



Universiteit
Leiden
The Netherlands

The Lambert-Eaton myasthenic syndrome

Wirtz, P.W.

Citation

Wirtz, P. W. (2005, November 7). *The Lambert-Eaton myasthenic syndrome*. Febodruk B.V.
Retrieved from <https://hdl.handle.net/1887/4275>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/4275>

Note: To cite this publication please use the final published version (if applicable).

Chapter 9

High innate production of interleukin-10 and tumour necrosis factor- α contributes to susceptibility for non-paraneoplastic Lambert-Eaton myasthenic syndrome

P.W. Wirtz,¹ T.W.J. Huizinga,² D.-J. Stoeken,³
A.R. Wintzen,¹ J.J. Verschuuren¹

Departments of ¹Neurology, ²Rheumatology and ³Clinical Chemistry
Leiden University Medical Centre
Leiden, The Netherlands

Abstract

Non-paraneoplastic Lambert-Eaton myasthenic syndrome (LEMS) is an antibody-mediated autoimmune disorder, in which genetically determined interleukin-10 (IL-10) and tumour necrosis factor- α (TNF- α) could play a role in the susceptibility for the disease. Therefore, we analyzed the production of IL-10 and TNF- α after whole-blood stimulation in first-degree family members of patients with LEMS without malignancy, as a measure of innate production in the patients. Thirty-six first-degree family members of ten patients and 80 healthy controls were studied. Both IL-10 ($p=0.037$) and TNF- α ($p=0.0016$) production were increased in the family members, but had no relation with the severity of LEMS or HLA-B8DR3 carriership. Our findings suggest that high innate production of IL-10 and TNF- α is a susceptibility factor for non-paraneoplastic LEMS.

Introduction

The Lambert-Eaton myasthenic syndrome (LEMS) is a strictly antibody-mediated autoimmune disorder, clinically characterized by proximal muscle weakness and autonomic dysfunction. These clinical features arise from a decreased release of acetylcholine from nerve terminals, which is caused by antibodies directed against voltage-gated calcium channels (VGCCs). In approximately half of the patients a tumour, in most cases small cell lung carcinoma (SCLC), is found, which is thought to be the initiating factor of LEMS.¹

In patients in whom no tumour is found, initiating factors are unknown, but a highly significant association with the HLA-B8DR3 haplotype is reported.²⁻⁴ This suggests a possible role for HLA-DR3 in antigen presentation in non-paraneoplastic LEMS. In the subsequent immune reaction the cytokines tumour necrosis factor- α (TNF- α) and interleukin-10 (IL-10) could play an important role. Tumour necrosis factor- α , the gene of which maps within the MHC, enhances the immune reaction, and interleukin-10 stimulates proliferation of activated B-cells and their production of antibodies.⁵

Therefore, we investigated whether the innate production of IL-10 and TNF- α is a susceptibility factor for developing non-paraneoplastic LEMS. As the cytokine production of patients themselves could have been disturbed by disease activity or immunomodulatory therapy, and the production of IL-10 and TNF- α is largely determined by genetic factors, we analyzed cytokine production after whole-blood stimulation in first-degree family members of patients.⁶⁻⁹ This provides a measure of the innate production in the patients with non-paraneoplastic LEMS.

Materials and methods

Subjects

All patients were diagnosed with LEMS as confirmed by a repetitive nerve stimulation test, showing a low compound muscle action potential amplitude as well as an increase of this amplitude of more than 100% following high frequency repetitive nerve stimulation or following maximal voluntary contraction.¹⁰ In all patients, repeated extensive search for an underlying malignancy, especially SCLC, was negative for at least four years. A history of the course of the disease and additional autoimmune disorders in the patients themselves and their first-degree family members was taken. Maximum disability during the course of LEMS was scored with a six-point score for myasthenic severity.¹¹ In all patients, the levels of anti-P/Q-type VGCC antibodies were investigated using a precipitation assay.¹² HLA-B typing was performed using the standard microcytotoxicity technique, and HLA-DR typing using the propidium

iodide staining and automated reading method.¹³ Results of an HLA-study of patients with LEMS, which included patients from this study, were recently published.⁴

First-degree family members of the LEMS patients were invited to participate. Their health status was evaluated using a semistructured questionnaire. Relatives with a history of an infection two weeks prior to blood sampling, diagnosed cancer, an immunological disorder or use of immunosuppressive agents were excluded.

The control group consisted of healthy blood donors, for whom exclusion criteria were the same as for the first-degree relatives.

This study was approved by the institutional Medical Ethical Board of the Leiden University Medical Centre. Informed consent was obtained from all subjects.

Whole-blood stimulation

Whole-blood samples were obtained and stimulated with 1000 ng/ml of lipopolysaccharide during 24 hours (IL-10) and 4 hours (TNF- α) according to previously described methods.¹⁴ Unstimulated baseline samples were obtained to serve as a control for contamination. IL-10 and TNF- α productions were measured using enzyme linked immunosorbent assay with a detection limit of 4 pg/ml. From one family member sufficient blood samples could not be obtained. Another family member had a baseline level of both IL-10 and TNF- α higher than 100 pg/ml and was excluded.

Statistical analysis

Production of IL-10 and TNF- α was compared between family members and controls with a mixed model analysis to account for clustering within families. The relation of production of IL-10 and TNF- α of family members with clinical parameters of the patients was analyzed with a mixed model analysis. Significance was defined as a probability value of less than 0.05.

Results

A total of 36 first-degree family members of 10 LEMS patients was studied (table 1). The control group consisted of 80 healthy donors. In the 10 analyzed patients (male: female = 6: 4) median age at onset of LEMS was 53 years (range 11-64 years). Median maximum disability score was 3 (range 1-4). Median anti-VGCC antibody titer was 117 pmol/l (range 0-738 pmol/l). Four patients were HLA-B8-DR3 positive. Three patients had an additional autoimmune disease (type I diabetes mellitus, rheumatoid arthritis, thyroid disorder). Three patients had one or more first-degree family members with an autoimmune disorder.

Table 1. Demographic characteristics, and IL-10 and TNF- α production of first-degree family members of patients with the Lambert-Eaton myasthenic syndrome, and controls

	family members	controls ^a
Number	36	80
Sex (male: female)	17:19	49:31
Mean age (years) $\pm$ SD	47 $\pm$ 17	45 $\pm$ 12
Mean (pg/ml) TNF- α production $\pm$ SD	16222 $\pm$ 6445	12827 $\pm$ 4394
Mean (pg/ml) IL-10 production $\pm$ SD	4900 $\pm$ 1993	3830 $\pm$ 1844

^aProduction of both cytokines was not related to sex in controls (data not shown)

Results of IL-10 and TNF- α production after whole blood stimulation are shown in table 1. In the family members, production of both IL-10 (mean difference 1071 pg/ml $\pm$ SE 506; $p=0.037$) and TNF- α (mean difference 3396 pg/ml $\pm$ SE 1096; $p=0.0016$) was significantly higher than in controls (Figure 1). Production levels were not predicted by the age or sex of the family members, the presence of additional autoimmune disorders in the family, or age at onset of LEMS, HLA-B8DR3, anti-P/Q-type VGCC antibody level and maximum disability score in their related patient.

Discussion

This study shows that both IL-10 and TNF- α production after whole blood stimulation is increased in family members of patients with non-paraneoplastic LEMS, suggesting that high innate production of these cytokines is related to the susceptibility for LEMS. No relation was found between the innate cytokine production in the family members and the severity of LEMS. In both systemic lupus erythematosus and multiple sclerosis the high innate IL-10 production had no relation with disease severity either.^{15,16} Furthermore, we could not demonstrate a relation with HLA-B8DR3 carriership of the patients. Accordingly, the HLA haplotype and innate production of IL-10 and TNF- α seem to be related to the risk of LEMS as independent variables.

We do not know of other studies investigating the role of cytokines in the pathogenesis of LEMS. In patients with myasthenia gravis (MG), another antibody-mediated autoimmune disease of the neuromuscular synapse, several studies suggest a stimulatory role of these cytokines in the disease as well. Expression of IL-10 mRNA was significantly upregulated in mononuclear cells of MG patients.¹⁷ Serum specimens from patients with MG contain increased levels of TNF- α .¹⁸ Cultivation of mononuclear cells of MG patients with autoantigen induced elevation of TNF- α mRNA expressing monocytes.¹⁹

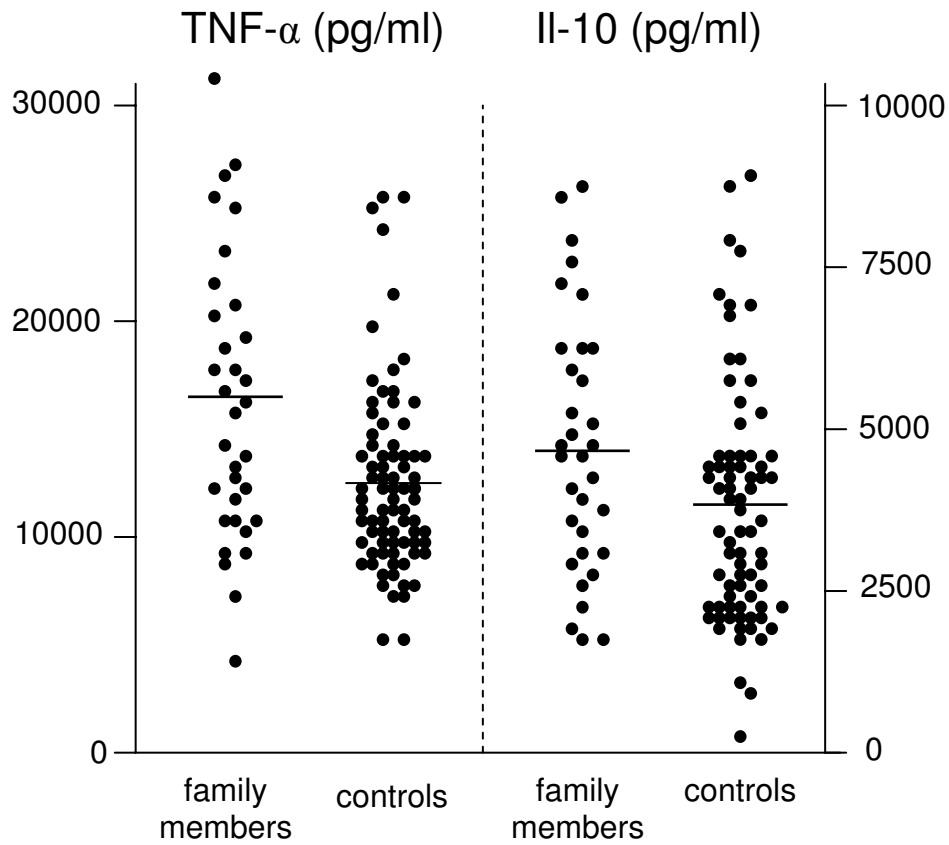


Figure 1. Production of tumour necrosis factor- α (TNF- α) and interleukin-10 (IL-10) in first-degree family members of patients with the Lambert-Eaton myasthenic syndrome and healthy controls. The horizontal lines represent the mean production for each group.

While the genetic basis of the production level of IL-10 and TNF- α is unknown, our functional assay performed in family members is the best measure of innate production of these cytokines in patients with LEMS themselves. Using this assay, we showed that innate production of IL-10 and TNF- α is a susceptibility factor for non-paraneoplastic LEMS.

Acknowledgements

M. Kersbergen and M. van Schie (Leiden University Medical Centre) are gratefully thanked for doing the whole-blood stimulations. B. Lang, (Oxford, United Kingdom) is acknowledged for performing the anti-VGCC antibody assay. H. Putter (Leiden University Medical Centre) is thanked for the statistical analysis. This study was supported by the Prinses Beatrix Fonds.

References

1. O'Neill, JH, Murray, NM, Newsom-Davis, J. The Lambert-Eaton myasthenic syndrome. A review of 50 cases. *Brain* 1988;111:577-596.
2. Willcox, N., Demaine, A.G., Newsom-Davis, J., Welsh, K.I., Robb, S.A., Spiro, S.G. Increased frequency of IgG heavy chain marker Glm(2) and of HLA-B8 in Lambert-Eaton myasthenic syndrome with and without associated lung carcinoma. *Hum Immunol* 1985;14: 29-36.
3. Parsons, KT, Kwok, WW, Gaur, LK, Nepom, GT. Increased frequency of HLA class II alleles DRB1*0301 and DQB1*0201 in Lambert-Eaton myasthenic syndrome without associated cancer. *Hum Immunol* 2000;61:828-833.
4. Wirtz, PW, Roep, BO, Schreuder, GM, van Doorn, PA, van Engelen, BG, Kuks, JB, Twijnstra, A, de Visser, M, Visser, LH, Wokke, JH, Wintzen, AR, Verschuuren, JJ. HLA class I and II in Lambert-Eaton myasthenic syndrome without associated tumor. *Hum Immunol* 2001;62:809-813.
5. Rousset, F, Garcia, E, Defrance, T, Peronne, C, Vezzio, N, Hsu, DH, Kastelein, R, Moore, KW, Banchereau, J. Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. *Proc Natl Acad Sci USA* 1992;89:1890-1893.
6. Westendorp, RG, Langermans, JA, Huizinga, TW, Elouali, AH, Verweij, CL, Boomsma, DI, Vandenbrouke, JP. Genetic influence on cytokine production and fatal meningococcal disease. *Lancet* 1997;349:170-173.
7. Park, YB, Lee, SK, Kim, DS, Lee, J, Lee, CH, Song, CH. Elevated interleukin-10 levels correlated with disease activity in systemic lupus erythematosus. *Clin Exp Rheumatol* 1998;16:283-288.
8. Rowland, TL, McHugh, SM, Deighton, J, Dearman, RJ, Ewan, PW, Kimber, I. Differential regulation by thalidomide and dexamethasone of cytokine expression in human peripheral blood mononuclear cells. *Immunopharmacology* 1998;40:11-20.
9. Reuss, E, Fimmers, R, Kruger, A, Becker, C, Rittner, C, Hohler, T. Differential regulation of interleukin-10 production by genetic and environmental factors - a twin study. *Genes Immun* 2002;3:407-413.
10. AAEM Quality Assurance Committee. American Association of Electrodiagnostic Medicine. Practice parameter for repetitive nerve stimulation and single fiber EMG evaluation of adults with suspected myasthenia gravis or Lambert-Eaton myasthenic syndrome: summary statement. *Muscle Nerve* 2001;24:1236-1238.
11. Beekman, R, Kuks, JB, Oosterhuis, HJ. Myasthenia gravis: diagnosis and follow-up of 100 consecutive patients. *J Neurol* 1997;244:112-118.
12. Motomura, M, Johnston, I, Lang, B, Vincent, A, Newsom-Davis, J. An improved diagnostic assay for Lambert-Eaton myasthenic syndrome. *J Neurol Neurosurg Psychiatry* 1995;58:85-87.
13. Naipal, AM, D'Amaro, J, Bruning, JW, van Leeuwen, A, van Rood, JJ. Automated reading of propidium iodide lymphocytotoxicity test for HLA-DR, MB, MT testing. *Tissue Antigens* 1984;24:302.

14. Van der Linden, MW, Huizinga, TW, Stoeken, TJ, Sturk, A, Westendorp, RG. Determination of tumour necrosis factor- α and interleukin-10 production in a whole blood stimulation system: Assessment of laboratory error and individual variation. *J Immunol Methods* 1998;218:63-71.
15. Van der Linden, MW, Westendorp, RG, Sturk, A, Bergman, W, Huizinga, TW. High interleukin-10 production in first-degree relatives of patients with generalized but not cutaneous lupus erythematosus. *J Investig Med* 2000;48:327-334.
16. De Jong, BA, Schrijver, HM, Huizinga, TW, Bollen, EL, Polman, CH, Uitdehaag, BM, Kersbergen, MC, Sturk, A, Westendorp, RG. Innate production of interleukin-10 and tumor necrosis factor affects the risk of multiple sclerosis. *Ann Neurol* 2000;48:641-646.
17. Matusevicius, D, Navikas, V, Palasik, W, Pirskanen, R, Fredrikson, S, Link, H. Tumor necrosis factor-, lymphotoxin, interleukin (IL)-6, IL-10, IL-12 and perforin mRNA expression in mononuclear cells in response to acetylcholine receptor is augmented in myasthenia gravis. *J Neuroimmunol* 1996;71:191-198.
18. Ahlberg, RE, Pirskanen, R, Lefvert, AK. Defective T lymphocyte function in nonthymectomized patients with myasthenia gravis. *Clin Immunol Immunopathol* 1991;60:93-105.
19. Link, J, He, B, Navikas, V, Palasik, W, Fredrikson, S, Soderstrom, M, Link, H. Transforming growth factor-beta 1 suppresses autoantigen-induced expression of pro-inflammatory cytokines but not of interleukin-10 in multiple sclerosis and myasthenia gravis. *J Neuroimmunol* 1995;58:21-35.

Chapter 9