

Effective humoral immunity against diphtheria and tetanus in patients with systemic lupus erythematosus or myasthenia gravis

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ABSTRACT

Introduction: Controversy exists about the effectiveness of vaccine-induced immune response in patients with immunoregulatory disorders. Our aim was to determine the antibody titers to diphtheria and tetanus in patients with either of two autoimmune diseases.

Methods: 279 patients with SLE (205 females, aged 45.0 ± 13.8 years), 158 patients with myasthenia gravis (MG) (101 females, aged 55 ± 18.7 years) and 208 healthy subjects (122 females, aged 48 ± 14.6 years) were enrolled. Serum concentrations of diphtheria-antitoxin-IgG (A-DIPHTH) and tetanus-antitoxin-IgG (A-TET) were determined with ELISA.

Results: Equal proportions of healthy subjects, as well as patients with SLE or MG exhibited proper antibody responses and immune protection against diphtheria and tetanus. In all three test groups, serum concentration of A-DIPHTH decreased significantly ($p < 0.001$) with age throughout the study population, while titers of A-TET dropped only in the elderly (>60-years-old) subjects. There were no significant differences among the groups in the age-related changes of A-TET and A-DIPHTH except that in <40-years-old subjects, A-DIPHTH level was significantly ($p = 0.029$) lower in SLE patients than in controls.

Conclusions: Our findings suggest that the level of vaccine-induced immunity against diphtheria and tetanus infections in patients with SLE or MG is comparable to the healthy population.

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1. Introduction

Patients with immunoregulatory disorders are at an increased risk of infection, due to the immune abnormalities associated with the autoimmune disease itself (e.g. leukopenia in patients with SLE), or to immunosuppressive therapy (Meyer-Olson et al., 2011). The widespread and common usage of immunosuppressive drugs and biological therapies increases the number of immunocompromised individuals. Despite this fact, much controversy exists about vaccination strategies in patients with autoimmune disease, and studies regarding vaccine-induced immunity in such patients are scarce (Bijl et al., 2011). Vaccination against diphtheria and tetanus

– two of the longest known vaccine-preventable diseases – is an absolute necessity for everyone, especially for the elderly who are often inadequately immunized in many countries.

In previous controlled studies, the response to tetanus toxoid was similar in patients with systemic lupus erythematosus (SLE), and in healthy controls. (Abe and Homma, 1971; Kashef et al., 2008) Likewise, in a large, uncontrolled study of 73 SLE patients, >90% of subjects achieved protection after vaccination (Battafarano et al., 1998). Recently, however, two groups presented different results analyzing the efficacy of vaccination in SLE (Marchand-Janssen et al., 2011; Miyamoto et al., 2011). Miyamoto et al. described significantly lower levels of tetanus antibodies in SLE patients than in controls. (Miyamoto et al., 2011) In their study conducted in 186 patients with systemic inflammatory and/or autoimmune diseases, Marchand-Janssen et al. concluded that these patients were at risk of vaccine-preventable illnesses (Marchand-Janssen et al., 2011). Owing to the apparent contradiction between these and previous findings and the uncomparable patient groups, as well as in view of the high clinical importance of the conclusion, we deemed it necessary to determine the antibody levels to diphtheria and tetanus in SLE patients accurately. We performed our study in

Abbreviations: A-DIPHTH, diphtheria-antitoxin-IgG; A-TET, tetanus-antitoxin-IgG; anti-AChR, antibodies against the acetylcholine-receptor; MG, myasthenia gravis; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index.

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Hungary, where vaccination coverage against these infectious diseases is close to 100% among children and adults, based on WHO estimates. (WHO, 2012) We extended the study to patients with another autoantibody-mediated disease, myasthenia gravis (MG), an autoimmune disorder in which – to our knowledge – the efficiency of vaccination against diphtheria and tetanus has never been reported.

This complete DTP vaccination coverage and the high number of samples available from well-documented SLE and MG patients made it possible to analyze the humoral immunity of patients with autoimmune disorders, in comparison to a large number of healthy subjects.

2. Patients and methods

2.1. Patients and controls

Two hundred seventy-nine patients with SLE (205 females, aged 45.0 ± 13.8 years) and 158 patients with MG (101 females, aged 55 ± 18.7 years) were enrolled. Both diseases were diagnosed according to accepted clinical and laboratory criteria. (Hochberg, 1997; Angelini, 2011)

Two-hundred and eight healthy volunteers (122 females, aged 48 ± 14.6 years), attending a routine health check-up served as controls.

Serum samples from the patients and the controls were stored at -80°C . The study protocol had been approved by the competent Ethics Committee, and written informed consent was obtained from each patients and control subjects. The study was implemented in accordance with the Declaration of Helsinki.

2.2. Vaccination strategy against diphtheria and tetanus in Hungary

In Hungary, primary vaccination against DTP is mandatory during childhood. At the age of 3–5 months, three injections are given for the purpose of basic immunization. The first booster is administered at 3 years of age, and the second one at the age of six. At 11 years of age, children receive the third DT booster (Control, 2010).

2.3. Measurement of diphtheria-antitoxin-IgG and tetanus-antitoxin-IgG

The serum concentrations of diphtheria-antitoxin-IgG (A-DIPHTH) or tetanus-antitoxin-IgG (A-TET) were determined with the Diphtheria ELISA IgG Testkit and the Tetanus ELISA IgG Testkit

(Sekisui Virotech GmbH, Rüsselsheim, Germany), according to the manufacturer's instructions. Concentrations were expressed as International Units (IU/ml) in conformity with the relevant WHO standards. According to the test instructions, humoral immunity against diphtheria or tetanus is indicated by a ≥ 0.1 IU/ml concentration of diphtheria- or tetanus-antitoxin-IgG.

2.4. Quantitation of antibodies against acetylcholine-receptor

Antibodies against the acetylcholine-receptor (anti-AchR) were measured using anti-AChR antibody radioimmunoassay (IBL GmbH, Hamburg, Germany), according to the manufacturer's instructions. The cut-off value for this assay was 0.4 nM.

2.5. Statistical analysis

Statistical calculations were performed with the Graph-Pad Prism software (v4.02, GraphPad Software Inc., San Diego, CA, www.graphpad.com). We calculated the differences of antibody titers between the groups of patients and healthy subjects using Mann-Whitney's *t*-test, and the differences in immune protection with the Chi-square test. In all tests, $p < 0.05$ was considered statistically significant.

3. Results

3.1. Comparison of vaccine-induced protection against diphtheria or tetanus in the study groups

We determined the effectiveness of vaccine-induced immunity in 208 healthy controls, in 279 patients with SLE, and in 158 patients with MG as advised by the test kits' instructions. Analyzing A-DIPHTH levels, there was no difference between healthy subjects and SLE patients (0.14 [0.06–0.33] vs. 0.13 [0.06–0.32], $p = 0.88$), and only a slight difference could be detected in comparison to MG patients (0.14 [0.06–0.33] vs. 0.10 [0.04–0.29], $p = 0.02$) (Fig. 1). Making the same comparisons with A-TET concentrations revealed no difference between healthy subjects (1.01 [0.37–2.49]), and patients with SLE (0.98 [0.38–1.93], $p = 0.56$) or MG (1.00 [0.38–2.65], $p = 0.73$) (Fig. 1).

Thereafter we compared the percentage of protected subjects in the three groups. Subjects with an A-DIPHTH titer of < 0.1 IU/ml were considered unprotected, whereas those with a titer of ≥ 0.10 IU/ml were regarded as protected against diphtheria. We applied the same limit for distinguishing between non-protection/protection against tetanus (Table 1).

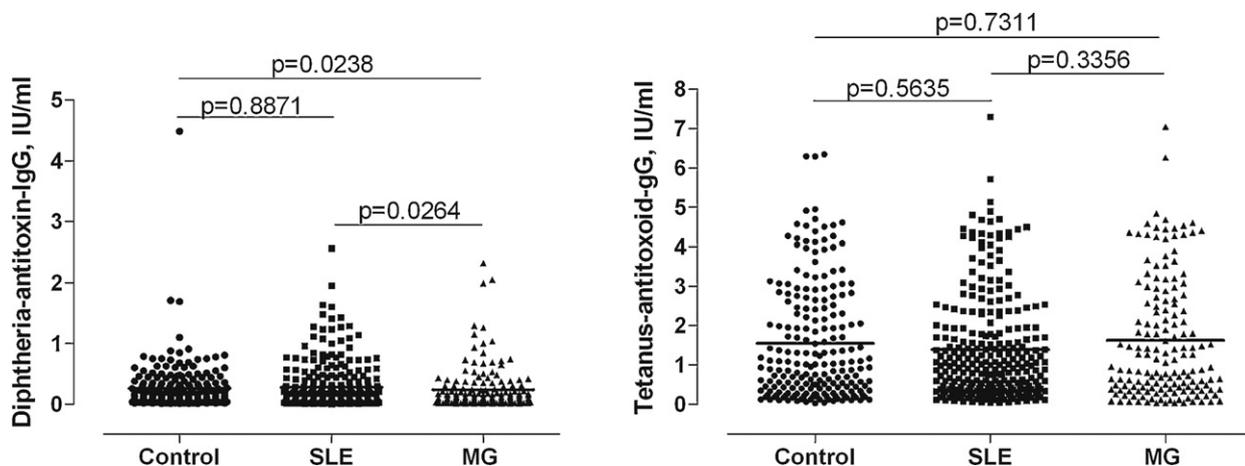


Fig. 1. The concentration of diphtheria-antitoxin-IgG and of tetanus-antitoxin-IgG in the sera of healthy subjects and of patients with SLE or MG.

Table 1

The distribution of vaccine-induced immune protection among healthy subjects and in patients with SLE or MG.

	Vaccine-induced protection against diphtheria			Vaccine-induced protection against tetanus		
	Unprotected <0.1 IU/mL diphtheria-antitoxin-IgG	Protected \geq 0.1 IU/mL diphtheria-antitoxin-IgG	p-Value (χ^2 test)	Unprotected <0.1 IU/mL tetanus-antitoxin-IgG	Protected \geq 0.1 IU/mL tetanus-antitoxin-IgG	p-Value (χ^2 test)
Healthy controls <i>n</i> = 208	81 (38.9%)	127 (61.1%)	<i>p</i> = 0.1863	7 (3.4%)	201 (96.6%)	<i>p</i> = 0.0335
Patients with SLE <i>n</i> = 279	110 (39.4%)	169 (60.6%)		9 (3.2%)	270 (96.8%)	
Patients with MG <i>n</i> = 158	75 (47.5%)	83 (52.5%)		13 (8.2%)	145 (91.8%)	

Table 2

The titers of diphtheria-antitoxin-IgG in various age-groups. Titers are expressed as U/mL with medians (IQ ranges).

Age-groups	Control subjects	MG patients	SLE patients	Total
<30	0.45 (0.25–0.75)	0.4 (0.18–0.89)	0.33 (0.12–0.82)	0.43 (0.18–0.81)
30–39	0.33 (0.14–0.47)	0.205 (0.07–0.49)	0.18 (0.10–0.39)	0.23 (0.11–0.43)
40–49	0.15 (0.07–0.30)	0.165 (0.07–0.22)	0.13 (0.07–0.19)	0.14 (0.07–0.25)
50–59	0.08 (0.04–0.17)	0.135 (0.05–0.31)	0.07 (0.04–0.18)	0.08 (0.04–0.18)
60–69	0.07 (0.05–0.17)	0.05 (0.02–0.20)	0.08 (0.03–0.25)	0.07 (0.03–0.19)
\geq 70	0.045 (0.03–0.14)	0.04 (0.02–0.16)	0.12 (0.04–0.50)	0.05 (0.03–0.17)

Immune protection against diphtheria was observed in 61.1% of healthy controls, in 60.6% of SLE patients, and in 52.5% of MG patients, whereas the proportions of patients protected against tetanus were 96.6%, 96.8%, and 91.8%, respectively (Table 1). The differences among the three groups were not significant.

3.2. Titers of diphtheria-antitoxin-IgG and tetanus-antitoxin-IgG among age-groups

In all three test groups, the serum concentration of A-DIPHTH significantly ($p < 0.001$) decreased with age throughout the study population (Table 2), while that of A-TET dropped only in elderly (>60-years-old) subjects (Table 3). We found age-related differences in the <40-years-old subjects as regards A-DIPHTH levels, which were significantly ($p = 0.029$) lower in SLE patients than in controls. However, no significant difference was found in \geq 40-years-old subjects (Table 2). The levels of these antibodies were about the same in MG patients and in control subjects, regardless of age. Similarly, no significant difference was observed among SLE patients, MG patients, and controls – their age notwithstanding – in the concentration of A-TET (Table 3).

3.3. Clinical correlations

In patients with SLE, we studied the relationship between the disease activity index (SLEDAI), as well as main organ involvement on one hand, and the serum concentration of the A-DIPHTH and A-TET antibodies on the other. SLEDAI values did not correlate significantly with A-DIPHTH and A-TET antibody titers (Spearman's correlation coefficient; $R = 0.0650$, $p = 0.321$ and $R = 0.013$, $p = 0.828$; respectively). Patients with SLEDAI in the highest quartile (≥ 8) had the same level of A-DIPHTH and A-TET antibodies as the rest of the patients (Mann-Whitney test; $p = 0.963$ and $p = 0.435$, respectively).

We did not find any difference between patients with diverse organ manifestations and concentration of A-DIPHTH (Kruskal-Wallis test, $p = 0.8929$) or A-TET ($p = 0.4193$) antibody titers.

In the group of patients with myasthenia gravis, there was no significant difference between patients with or without detectable anti-acetylcholine-receptor antibodies either in the titers of A-DIPHTH (Mann-Whitney test, $p = 0.196$) or those of A-TET ($p = 0.112$) antibodies.

4. Discussion

In our present study, we did not find any difference between healthy subjects and patients with SLE by comparing their levels of diphtheria- or tetanus-antitoxin-IgG, and only a slight difference could be demonstrated in comparison to MG patients. Moreover, not only SLE patients, but also patients with MG exhibited a comparable level of protection against both diphtheria and tetanus as the healthy control subjects. The similar median age of the three study groups, as well as the lack of significant differences among them as regards the age-related changes of the antibodies (except A-DIPHTH antibody levels in younger age) also seem to support the validity of these findings.

Our findings (based on more homologous patient groups) may complement those of two recently published papers, the authors of which reported impaired vaccine-induced immunity in autoimmune diseases. Miyamoto et al. (2011) reported decreased tetanus antibody levels in 30 SLE patients, as compared to 14 age-matched healthy subjects. Their study, however, recruited only children and adolescents, and the study population was very small. On the other hand, we found a similar observation: significantly lower A-DIPHTH antibody concentration was observed in young (<40 years old) SLE patients as compared to the healthy controls of the same age, although the findings of the Japanese group for A-TET antibodies

Table 3

The titers of tetanus-antitoxin-IgG in various age-groups. The titers expressed as U/mL, with medians (IQ ranges).

Age-groups	Control subjects	MG patients	SLE patients	TOTAL
<30	1.05 (0.39–2.13)	3.08 (1.47–4.31)	1.275 (0.76–1.91)	1.42 (0.64–3.08)
30–39	0.93 (0.43–2.97)	0.825 (0.36–2.80)	0.81 (0.33–1.59)	0.83 (0.37–2.35)
40–49	1.78 (0.52–2.98)	0.8 (0.54–1.56)	1.06 (0.51–2.41)	1.09 (0.53–2.69)
50–59	1.33 (0.34–2.65)	2.065 (0.37–3.06)	0.98 (0.48–1.93)	1.23 (0.46–2.39)
60–69	0.795 (0.25–1.61)	0.69 (0.38–1.40)	0.53 (0.24–2.50)	0.69 (0.25–1.69)
\geq 70	0.33 (0.12–1.42)	0.93 (0.23–1.73)	0.47 (0.07–1.72)	0.5 (0.12–1.585)

were not reproduced. In their study of 186 patients with systemic inflammatory and/or autoimmune diseases (mean age 51 years), Marchand-Janssen et al. (2011) concluded that these patients are at risk of vaccine-preventable illnesses, as vaccination coverage was only 29% for diphtheria and 48% for tetanus – that is, much lower than in our study (100%). Moreover, their study population was very heterogeneous in terms of autoimmune disorders, and they did not compare the antibody titers of the patients to those of healthy controls. In a Chinese study of 210 healthy individuals, anti-diphtheria antibody concentration was ≥ 0.1 IU/mL in 76.6% of the subjects and < 0.1 IU/mL in the remaining 23.3%. This shows a somewhat higher rate of protection against diphtheria, as compared to our population (61.1% protected vs. 38.9% not protected) (Zhang et al., 2011). In accordance with the findings of this study and of previous studies (Simonsen and Kristiansen, 1996; Matouskova and Matlerova, 2005), we also observed that antibody titers decreased with advancing age in adults, although a great proportion of cases still had a protective level of vaccine-induced immunity against diphtheria. Previous studies (Simonsen and Kristiansen, 1996; Matouskova and Matlerova, 2005) had estimated the decline of diphtheria and tetanus antitoxin concentrations at 5–10% per annum; our study also confirmed this rate.

In the study by Olander et al. in the Finnish population the geometric mean of tetanus antitoxin concentration declined to 0.06 IU/mL in older age groups. In the eldest age group (≥ 70 years of age), about 50% of the individuals were unprotected (< 0.01 IU/mL) (Olander et al., 2009). By contrast, according to the same criterion, all of our study subjects aged ≥ 70 years were protected against tetanus.

Our findings on the lack of any significant correlation between the activity index of organ manifestations in SLE patients and the levels of A-DIPHTH and A-TET antibodies indicate that the severity of the autoimmune disease does not influence the vaccine-induced immune response. Similarly, in MG patients we did not find any correlation between the hallmark of the disease, anti-acetylcholine receptor antibodies and the titers of vaccine-induced A-DIPHTH and A-TET antibodies.

To our knowledge, ours is the first study exploring humoral immunity against diphtheria and tetanus in patients with myasthenia gravis. Its strengths include the large numbers of healthy controls and patients with two antibody-mediated autoimmune diseases, SLE or MG, as this allowed analyzing the vaccine-induced humoral immune response accurately. Our findings suggest that the level of vaccine-induced immunity against diphtheria and tetanus in adults with either of the two studied autoimmune

diseases is comparable to that seen in the age-matched healthy population, at least in countries with a policy of mandatory vaccination.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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