and their differentiation to CD4 + CD8 + 15 thymocytes. In addition, PRL participates in the activation of T lymphocytes, mainly increasing their intensity 16, 17 and increasing the proliferative response of T lymphocytes against mitogens and the production

of IL-2 (Xu, Lin, Lin, Huang, and Lei, 2010). Additionally, PRL has antiapoptotic effects on B lymphocytes with increased production of antibodies (M. V. Legorreta-Haquet *et al.*, 2016; Maria Victoria Legorreta-Haquet *et al.*, 2013).

In the case of SLE, pre-clinical studies indicate that a hyperPRL would correlate with the degree of Lupus disease activity and with high autoantibody titers (Rezaieyazdi and Hesamifard, 2006).

The actions of PRL are mediated by its receptor, which is made up of an extracellular domain, a transmembrane domain, and a highly conserved intracellular signal transducer domain between species (Bole-Feysot, Goffin, Edery, Binart, and Kelly, 1998). The PRL-R gene contains different exons that can be spliced by alternative splicing. Generate various isoforms. These isoforms have an identical extracellular domain but differ in size and sequence from the intracellular portion, which can be short or long. The short isoform of PRL-R (PRL-RC) lacks the cytoplasmic domain and therefore does not have intracellular signaling. The long isoform of PRL-R (PRL-RL) is dimerized constitutively and, after binding a single molecule of the ligand (PRL), transmits a signal through associated cytoplasmic proteins. On the other hand, heterodimers of PRL-RL and PRL-RS1b are functionally inactive and cannot conduct signal transmission. In addition to the different isoforms bound to the membrane, we can find a soluble isoform of PRL-R (PRL-RS) composed of the extracellular portion (Dagil *et al.*, 2012).

It could then be speculated that due to failures in the regulation of the expression of PRL-RS short isoform, which lacks signaling, it would lead to a lesser effect on the modulation of activation mediated by PRL and PRL-RL during the activation of T lymphocytes. Thus, in susceptible individuals, these failures in regulation could favor the initiation and progression of autoimmune pathologies. On the contrary, the pharmacological induction of PRL-RS could suppress exacerbated inflammatory responses, being a potential therapeutic strategy for autoimmune diseases. Therefore, the focus of this work was to evaluate the expression of PRL-R isoforms during autoimmunity. For this, we used PBMCs from lupus patients and healthy controls to evaluate mRNA expression of PRL-RL and PRL-RS. We show these both isoforms displayed similar expression in all samples, and only PRL-RL/PRL-RS ratio from lupus samples set out differences from healthy controls.

2. MATERIALS AND METHODS

2.1. Patients

Patients For the development of the specific objectives, individuals belonging to a control group and an SLE group were recruited according to the following inclusion specifications:

Control group: composed of 5 control patients. They will be healthy female patients. With ages between 20 and 65 years. No known diseases and who are not undergoing any pharmacological treatment. Pregnant, menopausal, or lactating women were excluded. SLE group comprises 9 patients with SLE, female, who attend the rheumatology service and are enrolled by doctors belonging to the Immunology Institute of the Central Hospital of Mendoza. Inclusion criteria for SLE group:

1. Over 18 years old and under 65 years old;
2. Patients who meet the revised criteria of the American College of Rheumatology for the diagnosis of Systemic Lupus Erythematosus and Rheumatoid Arthritis;
3. Patients who, in the judgment of the treating rheumatologist, have "clinically stable" disease that makes the need for steroid dose adjustment or any initiation of a new immunosuppressive treatment during the course of the study, or both, unlikely;
4. Onset of the disease at least 6 months before inclusion in the study;
5. Active disease with SLEDAI-2k ≥ 6 ;
6. Use of hydroxychloroquine stable dose for 3 months;
7. Use of stable mycophenolate or azathioprine or methotrexate;
8. In the case of women of childbearing age, the use of contraception will be consulted.

Exclusion Criteria for SLE group:

1. Concomitant diagnosis of another autoimmune disease, such as Multiple Sclerosis, Type 1 Diabetes Mellitus, Scleroderma, Juvenile Idiopathic Arthritis;
2. Concomitant diagnosis of any chronic disease such as, but not exclusively, Type 2 Diabetes Mellitus, Chronic Renal Failure, Liver Cirrhosis, Acquired Immune Deficiency Syndrome, solid tumors, and hematological neoplasms, Heart Failure, Chronic Airflow Limitation;
3. Pregnancy or pregnancy intention in the year of the study;
4. Manifestations of disease that, in the opinion of the investigators, are serious and make entry into the study inappropriate. Examples: central nervous system vasculitis, lupus psychosis, active lupus nephritis, thrombocytopenia $< 50,000$, Hb < 8 gr/dL Leukopenia < 3000 , lymphopenia < 400 ;
5. Life-threatening manifestations of the disease;
6. Use of prednisone doses > 30 mg daily;
7. Use of biological therapy in the 6 months prior to the

baseline visit. Cyclosporine, chlorambucil, cyclophosphamide in the previous 6 months are excluded; 8. Serious infections (use of IV antibiotics, opportunistic, latent tuberculosis, herpes zoster) six months before the baseline visit; 9. Low normal plasma immunoglobulin levels; 10. Liver tests > 3 times normal. Abnormal EKG or abnormal chest X-ray; 11. Use of anticoagulant therapy or antiphospholipid syndrome associated with SLE; 12. Have received attenuated virus vaccines during the last year; 13. Have HIV, HBV, or HCV.

2.2. Bioethics and biosafety

The patients were communicated in a simple way about the study to ensure their correct understanding. They have been informed about the use of the study data and have signed an informed consent individually. The bioethics committee approves the protocols and the informed consent document developed in this project of the Faculty of Medical Sciences of UNCuyo and the teaching and research committee of the Hospital Central Mendoza. Likewise, all sample processing mechanisms are strictly supervised by biosafety protocols.

2.3. Samples

Peripheral blood mononuclear cells (PBMC) were separated by the conventional Ficoll-Hypaque method. First, 10 ml of blood was gently poured over 3 ml of Ficoll-Hypaque previously placed in a sterile 15 ml conical tube. Next, cells are carefully aspirated using sterile 3 ml Pasteur pipettes and placed in a new 15 ml tube.

2.4. Real-time PCR

RNA was obtained using the TRIZOL reagent (Invitrogen). Subsequently, 2 micrograms of total RNA were used to synthesize DNA copy using the enzyme MMLV reverse transcriptase and oligodT as specific primers for total mRNA (poly A) according to the reaction specifications of the merchant. Real-time PCR assays were performed to quantify the mRNA of the different PRL-R isoforms. Real-time PCR assays were performed on the Corbett 6000 equipment (Quiagen, Inc) using three technical replicas for each sample tested). The abundance of mRNA expression levels was performed by the $2^{-\Delta\Delta Ct}$ analysis used widely for a comparison of mRNA abundance between two groups using actin as a reference gene (or housekeeping gene). The sequences used were: human ACTIN Fw AAAGACCTGTACGCCAACAC and Rv GTCATACTCCTGCTTGCTGAT; PRL-RL Fw CCTTGTCAGGTTTCGCTGCAA and Rv

AGATGAGCATCAAATCCTTTTA; PRL-R1b (short) Fw TAAATGGTCTCCACCTACCCTGAT and Rv CACCTCCAACAGATGAGCATCAAATCC. The cycles used were 3 steps using 95° 30 s - 60° 30 s - 72° 30 s in the 3 genes studied. The products generated by the PCRs were previously sequenced to corroborate their identity and correspond specifically to the PRL-R sequences. The data obtained were analyzed with the GraphPad program and Microsoft Excel.

3. RESULTS AND DISCUSSION:

The average value of the relative expression of PRL-RS1b in healthy women is 1.0630 ± 0.3647 and in patients with SLE 0.8630 ± 0.4115 , this being the one with the most variability, CV = 47.69%. No statistically significant differences were observed between the means of the SLE and control groups.

The PRL-RL in the control group presents an average value of 1.063 ± 0.3647 in control patients and 0.8630 ± 0.4115 in patients with SLE and presents greater CV variability = 47.69%. No statistically significant differences were observed between the means of the SLE and control groups. The control group's PRL-RL / PRL-RS1b index presents an average value of 1.007 ± 0.1311 in control patients and 1.180 ± 0.1710 in patients with SLE and presents greater CV variability = 14.49%. Interestingly, it is observed that the SLE group presents an increase in this activation/inhibition index ($p < 0.05$ t-test) (Figure 1).

Figure 1. Relative RNA expression of PRL-R

Relative mRNA expression of PRL-RL (A), PRL-RS1b (B), and PRL-RL / PRL-RS1b (C) index in PBMC. * $p < 0.05$ "t student" test control vs SLE. +/- "T" bars show minimums and maximums. Box represents 95% confidence interval, including median represented as a line inside the box. The symbol "+" represents the mean.

Finally, intending to deepen the knowledge about the behavior of the different PRL-R isoforms, we evaluated the correlation between PRL-RL and PRL-RS1b in patient samples (Figure 2).

Figure 2. PRL-RL vs PRL-RS correlation

Correlation between the mRNA expression of the two PRL-R isoforms in samples from healthy individuals and patients with SLE. The statistical test used was Spearman r, taking as a statistically significant correlation when $r > 0.69$ and $p > 0.05$. the expression on PRL-RL ($r \approx 0.8945$; $p < 0.001$). These results indicate that the expression of PRL-RS1b could be regulated in a PRL-RL-dependent manner.

Surprisingly, the results showed a positive correlation of PRL-RS1b expression on PRL-RL ($r \approx 0.8945$; $p < 0.001$). These results indicate that the expression of PRL-RS1b could be regulated in a PRL-RL-dependent manner.

4. CONCLUSIONS

To the knowledge of the authors, this is the first report where a correlation between PRL-RS1b, SLEDAI, and duration was evaluated. Similarly, the study of the expression of PRL-R isoforms in the different purified lymphocyte populations is crucial to continuing this study. In summary, the data arising from the current study do not show a significant advantage in the use of the study of the different isoforms of PRL-R as a marker of SLE. However, the use of the PRL-RL/PRL-RS1b index could contribute to a future detailed classification of patients with SLE, although in this study, the authors were unable to associate this index with clinical parameters of the disease. Therefore, the study of PRL-R expression and epigenetic parameters related to the regulation of its transcription in purified leukocyte populations could be more efficient and clinically relevant.

5. ACKNOWLEDGMENTS:

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6. REFERENCES:

1. Ben-Jonathan, N., LaPensee, C. R., and LaPensee, E. W. (2008). What Can We Learn from Rodents about Prolactin in Humans? *Endocr Rev*, 29(1), 1-41.
2. Bole-Feysot, C., Goffin, V., Edery, M., Binart, N., and Kelly, P. A. (1998). Prolactin (PRL) and Its Receptor: Actions, Signal Transduction Pathways, and Phenotypes Observed in PRL Receptor Knockout Mice. *Endocr Rev*, 19(3), 225-268.
3. Dagil, R., Knudsen, Maiken J., Olsen, Johan G., O'Shea, C., Franzmann, M., Goffin, V., *et al.* (2012). The WSXWS Motif in Cytokine Receptors Is a Molecular Switch Involved in Receptor Activation: Insight from Structures of the Prolactin Receptor. *Structure*, 20(2), 270-282.
4. Houssiau, F., and Ginzler, E. (2008). Current treatment of lupus nephritis. *Lupus*, 17(5), 426-430.
5. Legorreta-Haquet, M. V., Chávez-Rueda, K., Chávez-Sánchez, L., Cervera-Castillo, H., Zenteno-Galindo, E., Barile-Fabris, L., *et al.* (2016). Function of Treg Cells Decreased in Patients With Systemic Lupus Erythematosus Due To the Effect of Prolactin. *Medicine (Baltimore)*, 95(5), e2384.
6. Legorreta-Haquet, M. V., Flores-Fernandez, R., Blanco-Favela, F., Fuentes-Panan, E. M., Chavez-Sanchez, L., Hernandez-Gonzalez, R., *et al.* (2013). Prolactin Levels Correlate with Abnormal B Cell Maturation in MRL and MRL/lpr Mouse Models of Systemic Lupus Erythematosus-Like Disease. *Clin Dev Immunol*, 2013, 11.
7. Li, P. H., Wong, W. H. S., Lee, T. L., Lau, C. S., Chan, T. M., Leung, A. M. H., *et al.* (2013). Relationship between autoantibody clustering and clinical subsets in SLE: cluster and association analyses in Hong Kong Chinese. *Rheumatology*, 52(2), 337-345.
8. Rezaieyazdi, Z., and Hesamifard, A. (2006). Correlation between serum prolactin levels and lupus activity. *Rheumatology International*, 26(11), 1036-1039.
9. Suurmond, J., Zou, Y. R., Kim, S. J., and Diamond, B. (2015). Therapeutics to block autoantibody initiation and propagation in systemic lupus erythematosus and rheumatoid arthritis. [Journal Article]. *Sci Transl Med*, 7(280), 280-285.
10. Xu, D., Lin, L., Lin, X., Huang, Z., and Lei, Z. (2010). Immunoregulation of autocrine prolactin: Suppressing the expression of costimulatory molecules and cytokines in T lymphocytes by prolactin receptor knockdown. *Cell Immunol*, 263(1), 71-78.