ARTHRITIS & RHEUMATISM
Vol. 58, No. 12, December 2008, pp 3873–3883
DOI 10.1002/art.24027

Spliceosomal Peptide P140 for Immunotherapy of Systemic Lupus Erythematosus

Results of an Early Phase II Clinical Trial

Sylviane Muller,[1] Fanny Monneaux,[1] Nicolas Schall,[1] Rasho K. Rashkov,[2] Boycho A. Oparanov,[3] Philippe Wiesel,[4] Jean-Marie Geiger,[5] and Robert Zimmer[5]

Objective. **To assess the safety, tolerability, and efficacy of spliceosomal peptide P140 (IPP-201101; sequence 131–151 of the U1-70K protein phosphorylated at Ser^{140}), which is recognized by lupus CD4+ T cells, in the treatment of patients with systemic lupus erythematosus (SLE).**

Methods. **An open-label, dose-escalation phase II study was conducted in two centers in Bulgaria. Twenty patients (2 male and 18 female) with moderately active SLE received 3 subcutaneous (SC) administrations of a clinical batch of P140 peptide at 2-week intervals. Clinical evaluation was performed using approved scales. A panel of autoantibodies, including antinuclear antibodies, antibodies to extractable nuclear antigens (U1 RNP, SmD1, Ro/SSA, La/SSB), and antibodies to double-stranded DNA (anti-dsDNA), chromatin, cardiolipin, and peptides of the U1-70K protein, was tested by enzyme-linked immunosorbent assay (ELISA). The plasma levels of C-reactive protein, total Ig, IgG, IgG subclasses, IgM, IgA, and IgE, and of the cytokines interleukin-2 and tumor necrosis factor α were measured by ELISA and nephelometry.**

Results. **IgG anti-dsDNA antibody levels decreased by at least 20% in 7 of 10 patients who received 3 × 200 μg IPP-201101 (group 1), but only in 1 patient in the group receiving 3 × 1,000 μg IPP-201101 (group 2). Physician's global assessment of disease activity scores and scores on the SLE Disease Activity Index were significantly decreased in group 1. The changes occurred progressively in the population of responders, increased in magnitude during the treatment period, and were sustained. No clinical or biologic adverse effects were observed in the individuals, except for some local irritation at the highest concentration.**

Conclusion. **IPP-201101 was found to be safe and well tolerated by subjects. Three SC doses of IPP-201101 at 200 μg significantly improved the clinical and biologic status of lupus patients.**

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease of multifactorial etiology. It is a prototypic autoimmune disease characterized by increased production of autoantibodies, immune complex deposition in the microvasculature, leukocyte infiltration, and, finally, progressive tissue damage in certain organs (1). The clinical course of the disease is episodic with unpredictable periods of activity flares. Current treatments of the disease are mainly based on immunosuppressive drugs such as corticosteroids and cyclophosphamide, which are often administered at high doses in

Clinical trial approval no. 143/14.06.2006 (Bulgaria).

Supported by ImmuPharma France, an affiliate of ImmuPharma PLC, as part of a full clinical development program.

[1]Sylviane Muller, PhD, Fanny Monneaux, PhD, Nicolas Schall, BSc: CNRS, Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France; [2]Rasho K. Rashkov, MD: Medical University Sofia, Sofia, Bulgaria; [3]Boycho A. Oparanov, MD: Medical Military Academy, Sofia, Bulgaria; [4]Philippe Wiesel, MD: Genexion SA, Geneva, Switzerland; [5]Jean-Marie Geiger, MD, PharmD, Robert Zimmer, MD, PhD: ImmuPharma France SA, Mulhouse, France.

Dr. Muller has received consulting fees, speaking fees, and/or honoraria from ImmuPharma (less than $10,000); she owns stock or stock options in ImmuPharma PLC and is coinventor of P140 peptide. Dr. Wiesel is Managing Director of Genexion SA; he owns stock or stock options in Genexion SA. Dr. Geiger is Vice President of clinical development with ImmuPharma; he owns stock or stock options in ImmuPharma PLC. Dr. Zimmer is President and Chief Scientific Officer of ImmuPharma PLC; he owns stock or stock options in ImmuPharma PLC.

Address correspondence and reprint requests to Sylviane Muller, PhD, Institut de Biologie Moléculaire et Cellulaire, UPR9021, Centre National de la Recherche Scientifique, 15 Rue René Descartes, 67000 Strasbourg, France. E-mail: S.Muller@ibmc.u-strasbg.fr.

Submitted for publication May 13, 2008; accepted in revised form August 1, 2008.

acute exacerbation phases (1–3). Although these treatments have significantly reduced mortality and lengthened patients' life expectancies, they have important side effects, particularly when they are applied for long-term management of disease. Adverse effects such as obesity, diabetes mellitus, hyperlipidemia, and hypertension are reversible and generally improve after reducing the corticoid dosage. These drugs may also contribute to late irreversible complications such as bone marrow depression, ovarian failure, or enhanced risk of bladder cancer. To avoid such important side effects, we focus our efforts on the development of alternative therapeutic strategies, which are specific, targeted, and less toxic.

An epitope has been identified that is present in residues 131–151 of the 70K spliceosomal protein within the U1 small nuclear RNP (the U1-70K spliceosomal protein) and that is recognized very early by IgG antibodies and CD4+ lymph node (LN) T cells from both H-2^k MRL/*lpr* and H-$2^{d/z}$ (NZB × NZW)F_1 lupus-prone mice (4,5). It was shown further that an analog of this sequence, phosphorylated on Ser^{140} (named peptide P140), was also recognized by LN and peripheral CD4+ T cells as well as by IgG antibodies from MRL/*lpr* mice (6,7). In a therapeutic protocol in which peptides were administered intravenously (IV) in saline (three injections at 2-week intervals and a fourth injection 1 month later), the P140 peptide, but not the cognate nonphosphorylated peptide 131–151, significantly reduced proteinuria and anti–double-stranded DNA (anti-dsDNA) IgG antibody levels, delayed mortality, and enhanced the survival rate of treated MRL/*lpr* mice (6). Unit peptide doses of 100 μg and 50 μg were effective, whereas the effectiveness of the 25-μg dose was similar to that of saline (Monneaux F, Muller S: unpublished observations).

Peptide 131–151, the sequence of which is completely conserved in mice and humans, was able to induce ex vivo the proliferation of CD4+ T cells from lupus patients (8). Interestingly, however, when lupus patients' peripheral CD4+ T cells were incubated in the presence of the P140 analog, the phosphorylation of Ser^{140} prevented the proliferation while favoring the secretion of high levels of interleukin-10 (IL-10).

Although the precise mode of action of P140 peptide is not fully understood, repeated administration of P140 peptide in saline into preautoimmune MRL/*lpr* mice transiently abolishes both T cell intramolecular spreading to other regions of the U1-70K protein (7) and T cell intermolecular spreading to regions of other spliceosomal proteins such as SmD1 (9). These important observations suggest that the P140 analog might initiate a mechanism of so-called tolerance spreading (10) and reorient, at least transiently, the deleterious autoimmune response by a mechanism that remains to be fully elucidated.

The recognition of P140 or peptide 131–151 by T cells from patients with other autoimmune diseases (rheumatoid arthritis, primary Sjögren's syndrome, polymyositis, primary biliary cirrhosis, autoimmune hepatitis) could not be demonstrated (8). No increase in IL-10 was observed. Further, it was demonstrated that P140-treated and untreated lupus mice behaved similarly when infected by a flu virus (9). This indicates that the tolerogenic effect of P140 peptide was limited to certain autoreactive T cell clones and that the overall immune system was maintained unaffected. The present study was designed to examine the safety, tolerability, and efficacy of peptide P140 (IPP-201101) in lupus patients.

PATIENTS AND METHODS

Study design. This phase II study was an open-label, dose-escalation, add-on study in two centers in Bulgaria including 20 patients with moderately active SLE who received 3 subcutaneous (SC) administrations of IPP-201101 2 weeks apart. In addition to standard care, patients received 3 × 200 μg of IPP-201101 (group 1) or 3 × 1,000 μg of IPP-201101 (group 2). The primary objective was to evaluate the effect of treatment on anti-dsDNA antibodies in the plasma of lupus patients. In this initial phase II study, a positive response was defined as a decrease in levels of anti-dsDNA antibodies by 20% compared with the respective baseline level in each patient enrolled in the study. Secondary objectives were to ascertain the effectiveness of IPP-201101 on other biologic parameters as well as on the clinical signs of lupus patients as determined by the SLE Disease Activity Index (SLEDAI) (11). The physician's global assessment of disease activity was also recorded using a 100-mm visual analog scale. Values are expressed in length (0–25 mm = no active disease, >25–50 mm = mild disease, >50–75 mm = moderate disease, >75–100 mm = severe disease). Detailed informed consent was obtained from all patients in accordance with the Declaration of Helsinki, the International Conference of Harmonization guideline for Good Clinical Practice, and application of local regulations.

Patients. Male and female subjects (age 18–70 years) with an established diagnosis of SLE according to American College of Rheumatology (ACR) classification criteria (12) and with elevated titers of anti-dsDNA antibodies (>50 IU/ml at screening visit) were eligible. If subjects were receiving oral corticosteroids, the daily dose could not exceed 10 mg of prednisolone or equivalent, the start date had to be at least 3 months prior to the start of study treatment, and the daily dose had to be stable during the 4 weeks preceding the start of study treatment. If subjects were receiving antimalarials, methotrexate, or azathioprine, the start date had to be at least 3 months prior to the start of study treatment, and the daily dose had to be stable during the 4 weeks preceding the start of study treatment.

Patients were excluded if they were undergoing a flare

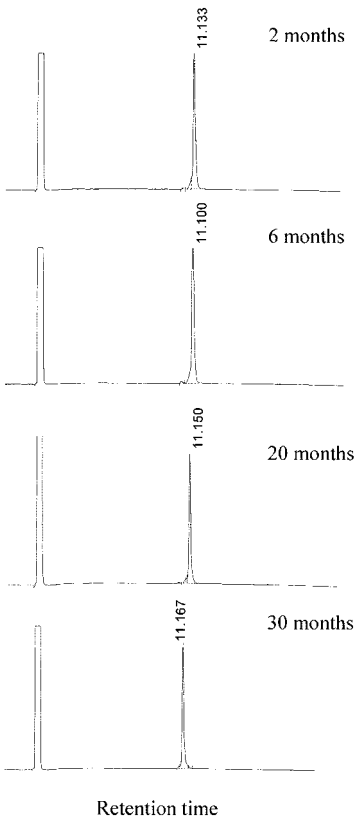

Figure 1. Shelf life of P140 peptide. Each batch of P140 peptide was stored lyophilized at −20°C in glass tubes. Peptide integrity was checked by analytic reverse-phase high-performance liquid chromatography on a Beckman Coulter instrument using a Nucleosil C18 5-μm column (150 × 4.6 mm). Linear gradients detected at 215 nm were 5–65% solvent B (acetonitrile/0.08% trifluoroacetic acid). Retention time is indicated in minutes.

of disease activity (SLEDAI score >15) requiring treatment with immunosuppressive drugs, as were patients with severe central nervous system, hematologic, cardiac, or renal manifestations of SLE, patients with cytopenia with hemoglobin <7.5 gm/dl, white blood cell (WBC) count <2,000/ml, and/or platelet count <50,000/ml, and patients undergoing hemodialysis. We also excluded patients who were previously being treated with immunosuppressives (cyclophosphamide, mycophenolate mofetil, rituximab, or any other immunosuppressive drug) within 4 months of the start of study treatment as well as patients with any concomitant medical condition which, in the opinion of the investigator, might have interfered with the safety or with the evaluation of the study.

Investigational drug. The peptide IPP-201101 was synthesized by NeoMPS (Strasbourg, France) and manufactured by Innotech Labor (Basel, Switzerland), all in compliance with current Good Manufacturing Practice conditions. It is soluble in water (>25 gm/liter), 5% (weight/volume) sucrose (12 gm/liter), and saline (between 0.5 gm/liter and 1 gm/liter). Several stability studies have been performed with different batches of P140 peptide. Peptide integrity was checked by analytic reverse-phase high-performance liquid chromatography on a Beckman Coulter (Roissy, France) instrument. The linear gradient was from 5% to 65% solvent B (acetonitrile/0.08% trifluoroacetic acid). Data have shown that the shelf life of the product is at least 30 months (Figure 1). Prior to administration to patients, a subchronic toxicity study was conducted in rats and dogs under standard Good Laboratory Practice conditions. No adverse reactions were recorded, and the no observable effects limit level was set at >1 mg/kg body weight. Segment 2 studies were also performed in rats and rabbits. No deleterious events were recorded. The total exposure during this study was equivalent to 130 years of the anticipated human therapeutic exposure on a body weight basis.

Measurements. Patients were assessed for adverse effects. The complete blood cell counts were checked, as were chemistry parameters (blood/urine), blood pressure, heart rate, body temperature, and electrocardiographic parameters. The same laboratory carried out the serologic measurements performed with the samples obtained from patients in groups 1 and 2. Anti-dsDNA antibodies were determined by enzyme-linked immunosorbent assay (ELISA) (Varelisa and EliA; Pharmacia, Uppsala, Sweden). The total levels of Ig as well as the individual levels of IgG, IgG subclasses (from IgG1 to IgG4), and IgE were measured by ELISA. A nephelometric method was also used to measure IgG, IgM, and IgA levels (MININEPH Human IgG, IgM, IgA kits; The Binding Site, Birmingham, UK). The serum samples were tested by ELISA for antinuclear antibodies (ANAs) (ReCombi ANA Screen

Table 1. Characteristics of the SLE patients at baseline*

Characteristic	Group 1 (n = 10)†	Group 2 (n = 10)†
Men/women	2/8	0/10
Age, mean years	32.6	37
Weight, mean kg	66.1	63.0
BMI, mean kg/m^2	23.6	22.0
Malar rash	3	5
Discoid rash	1	1
Photosensitivity	9	8
Oral ulcers	1	2
Arthritis	9	10
Serositis	1	2
Renal disorders	0	3
Neurologic disorder	0	3
Hematologic disorder	3	3
Immunologic disorder	10	10
ANAs	10	9
SLEDAI, mean score (0–94)	7.8	9.0
Disease duration, years		
Median	5	4.5
Mean (range)	6.5 (1–14)	5.9 (2–12)
Concomitant therapy for SLE		
MP	2	0
MP + AZA	2	0
MP + HCQ	5	8
HCQ	1	2

* Except where indicated otherwise, values are the number of patients. SLE = systemic lupus erythematosus; BMI = body mass index; ANAs = antinuclear antibodies; SLEDAI = SLE Disease Activity Index; MP = methylprednisolone; AZA = azathioprine; HCQ = hydroxychloroquine.

† Patients in group 1 received 3 × 200 μg IPP-201101. Patients in group 2 received 3 × 1,000 μg IPP-201101.

Table 2. Evolution of anti-dsDNA antibody levels during the study period*

	Anti-dsDNA antibody levels, IU/ml					
	Day 1	Day 8	Day 15	Day 29	Day 43	Day 57
Patients receiving 200 μg IPP-201101						
1	141.0	176.8	140.3	158.2	162.1	192.8
2	51.4	37.1	31.2	28.6	31.3	36.1
3	316.6	318.3	237.0	251.1	237.0	343.5
4	121.0	115.7	107.8	70.1	79.4	64.6
5	178.2	138.5	149.8	123.3	129.6	140.8
6	161.6	154.0	138.8	142.8	102.1	114.3
7	138.9	136.4	121.8	109.5	122.4	125.7
8	60.3	55.9	52.2	51.7	49.2	51.2
9	156.2	132.6	132.4	138.4	124.0	144.1
10	138.9	127.0	106.4	87.2	80.7	78.1
Mean	146.4 (0.0)	139.2 (−6.6)†	121.8 (−17.0)‡	116.1 (−22.1)§	111.8 (−24.1)¶	129.1 (−15.8)#
Median	140.0	134.5	127.1	116.4	112.3	120.0
SD	72.7 (0.0)	75.9 (14.4)	56.1 (10.3)	63.3 (17.2)	59.0 (16.8)	89.5 (24.9)
Minimum	51.4	37.1	31.2	28.6	31.3	36.1
Maximum	316.6	318.3	237.0	251.1	237.0	343.5
SEM	23.0	24.0	17.7	20.0	18.6	28.3
Patients receiving 1,000 μg IPP-201101						
11	144.6	136.9	129.1	152.5	121.3	155.7
12	135.7	122.3	118.3	133.3	149.3	144.2
13	209.3	219.0	176.0	197.4	180.0	185.6
14	114.5	105.5	95.0	127.0	112.7	123.0
15	139.6	154.4	79.4	80.7	92.0	101.2
16	239.6	240.6	249.1	292.9	344.4	267.4
17	150.6	167.9	163.7	180.3	185.5	215.8
18	132.4	120.4	116.6	145.6	106.9	106.3
19	183.5	191.3	166.2	208.9	179.2	179.5
20	156.6	154.0	99.0	141.8	152.1	138.1
Mean	160.6 (0.0)	161.2 (−0.2)	139.2 (−14.5)**	166.0 (2.3)	162.3 (−1.3)	161.7 (0.4)
Median	147.6	154.2	123.7	149.1	150.7	150.0
SD	38.7 (0.0)	44.2 (7.9)	50.5 (15.8)	57.9 (18.8)	72.1 (22.5)	51.8 (19.9)
Minimum	114.5	105.5	79.4	80.7	92.0	101.2
Maximum	239.6	240.6	249.1	292.9	344.4	267.4
SEM	12.2	14.0	16.0	18.3	22.8	16.4

* Values in parentheses are the mean of differences, expressed as percent change versus day 1. In the group receiving 200 μg IPP-201101, there were 7 patients with a ≥20% decrease of anti–double-stranded DNA (anti-dsDNA) antibody levels on day 43 (patients 2–6, 9, and 10), termed responders. In the group receiving 1,000 μg IPP-201101, there was 1 responder (patient 15).
† $P = 0.1823$ versus day 1.
‡ $P = 0.0006$ versus day 1.
§ $P = 0.0028$ versus day 1.
¶ $P = 0.0014$ versus day 1.
# $P = 0.0767$ versus day 1.
** $P = 0.0176$ versus day 1.

Varelisa) and for antibodies to U1 RNP, SmD1, Ro/SSA, and La/SSB antigens (ReCombi ANA 4-Profile Varelisa). Specific ELISAs were used to measure levels of antibody to cardiolipin (antigen ref. C-0563; Sigma, St. Louis, MO), chromatin (QUANTA Lite; Inova Diagnostics, San Diego, CA), and peptides of the U1-70K protein (adapted for human sera [6]).

Plasma levels of C-reactive protein (CRP) were measured using a nephelometric method (MININEPH human C-reactive protein kit). Plasma levels of IL-2 and tumor necrosis factor α (TNFα) were measured by double-sandwich ELISA. Standard curves performed with known concentrations of cytokines (PharMingen, San Diego, CA) were used for the test calibration. In the test conditions, the minimal levels of detectable cytokines were 8 pg/ml IL-2 and 2 pg/ml TNFα.

Statistical analysis. Percent reduction in levels of anti-dsDNA antibodies compared with baseline was computed by subject and by study day. Student's *t*-test was applied to detect a positive mean reduction compared with baseline. The Mann-Whitney nonparametric test was used to evaluate the number of responders in the 2 groups of patients. *P* values less than 0.05 were considered significant.

RESULTS

Patient characteristics. The study population consisted of 20 white patients (2 male, 18 female) age >18 years (mean ± SD 34.8 ± 10.0 years, median 33.5

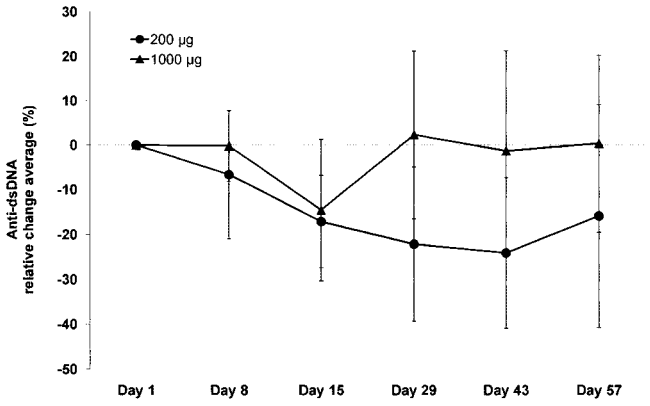

Figure 2. Evolution of anti–double-stranded DNA (anti-dsDNA) antibody levels during the study period. Administration of IPP-201101 was on days 1, 15, and 29. The results are expressed as the mean percent reduction with regard to baseline values (horizontal bar at the zero level). Error bars are shown.

years) with a mean ± SD body mass index of 22.8 ± 4.5 kg/m^2 (median 21.3). All patients met the ACR criteria for the classification of SLE (12) and had moderately active disease as defined by SLEDAI scores between 2 and 14 (Table 1). Duration of IPP-201101 treatment was 4 weeks (SC injections on days 1, 15, and 29), and followup visits were on days 8, 43, and 57. Long-term followup visits were at months 4, 5, and 6. The concomitant treatment received by patients in groups 1 and 2 is indicated in Table 1. There was no change in doses of medication for lupus disease during the study period (57 days).

Anti-dsDNA antibody levels. The efficacy objective of this open-label add-on phase II study was to examine the relationship between IPP-201101 treatment and biologic and clinical markers of disease activity. Specifically, the predefined primary efficacy end point was a significant reduction in anti-dsDNA antibody titers. Anti-dsDNA antibody levels, as measured by ELISA, decreased during the treatment period in group 1, with a nadir of 112 IU/ml on day 43 (Table 2). When expressed as the mean percent reduction from baseline, the changes in anti-dsDNA antibody levels seen in group 1 were time dependent and statistically significant (Table 2 and Figure 2). A 24% reduction was observed on day 43 ($P = 0.0014$ compared with day 1). Remarkably, a 17% decrease ($P = 0.0006$) was already observed after the first administration of IPP-201101. A more limited reduction was observed in group 2 (a transient but statistically significant decrease was observed on day 15 only) (Table 2). Thus, in the course of treatment with the 200 μg IPP-201101 dose, 7 of 10 patients had a reduction of at least 20% in anti-dsDNA antibody levels (Table 2) compared with only 1 of 10 patients in group 2 ($P < 0.03$).

Other immunologic parameters. During the observation period, total Ig (IgM, IgG, and IgA) and IgG levels slowly decreased in all group 1 patients except in patient 1, in whom the total Ig and IgG levels remained stable (not shown). A similar kinetic was observed when IgG1 levels were measured in the consecutive serum samples. In some patients, the level of Ig increased again after the last administration on day 43 and/or day 57. Good positive correlations ($R^2 = 0.752$, 0.733, 0.685, and 0.579) between the levels of IgG and anti-dsDNA antibody were observed in 4 patients (patients 2, 3, 6, and 9, respectively). IgM and IgA levels decreased slightly or showed no change during the study period. IgE levels remained very low and largely below the limit of positivity in all samples (not shown).

A panel of typical autoantibodies was measured in the samples. The levels of ANAs present in all 10 patients of group 1 did not change significantly during the study period except in patient 3 (Figure 3A). The levels of antibodies to U1 RNP and SmD1 present at low levels in 3 patients and 1 patient, respectively, also remained unchanged (Figures 3B and C). Antibodies to Ro (positive in 3 patients) and La (positive in 1 patient) also remained at their initial levels during the study period (Figures 3D and E). Antichromatin IgG antibody levels, which were high in 6 of 10 patients in group 1, slightly decreased with time or remained unchanged (Figure 4A). Anticardiolipin antibody levels were weakly positive in 3 of 10 patients and showed no significant fluctuation in these patients (Figure 4B). IgG antibodies to peptide 131–151 and P140 were absent on day 1 in the serum of our entire cohort, and their titers remained unchanged during the treatment period (not shown). Plasma levels of IL-2 remained below the threshold of detection during the entire study. TNFα levels remained ~15 pg/ml in all samples and were not different from those measured in normal individuals tested in parallel (not shown).

SLEDAI score and physician's global assessment of disease activity score. SLEDAI data were collected and assessed in order to explore the initial therapeutic profile of IPP-201101 in SLE patients. In this analysis, we discarded data from patient 13 (group 2), who had a low SLEDAI score of 2.0 at entry. Regarding the other patients (SLEDAI scores ≥6), the mean SLEDAI score was found to decrease from 7.8 to 4.8 in a progressive and sustained manner in the 200 μg dose group (group 1) during the course of the study, while it decreased only

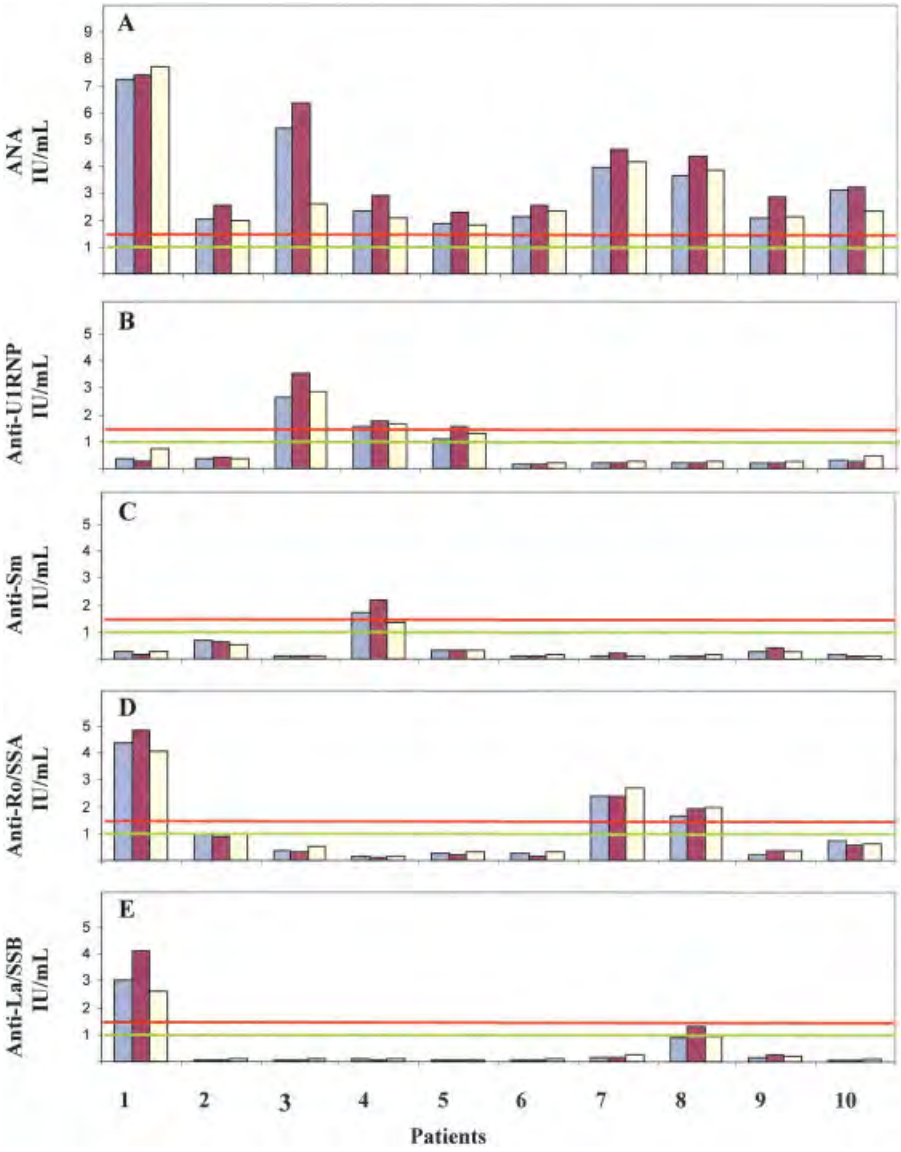

Figure 3. Evolution of levels of antinuclear antibodies (ANAs) (**A**) and of levels of anti–U1 RNP (**B**), anti-SmD1 (anti-Sm) (**C**), anti-Ro/SSA (**D**), and anti-La/SSB (**E**) antibodies in the 10 patients of group 1 during the study period. Administration of IPP-201101 was on days 1, 15, and 29; the 3 consecutive samples were obtained from each patient on days 1, 29, and 57 (gray, red, and yellow bars, respectively). An enzyme-linked immunosorbent assay (ELISA) was used to measure levels of ANAs (ReCombi ANA Screen Varelisa) and levels of antibodies to U1 RNP, SmD1, Ro/SSA, and La/SSB antigens (ReCombi ANA 4-Profile Varelisa). The lower horizontal line corresponds to the cutoff value of positivity <1.0 IU/ml); between the lower and upper horizontal lines are serum sample data with positivity considered equivocal (1.0 < value < 1.4) according to the ELISA manufacturer's recommendations.

slightly from 9.0 to 7.0 in the 1,000 μg dose group (group 2) (Table 3). When expressed as the mean percent reduction from baseline, these changes represented a 40% reduction at the end of the observation period (days 43 and 57) in group 1. In contrast, but in accordance with the data obtained for anti-dsDNA antibody titers, a more limited improvement in the SLEDAI score was observed in group 2 (19%). Similarly, the proportion of patients achieving a reduction of at least 4 points in the SLEDAI score was 60% (6 of 10 patients) and 44% (4 of 9 patients) in the 200 μg and 1,000 μg IPP-201101 dose groups, respectively (Table 3).

It is worth noting that 5 of 6 patients in group 1 showed both a decrease of anti-dsDNA antibody levels

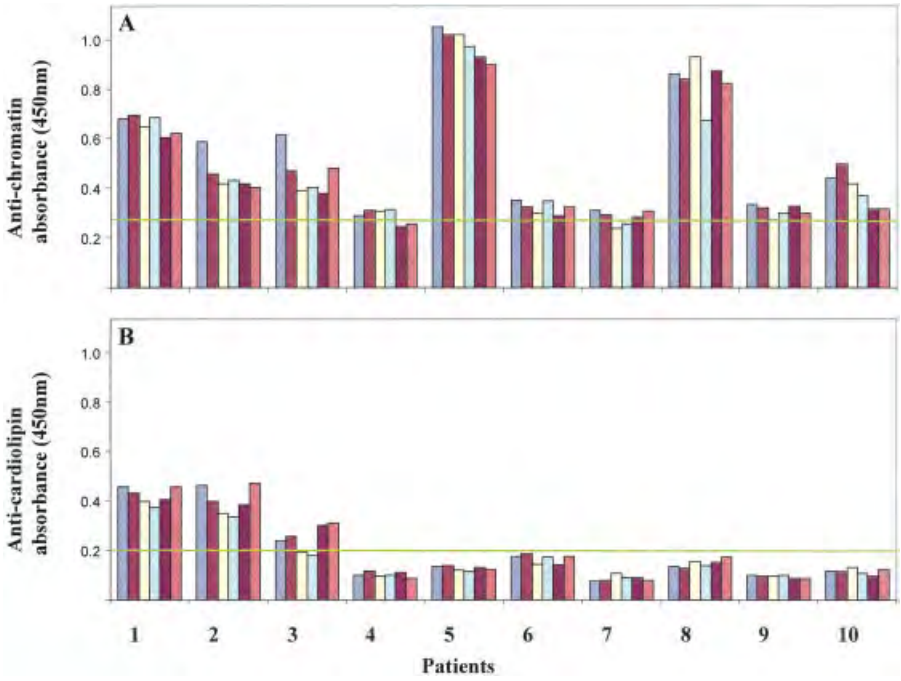

Figure 4. Evolution of antichromatin (**A**) and anticardiolipin (**B**) IgG antibody levels in the 10 patients of group 1 during the study period. Administration of IPP-201101 was on days 1, 15, and 29; the 6 consecutive samples were obtained from each patient on days 1, 8, 15, 29, 43, and 57 (gray, dark red, yellow, light green, dark red, light red bars, respectively). Antichromatin and anticardiolipin IgG levels were measured by enzyme-linked immunosorbent assay. The horizontal line corresponds to the cutoff value for positivity calculated using a series of 24 serum samples from normal individuals.

by at least 20% and a decrease in SLEDAI score by at least 4 points. The anti-dsDNA antibody level of the sixth "SLEDAI responder" of this group (patient 9) had decreased by 20.6% on day 43. The only anti-dsDNA responder in group 2 (patient 15) showed a 4-point decrease in the SLEDAI score. Altogether, the SLEDAI data were generally in accordance with the anti-dsDNA antibody data.

Changes in the physician's global assessment scores mirrored the changes observed for the SLEDAI score. In group 1, the physician's global assessment score decreased from 31.3 at baseline to 21.6 on days 43 and 57. In group 2, it was only mildly reduced, from 29.9 at baseline to 26.6 on day 57. When expressed as the mean percent reduction, the changes in physician's global assessment score were >30% in group 1 (31.9% and 34.4% on days 43 and 57, respectively). In group 2, a mild reduction of <15% was observed.

CRP levels. Plasma levels of CRP were measured by ELISA. As anticipated, the CRP data were characterized by marked variability among lupus patients and throughout the study period. We noticed that compared with baseline, median plasma levels of CRP decreased in both treatment groups on day 57, from 4.2 mg/dl to 2.2 mg/dl in group 1 and from 10.5 mg/dl to 1.9 mg/dl in group 2 (Table 4).

Safety. All patients included completed the study. No subjects discontinued study treatment prematurely due to adverse effects. There were no serious adverse effects reported during the course of the study. Overall, the incidence of nonserious adverse effects was 45% (9 subjects) experiencing 12 nonserious adverse effects during the course of the study. All 12 of the reported nonserious adverse effects were assessed as mild in severity, and the only drug-related event was a mild erythema at the site of injection which resolved within 10 minutes to 1 hour, according to individuals. In the 200 μg group, only 1 subject experienced such a mild erythema at the site of injection, compared with 6 subjects in the 1,000 μg group. Two subjects in the 1,000 μg group experienced nonserious adverse effects other than injection site reactions. There was 1 case of muscle pain and 1 case of nausea. A causal relationship to the test drug could not be established. No clinically significant changes in hematology (in particular, the WBC counts remained unchanged), blood chemistry, or urinalysis

Table 3. Evolution of SLEDAI scores during the study period*

	SLEDAI score					
	Day 1	Day 8	Day 15	Day 29	Day 43	Day 57
Patients receiving 200 μg IPP-201101						
1	6	6	6	6	6	6
2	6	6	6	6	6	6
3	6	6	2	2	2	2
4	10	10	10	10	10	10
5	10	10	2	10	2	2
6	6	6	6	6	2	2
7	14	10	10	10	10	10
8	6	6	6	6	2	2
9	6	6	2	2	2	2
10	8	6	6	6	6	6
Mean	7.8 (0.0)	7.2 (−5.4)	5.6 (−26.7)	6.4 (−18.7)	4.8 (−40.0)	4.8 (−40.0)
Median	6.0	6.0	6.0	6.0	4.0	4.0
SD	2.7 (0.0)	1.9 (11.3)	3.0 (32.7)	3.0 (27.6)	3.3 (32.7)	3.3 (32.7)
Minimum	6.0	6.0	2.0	2.0	2.0	2.0
Maximum	14.0	10.0	10.0	10.0	10.0	10.0
SEM	0.9	0.6	0.9	1.0	1.0	1.0
Patients receiving 1,000 μg IPP-201101						
11	14	14	10	10	10	10
12	6	6	6	2	2	2
13	2	2	2	2	2	2
14	6	6	6	6	6	6
15	14	14	10	10	10	10
16	6	6	6	6	6	6
17	10	10	10	10	10	10
18	12	12	12	12	10	6
19	8	8	8	8	8	8
20	12	12	12	10	10	10
Mean	9.0 (0.0)	9.0 (0.0)	8.2 (−5.7)	7.6 (−14.0)	7.4 (−15.7)	7.0 (−19.0)
Median	9.0	9.0	9.0	9.0	9.0	7.0
SD	4.0 (0.0)	4.0 (0.0)	3.2 (12.0)	3.5 (22.1)	3.3 (21.5)	3.2 (24.1)
Minimum	2.0	2.0	2.0	2.0	2.0	2.0
Maximum	14.0	14.0	12.0	12.0	10.0	10.0
SEM	0.9	0.6	0.9	0.9	1.0	1.0

* Values in parentheses are the mean of differences, expressed as percent change versus day 1. In the group receiving 200 μg IPP-201101, there were 6 patients with a decrease in the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score of ≥4 points during the study period (patients 3 and 5–9), termed responders. In the group receiving 1,000 μg IPP-201101, there were 4 responders (patients 11, 12, 15, and 18). Patient 13 was not evaluable. There was no statistically significant difference in the percent of responders between the 2 groups. The overall proportion of responders was significantly greater than 20% ($P < 0.002$).

were reported during the course of the study. No clinically significant changes in vital signs, including arterial blood pressure, pulse rate, and body temperature, were reported.

DISCUSSION

The observed safety and tolerability profile of IPP-201101 in 20 SLE subjects did not suggest any safety concern with the tested doses. Therefore, based on the results of this study, there are no data suggesting that IPP-201101 might be unsafe when administered to subjects with SLE. These findings confirmed the results of a phase I study in healthy volunteers and were in accordance with the results of preclinical animal toxicology studies (ImmuPharma data on file). Furthermore, it is worth noting that the peptide is not immunogenic when administered IV to mice (Monneaux F, Muller S: unpublished observations) or injected SC into humans (healthy volunteers and lupus patients).

The study achieved its primary efficacy end point, since IPP-201101 caused a statistically significant reduction in anti-dsDNA antibody titers. While this short-term study was not designed or powered to evaluate clinical efficacy, IPP-201101 was also found to cause a statistically significant reduction in the SLEDAI score. In certain patients, this feature was already noted after the first administration of IPP-201101. The level of CRP, a major acute-phase reactant, was also decreased in the plasma of treated patients of both groups. The levels of total Ig, IgG, and IgG1 remained constant or decreased

Table 4. C-reactive protein levels (mg/dl) by day of visit and treatment group

	Day 1	Day 29	Day 57
Patients receiving 200 μg IPP-201101			
Median	4.2	3.6	2.2
Mean	4.9	17.6	7.6
SD	4.9	40.3	8.8
Minimum	0.5	0.4	0.6
Maximum	16.2	131.5	22.7
SEM	1.5	12.8	2.8
Patients receiving 1,000 μg IPP-201101			
Median	10.5	13.7	1.9
Mean	8.7	16.3	8.9
SD	5.8	15.8	12.2
Minimum	0.2	3.7	0.5
Maximum	19.8	56.0	34.1
SEM	1.8	5.0	3.9

during the treatment period and then often increased again after the last administration. The same kinetics were observed when IgG antibodies to chromatin and cardiolipin were measured. The levels of ANAs and antibodies to U1 RNP, SmD1, Ro/SSA, and La/SSB (infrequent and present in low amounts in the samples from group 1) remained unchanged during the study period. None of the treated patients developed an IgE response during the study period. Finally, biologic and clinical results were in good concordance. Taken together, these data warrant the further evaluation of IPP-201101 in a placebo-controlled clinical study, and a phase IIb clinical trial has recently been initiated including 120 patients from Latin America and Europe.

The mechanism by which P140 peptide ameliorates characteristic SLE manifestations is currently under investigation. It has been observed that repeated administration into preautoimmune MRL/*lpr* mice of peptide P140 in saline transiently abolishes T cell intramolecular spreading to other regions of the U1-70K protein and to regions of spliceosomal U1-A, heterogeneous nuclear RNP A2, and SmD1 antigens (7,9). These findings suggest that the phosphorylated sequence 131–151 might initiate a mechanism of so-called "tolerance spreading" that leads to the beneficial effect observed in MRL/*lpr* mice, and possibly also in patients, after treatment with the peptide analog. Whether this occurs via anergy or deletion of autoimmune T cells, via antagonism or partial agonism of the T cell receptor (TCR), or via a direct or indirect effect on Treg cells, for example, is currently being investigated. The observation that the higher dose of IPP-201101 (3 × 1,000 μg) was less effective than the lower dose (3 × 200 μg) is also puzzling and needs to be examined further. It is possible that the bioavailability of IPP-201101 and/or its capacity to reach and selectively interact with specific targets or receptors differ according to its concentration. We might also argue that if IPP-201101 behaves like a partial agonist of the TCR, for example, its concentration can affect the quality of serial engagement of the TCR by peptide–major histocompatibility complex and further signaling and modulation (13).

Several successful attempts of peptide-based therapy have been described in the murine model of lupus. Some peptides corresponding to antibody idiotypes have been used with remarkable efficacy in (NZB × NZW)F_1 mice. Examples include the pCONS peptide, a consensus peptide derived from the V_H region of (NZB × NZW)F_1 IgG antibodies to DNA and predicted to possess T cell stimulatory activity (14,15), or peptides derived from the sequence of complementarity-determining regions (CDRs) 1 and 3 (pCDR1 and pCDR3) of a human anti-DNA monoclonal antibody that bears the so-called 16/6 idiotype (16). An impressive protective effect was also observed in MRL/*lpr* mice with a peptide identified using combinatorial chemistry approaches and able to interfere with Fcγ receptor recognition (17). For therapeutic application, this immunoglobulin-binding peptide (called TG19320) was used as a protease-resistant tetrameric tripeptide containing D amino acid residues.

Regarding peptides from nuclear autoantigens, Kaliyaperumal and colleagues showed that repeated IV or intraperitoneal administration of a single peptide of histone H4 (sequence 16–39, which behaves like a "promiscuous" T cell epitope) into (SWR × NZB)F_1 lupus mice with established glomerulonephritis prolonged survival of treated animals and halted progression of renal disease (10). The protective properties of another peptide of histone H4 (sequence 71–93), accompanied by an increased level of IL-10 and suppression of interferon-γ (IFNγ) secreted by LN cells, were described in (SWR × NZB)F_1 mice that were administered the sequence intranasally (IN) (18). Following IN (but not intradermal [ID]) administration of histone H4 peptide 71–93, the number of CD4+CD25+ Treg cells, which is low in (NZB × NZW)F_1 and (SWR × NZB)F_1 mice compared with normal mice, was restored in both strains (19). Very low dose treatment of (SWR × NZB)F_1 mice with histone H4 peptide 71–94 was also found to induce CD8+ and CD4+CD25+ Treg cells including autoantigen-specific cells, to decrease levels of IFNγ secreted by pathogenic T cells, and to decrease the antibody levels by 90–100% (20). The histone H3 peptide 111–130 encompassing a T cell epitope in (NZB ×

NZW)F_1 mice was also used with success when administered ID in Freund's adjuvant into these mice (21).

Treatment of MRL/*lpr* mice with a 21-mer peptide of laminin α-chain targeted by lupus antibodies also prevented antibody deposition in the kidneys, ameliorated renal disease, decreased the weight gain caused by accumulating ascitic fluid, and markedly improved longevity of treated mice (22). Examples in other experimental models of autoimmunity such as experimental autoimmune encephalomyelitis, experimental myasthenia gravis, or diabetic NOD mice also show spectacular protective effects (23,24).

Two aspects of P140 peptide analog should be highlighted. First, this promiscuous peptide is recognized ex vivo and induces a strong IL-10 secretion by lupus patients' peripheral CD4+ T cells only and not by CD4+ T cells from patients with other related autoimmune diseases such as rheumatoid arthritis, primary Sjögren's syndrome, and polymyositis (8). Second, it is notable that in lupus-prone MRL/*lpr* mice, P140 therapy does not affect the resistance of mice challenged with infectious virus and has no consequence on the specific antibody and CD4+ T cell response to the pathogen (9). Although much has to be done to precisely understand the mode of action of P140 analog, this initial phase II clinical trial demonstrates that treatment with IPP-201101 seems to be beneficial in human lupus. This result confirms the findings of our previous protection experiments in a murine model of lupus (6). The promiscuous phosphorylated peptide IPP-201101 is therefore a novel potential candidate for the specific treatment of SLE patients of virtually any origin.

AUTHOR CONTRIBUTIONS

Dr. Muller had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Wiesel, Geiger, Zimmer.
Acquisition of data. Muller, Monneaux, Schall, Rashkov, Oparanov.
Analysis and interpretation of data. Muller, Wiesel, Geiger, Zimmer.
Manuscript preparation. Muller, Geiger.
Statistical analysis. Wiesel, Geiger.

ROLE OF THE STUDY SPONSOR

ImmuPharma is the sponsor of the study as part of a full clinical program to develop Lupuzor as a treatment for SLE. ImmuPharma hired Genexion SA as a contract research organization to run the study. The phase IIa study protocol design as well as the data analysis were performed by ImmuPharma, Genexion SA, and the authors, whereas Genexion SA handled the logistics. ImmuPharma had no influence on the publication of the study results.

REFERENCES

1. D'Cruz D, Khamashta M, Hughes G. Systemic lupus erythematosus. Lancet 2006;369:587–96.
2. Merrill JT, Erkan D, Byon JP. Challenge in bringing the bench to bedside in drug development for SLE. Nat Rev Drug Discov 2004;3:1036–46.
3. Sibilia J. Treatment of systemic lupus erythematosus in 2006. Joint Bone Spine 2006;73:591–8.
4. Monneaux F, Briand JP, Muller S. B and T cell immune response to snRNP in lupus mice: autoreactive $CD4^+$ T cells recognize a T cell epitope located within the conserved RNP consensus sequence of the 70K protein. Eur J Immunol 2000;20:2191–200.
5. Monneaux F, Dumortier H, Steiner G, Briand JP, Muller S. Murine models of systemic lupus erythematosus: B and T cell responses to spliceosomal ribonucleoproteins in MRL/Fas^{lpr} and (NZBxNZW)F_1 lupus mice. Int Immunol 2001;13:1155–63.
6. Monneaux F, Lozano JM, Patarroyo ME, Briand JP, Muller S. T cell recognition and therapeutic effects of a phosphorylated synthetic peptide of the 70K snRNP protein administered in MRL/lpr lupus mice. Eur J Immunol 2003;33:287–96.
7. Monneaux F, Parietti V, Briand JP, Muller S. Intramolecular T cell spreading in unprimed MRL/lpr mice: importance of the U1-70K protein sequence 131–151. Arthritis Rheum 2004;50: 3232–8.
8. Monneaux F, Hoebeke J, Sordet C, Nonn C, Briand JP, Maillere B, et al. Selective modulation of $CD4^+$ T cells from lupus patients by a promiscuous, protective peptide analogue. J Immunol 2005; 175:5839–47.
9. Monneaux F, Parietti V, Briand JP, Muller S. Importance of spliceosomal RNP1 motif for intermolecular T-B cell spreading and tolerance restoration in lupus. Arthritis Res Ther 2007;9: R111.
10. Kaliyaperumal A, Michaels MA, Datta SK. Antigen-specific therapy of murine lupus nephritis using nucleosomal peptides: tolerance spreading impairs pathogenic function of autoimmune T and B cells. J Immunol 1999;162:5775–83.
11. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH, and the Committee on Prognosis Studies in SLE. Derivation of the SLEDAI: a disease activity index for lupus patients. Arthritis Rheum 1992;35:630–40.
12. Hochberg MC, for the Diagnostic and Therapeutic Criteria Committee of the American College of Rheumatology. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus [letter]. Arthritis Rheum 1997;40:1725.
13. Itoh Y, Hemmer B, Martin R, Germain RN. Serial TCR engagement and down-modulation by peptide:MHC molecule ligands: relationship to the quality of individual TCR signaling events. J Immunol 1999;162:2073–80.
14. Hahn BH, Singh RR, Wong WK, Tsao BP, Bulpitt K, Ebling FM. Treatment with a consensus peptide based on amino acid sequences in autoantibodies prevents T cell activation by autoantigens and delays disease onset in murine lupus. Arthritis Rheum 2001;44:432–41.
15. Hahn BH, Singh RP, La Cava A, Ebling FM. Tolerogenic treatment of lupus mice with consensus peptide induces Foxp3-expressing, apoptosis-resistant, TGFβ-secreting $CD8+$ T cell suppressors. J Immunol 2005;175:7728–37.
16. Zinger H, Eilat E, Meshorer A, Mozes E. Peptides based on the complementarity-determining regions of a pathogenic autoantibody mitigate lupus manifestations of (NZB x NZW)F_1 mice via active suppression. Int Immunol 2003;15:205–14.
17. Marino M, Ruvo M, De Falco S, Fassina G. Prevention of systemic lupus erythematosus in MRL/lpr mice by administration of an immunoglobulin-binding peptide. Nat Biotechnol 2000;18:735–9.
18. Wu HY, Ward FJ, Staines NA. Histone peptide-induced nasal

tolerance: suppression of murine lupus. J Immunol 2002;169: 1126–34.
19. Wu HY, Staines NA. A deficiency of CD4+CD25+ T cells permits the development of spontaneous lupus-like disease in mice, and can be reversed by induction of mucosal tolerance to histone peptide autoantigen. Lupus 2004;13:192–200.
20. Kang HK, Michaels MA, Berner BR, Datta SK. Very low-dose tolerance with nucleosomal peptides controls lupus and induces potent regulatory T cell subsets. J Immunol 2005;174: 3247–55.
21. Suen JL, Chuang YH, Tsai BY, Yau PM, Chiang BL. Treatment of murine lupus using nucleosomal T cell epitopes identified by bone marrow–derived dendritic cells. Arthritis Rheum 2004;50: 3250–9.
22. Amital H, Heilweil M, Ulmansky R, Szafer F, Bar-Tana R, Morel L, et al. Treatment with a laminin-derived peptide suppresses lupus nephritis. J Immunol 2005;175:5516–23.
23. Larche M, Wraith DC. Peptide-based therapeutic vaccines for allergic and autoimmune diseases. Nat Med 2005;11:S69–76.
24. Leech MD, Chung CY, Culshaw A, Anderton SM. Peptide-based immunotherapy of experimental autoimmune encephalomyelitis without anaphylaxis. Eur J Immunol 2007;37:3576–81.