

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: <http://www.elsevier.com/locate/pjnns>

Original research article

Serum interleukin 15 levels in patients with seropositive myasthenia gravis do not correlate with disease severity

Ewa Sobieszczuk^a, Justyna Kubiszewska^a, Marta Lipowska^a,
Piotr Szczudlik^a, Beata Szyluk^a, Małgorzata Dutkiewicz^b,
Anna Kostera-Pruszczyk^{a,*}

^a Department of Neurology, Medical University of Warsaw, Warsaw, Poland

^b Department of Immunology, Biochemistry and Nutrition, Medical University of Warsaw Poland

ARTICLE INFO

Article history:

Received 24 February 2017

Accepted 30 December 2017

Available online 6 January 2018

Keywords:

Myasthenia gravis

Interleukin 15

Cytokine

Autoimmune disease

ABSTRACT

Aim: To assess interleukin 15 (IL-15) serum levels in patients with seropositive myasthenia gravis (MG); searching for potential relationship between IL-15 levels and clinical features such as gender, age at onset, clinical presentation or treatment received.

Background: IL-15 plays pivotal role in T-cell dependent autoimmunity. Increased IL-15 serum levels have been reported in several autoimmune diseases including MG patients from Japan.

Patients and methods: Sera of 42 seropositive MG patients (66.7% women), mean age 50.6 ± 23.7 years) have been tested by ELISA for IL-15 levels.

Results: There were no statistically significant differences between IL-15 serum levels in MG patients in comparison with controls as well as between subgroups of MG patients (early vs. late onset and thymoma MG). Mean/median IL-15 serum levels were similar in MG patients treated with corticosteroids (CS) and CS naïve. Outliers (very high values) were seen only in untreated generalized MG patients.

Conclusions: Serum interleukin 15 levels in patients with seropositive myasthenia gravis do not correlate with disease severity.

© 2018 Published by Elsevier Sp. z o.o. on behalf of Polish Neurological Society.

1. Introduction

Myasthenia gravis (MG) is a neuromuscular disease caused in 80–95% of patients by a T-cell dependent autoantibody response against receptor for acetylcholine at the postsynaptic

muscle membrane (AChRab). AChRab in the neuromuscular junction cause neuromuscular transmission disturbances and, as a consequence, muscle weakness and fatigability. AChRab serum levels show no correlation with the severity of MG course and no biological marker of MG severity was identified so far [1]. Also factors active in triggering and

* Corresponding author at: Department of Neurology, Medical University of Warsaw, Banacha 1a, 02-097 Warsaw, Poland.

E-mail address: akostera@wum.edu.pl (A. Kostera-Pruszczyk).

<https://doi.org/10.1016/j.pjnns.2017.12.011>

0028-3843/© 2018 Published by Elsevier Sp. z o.o. on behalf of Polish Neurological Society.

sustaining the autoimmune reaction in MG are unknown. Studies in experimental MG (EAMG) suggested that myocytes are not only passive participants or target of the immunologic cascade, but are active during the course of the disease by producing specific cytokines, including interleukin 15 (IL-15) [2,3]. IL-15 stimulates interferon gamma (INF gamma) production. INF gamma in turn was proved to enhance experimental MG symptoms [2,4–6]. IL-15 not only induces T lymphocytes proliferation, enhances NK cells cytotoxicity and leads to increased production of their cytokines and chemokines, but also facilitates survival of CD8+ memory T cells including self-reactive memory T-cells [7].

Increased IL-15 serum levels have been reported in several autoimmune diseases, including systemic lupus erythematosus, inflammatory bowel disease, multiple sclerosis, rheumatoid arthritis, diabetes type 1 or celiac disease [7,8]. Increased serum IL-15 levels were reported in Japanese MG patients not treated with immunosuppressants or thymectomy [8]. It prompted us to verify the hypothesis that IL-15 serum level is increased in a group of Caucasian seropositive MG patients and test its possible relationship with clinical features such as gender, age of onset, clinical presentation or treatment received.

2. Patients and methods

2.1. Subjects

42 patients with seropositive MG were enrolled in this study in the period between 2011 and 2014 after informed consent. Clinical diagnosis of MG was confirmed by positive result of repetitive nerve stimulation test or single-fibre electromyography (SFEMG) and elevated serum anti-AChR levels. 66.7% of tested patients were women; age of onset ranged from 15 to 85 years (mean 50.6 ± 23.7 years). By definition all subjects had seropositive MG. 42.9% of patients were classified as early onset MG (EOMG, <50 years), 45.2% as late onset MG (LOMG, ≥ 50 years); 11.9% had thymoma-MG (T-MG). The majority (78.6%) of the subjects were never treated with corticosteroids (CS) or other immunosuppressants. 19% of all patients underwent thymectomy. In 14.3% of the patients autoimmune thyroid disease coexisted with MG. The severity of symptoms was assessed according to Myasthenia Gravis Foundation of America (MGFA) clinical classification scale. 88.1% of subjects had generalized MG. Two patients (4.8%) were in complete stable remission. Sera of 16 subjects with no history of MG or other autoimmune diseases served as normal controls. All sera were frozen and stored at -73°C until analysis.

2.2. Methods

IL-15 serum levels were measured by ELISA (BioLegend IL-15 Pre-coated ELISA Kit, according to the manufacturer's instruction, by MD). The detection limit of ELISA Kit was 2 pg/ml.

2.3. Statistical analysis

Independent-Samples T test, after Levene's Test for Equality of Variances and U Mann Whitney test, were used to compare

respectively means and ranges of IL-15 serum levels within groups taken into comparison. Correlations between IL-15 and AChRAb serum levels and clinical and laboratory features were studied using Pearson's and Spearman's tests, as appropriate. Statistical significance was defined at $p < 0.05$.

3. Results

There was no significant difference between serum IL-15 levels in patients with MG as compared to controls (9.87 ± 9.37 (median 7.38; Q1 = 4.51, Q3 = 10.28) vs. 8.69 ± 3.61 pg/ml (median 8.69; Q1 = 5.13, Q3 = 11.38), respectively; $p = 0.449$). There was also no significant difference between women and men (9.29 ± 7.79 (median 7.38; Q1 = 4.62, Q3 = 9.43) vs. 11.21 ± 12.64 pg/ml (median 6.81; Q1 = 4.28, Q3 = 11.31); $p = 0.560$). Also the patients treated with corticosteroids within the last 3 months did not have lower IL-15 serum levels than patients who have received prednisone recently (7.67 ± 4.17 (median 8.02; Q1 = 4.04, Q3 = 11.31) vs. 10.19 ± 10.05 pg/ml (median 7.05; Q1 = 4.52, Q3 = 10.35), respectively; $p = 0.941$); there was also no differences in IL-15 levels in patients treated with corticosteroids in the past vs. untreated (Fig. 1).

Patients with generalized MG had similar mean serum IL-15 levels as patients with ocular MG only (9.92 ± 9.85 (median 6.67; Q1 = 4.48, Q3 = 10.52) vs. 8.45 ± 4.45 (median 8.97; Q1 = 4.72, Q3 = 11.92); $p = 0.641$) (Fig. 2). Patients with EOMG tended to have lower IL-15 serum levels from LOMG (8.53 ± 5.41 (median 6.62; Q1 = 4.58, Q3 = 10.89) vs. 11.65 ± 12.87 (median 8.07; Q1 = 4.33, Q3 = 11.66); $p = 0.380$) (Fig. 3). There were similar serum IL-15 levels in T-MG in comparison with other MG groups (9.25 ± 3.46 (median 8.59; Q1 = 6.17, Q3 = 13.00) vs. 9.71 ± 9.66 ; $p = 0.936$ (median 8.07; Q1 = 4.33, Q3 = 11.66), respectively). We have not found significant differences between patients with or without autoimmune thyroid disease (respectively 8.61 ± 2.96 (median 7.05; Q1 = 4.47, Q3 = 10.05) vs. 9.86 ± 10.03 ; (median 6.72; Q1 = 4.47, Q3 = 10.75) $p = 0.283$).

One patient had IL15 level below detection limit.

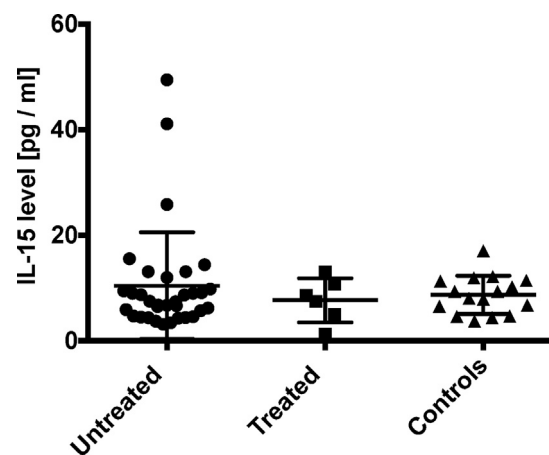


Fig. 1 – IL-15 serum levels in patients untreated vs. treated with corticosteroids (CS) within last 3 months. Patients treated with CS tend to have lower IL-15 serum levels (p ns).

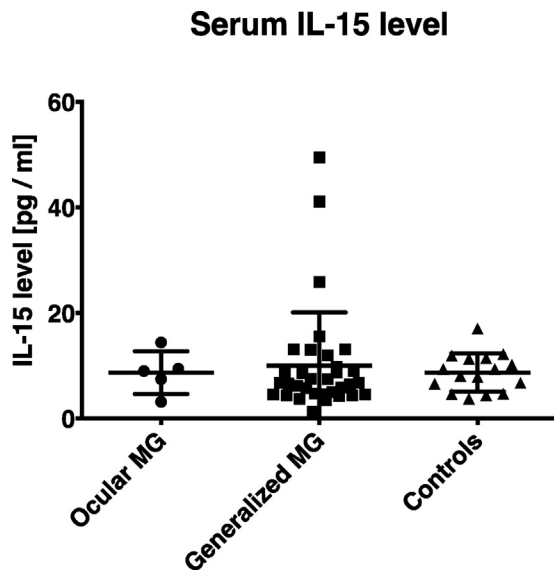


Fig. 2 – Serum IL-15 levels in ocular-MG vs. generalized-MG patients. There are no differences in IL-15 serum levels between groups (p ns).

IL-15 serum levels within different types of MG

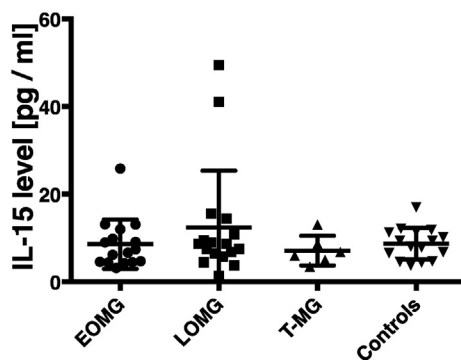


Fig. 3 – IL-15 serum levels within different types of MG. Patients with early onset MG (EOMG) and thymoma-MG (T-MG) tend to have lower serum IL-15 levels in comparison with late onset MG (LOMG) (p ns).

4. Discussion

In this study we have assessed IL-15 serum levels in patients with seropositive MG and searched for potential relationship between IL-15 levels and clinical features of the disease such as gender, age at onset, clinical presentation or treatment received. Although we have not found any statistically significant differences neither between MG patients and HC, nor among different categories of MG patients, the IL15 results in our study group were comparable to those reported before [8,9]. The main difference between our cohort and the MG patients reported by Uzawa were their treatment status. All of Uzawa et al. patients were treatment-naïve while 19% of ours

had history of immunosuppressive treatment. We have not found any significant differences among groups of MG patients, nor between CS-treated and CS-naïve in mean IL-15 serum level in patients treated with CS within last 3 months. All of the patients with highest IL15 values had generalized MG and were not treated with CS.

Ocular MG patients tended to have slightly lower mean IL-15 serum levels, although the difference was not statistically significant. This observation is consistent with Uzawa et al. study where only generalized MG patients had statistically significant higher IL-15 serum levels in comparison with HC and no such difference was noted for ocular MG patients.

Our series is the third study addressing IL-15 serum levels in MG patients, although numerous studies on the potential IL-15 role in the pathogenesis and course of MG were conducted so far. They led to the concept of immunological pathway, where AChRab lead to the activation of myocytes and increase of the IL-4R on their surface; activated myocytes increase IL-15 production that enhances MHC II and ICAM 1 expression leading to worsening MG symptoms [1-6]. IL-15 plays important role in facilitating survival of CD8+ memory T cells including self-reactive memory T-cells [7]. It has been proven that depletion of CD8+ cells suppresses the development of experimental MG [10,11]. Beneficial effects of the decrease of IL-15 serum levels has been also already observed in other autoimmune diseases such as rheumatoid arthritis (RA) [12]. The benefits resulting from IL-15 activity blocking have been also observed in experimental psoriasis model in mice [13]. Currently several clinical trials using monoclonal antibodies targeting the cytokine receptor subunit IL-2/IL-15R β for IL-15 (CD122) and blocking IL-15 transpresentation in autoimmune diseases are in progress. Promising findings resulted from using these substances in the study on transgenic mice with experimental celiac disease [7,14]. The role of IL15 in MG patients requires further studies.

We conclude that although we did not demonstrate relationship between IL15 serum levels and MG severity or MG type in a studied cohort.

Conflict of interest

None declared.

Acknowledgement and financial support

This study was supported by grant NCN DEC-2011/01/B/NZ5/05346.

REFERENCES

- [1] Shandley S, Martinez S, Krolick K. IL-4 receptor as a bridge between the immune system and muscle in experimental myasthenia gravis I: up-regulation of muscle IL-15 by IL-4. *Clin Immunol* 2009;132:246-56.
- [2] Stegall T, Krolick KA. Myocytes respond in vivo to an antibody reactive with the acetylcholine receptor by

- upregulating interleukin-15: an interferon-gamma activator with the potential to influence the severity and course of experimental myasthenia gravis. *J Neuroimmunol* 2001;119:377–86.
- [3] Stegall T, Krolick KA. Myocytes respond to both interleukin-4 and interferon- γ : cytokine responsiveness with the potential to influence the severity and course of experimental myasthenia gravis. *Clin Immunol* 2000;94:133–9.
- [4] Gu D, Wogensen L, Calcutt NA, Xia C, Zhu S, Merlie JP, et al. Myasthenia gravis-like syndrome induced by expression of interferon gamma in the neuromuscular junction. *J Exp Med* 1995;181:547–57.
- [5] Balasa B, Deng C, Lee J, Bradley LM, Dalton DK, Christadoss P, et al. Interferon γ (IFN- γ) is necessary for the genesis of acetylcholine receptor – induced clinical experimental autoimmune myasthenia gravis in mice. *J Exp Med* 1997;186:385–91.
- [6] Zhang G-X, Xiao B-G, Bai X-F, van der Meide PH, Örn A, Link H. Mice with IFN- γ receptor deficiency are less susceptible to experimental autoimmune myasthenia gravis. *J Immunol* 1999;162:3775–81.
- [7] Waldmann TA. The biology of IL-15: implications for cancer therapy and the treatment of autoimmune disorders. *J Invest Derm Symp Proc* 2013;16:S28–30.
- [8] Uzawa A, Kawaguchi N, Himuro K, Kanai T, Kuwabara S. Serum cytokine and chemokine profiles in patients with myasthenia gravis. *Clin Exp Immunol* 2014;176:232–7.
- [9] Shi L, Liu T, Zhang M, Guo Y, Song C, Song D, et al. miR-15b is downregulated in myasthenia gravis patients and directly regulates the expression of interleukin-15 (IL-15) in experimental myasthenia gravis mice. *Med Sci Monit* 2015;21:1774–80.
- [10] Zhang G-X, Ma C-G, Xiao B-G, Bakhiet M, Link H, Olsson T. Depletion of CD8+ T cells suppresses the development of experimental autoimmune myasthenia gravis in Lewis rats. *Eur J Immunol* 1995;25:1191–8.
- [11] Zhang GX, Xiao BG, Bakhiet M, van der Meide P, Wigzell H, Link H, et al. Both CD4+ and CD8+ T cells are essential to induce experimental autoimmune myasthenia gravis. *J Exp Med* 1996;184:349–56.
- [12] Díaz-Torné C, Ortiz de Juana MA, Geli C, Cantó E, Laiz A, Corominas H, et al. Rituximab-induced interleukin-15 reduction associated with clinical improvement in rheumatoid arthritis. *Immunology* 2014;142:354–62.
- [13] Villadsen LS, Schuurman J, Beurskens F, Dam TN, Dagnæs-Hansen F, Skov L, et al. Resolution of psoriasis upon blockade of IL-15 biological activity in a xenograft mouse model. *J Clin Investig* 2003;112:1571–80.
- [14] Yokoyama S, Perera P-Y, Waldmann TA, Hiroi T, Perera LP. Tofacitinib, a janus kinase inhibitor demonstrates efficacy in an IL-15 transgenic mouse model that recapitulates pathologic manifestations of celiac disease. *J Clin Immunol* 2013;33:586–94.