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SPECIAL ARTICLE

Resetting the autoreactive immune system with a therapeutic peptide in lupus

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Over the last decade there has been a rapid expansion in the use of peptides as drugs. Nowadays, they are being used therapeutically in such diverse areas as endocrinology, neurology, haematology and some types of allergies. In the field of autoimmunity, a few candidates have emerged. Thus, in the pipeline of novel strategies designed to treat patients with systemic lupus erythematosus, the 21-mer peptide P140/Lupuzor raises hopes for the generation of an efficient, specific and safe treatment. This phosphopeptide has successfully completed a phase IIb clinical trial and will enter into a multi-centre, double-blind, placebo-controlled phase III clinical trial. The phase IIb trial showed that after three months of therapy (three subcutaneous injections of 200 µg peptide/patient in addition to standard of care), Lupuzor improved Systemic Lupus Erythematosus Disease Activity Index score of lupus patients under active treatment by 67.6% versus 41.5% in the placebo group ($p < 0.025$). After three additional months of follow-up, the improvement rate was 84.2% versus 45.8% ($p < 0.025$). The side-effect profile was unproblematic and the drug was well tolerated as evidenced by a very low drop-out rate. P140 does not behave as an immunosuppressant, it acts primarily as a fine immunomodulator of autoreactive $CD4^+$ T cells. Its underlying mechanism of action involves autophagy, a cellular process that implicates lysosomal-dependent recycling of intracellular components and controls the pool of major histocompatibility complex class II-displayed peptides that is presented to $CD4^+$ T cells. *Lupus* (2015) **24,** 412–418.

Key words: Lupus; peptide P140; Lupuzor; HSPA8/HSC70; autophagy

Introduction

Developing a therapeutic peptide for treating patients with systemic lupus erythematosus (SLE) faces at least two science and technology challenges, one resulting from the continued poor perception of peptides as active pharmaceutical ingredients, and the other linked to the multifactorial and heterogeneous nature of the lupus syndrome that is a highly polymorphic disease with a complex autoimmune aetiology. The negative image of peptides for designing drug formulation is in fact outdated and should be reviewed. Over the last decades, peptide-based drug products have benefited from an intense research aimed at substantially improving peptide stability, bioavailability and metabolic efficiency. Furthermore, their toxicity and immunogenicity are generally very weak, a valuable asset over other classes of biologics. Scale-up production (multi-10 or multi-100 kg) has also been highly facilitated and their cost, in comparison with other therapeutics, remains largely affordable. As a consequence, the US Food and Drug Administration (FDA) accepts a growing number of therapeutic peptides, and nowadays about 70 peptide-drug products have reached approval. The released peptides are currently much more successful as therapeutics than small molecules, and when compared with therapeutic monoclonal antibodies, they occupy a prominent position.[1–7]

Lupus represents a complex disease; the precise immunological events that trigger the onset of clinical manifestation and those that perpetuate the disease over years are not fully understood.[8–10] Yet, important molecular and cellular pathways have been deciphered and some elements revealed as possible targets for efficient intervention. In this

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context, the P140 peptide/Lupuzor, which has received Special Protocol Assessment and Fast Track Designation from the FDA for a phase III trial, is a very promising therapeutic candidate. The key points of its potential are summarized in this short review, at both the mechanistic and the clinical levels.

The therapeutic value of P140 peptide in lupus mice

The P140 peptide is an amazing bioactive molecule. First described in 2003,[11] this 21-mer fragment contains a phosphoserine residue at position 140, a modification introduced during its synthesis, which was discovered several years later to occur specifically at an early stage of apoptosis.[12] The pioneer work that led us to identify the P140 peptide (sequence 131–151) was based on the test, with lymph node T cells from lupus-prone MRL/lpr mice, of a series of non-modified overlapping peptides covering the U1-70 K (52 kD) small nuclear ribonucleoprotein (snRNP).[13] This self-protein, which is an essential splicing factor, was selected because it corresponds to one of the major autoantigens in lupus.[14] Peptide 131–151, the sequence of which was highly conserved during evolution, was the only one in our series of peptides to be recognized by H-2^{k} MRL/lpr lymph node T cells. It was revealed in young mice when the pathological signs of the disease were yet to be declared. Recognition of the non-phosphorylated peptide 131–151 (and also that of P140) was not found to be MHC-restricted and T cells from H-$2^{d/z}$ (NZBxNZW)F1 lupus-prone mice also react with the 131–151 peptide.[15]

A number of modifications were introduced in the initial sequence 131–151. The first series included classical post-translational modifications of serine (PhosphoSer residues 137 and 140) and lysine residues (AcLys residues 138 and 142). While acetylated peptides were not recognized by MRL/lpr T cells, both peptide analogues containing PhophoSer residues efficiently induced the proliferation of MRL/lpr $CD4^{+}$ T cells and IL-2 secretion.[11] Because of its poor solubility, however, the so-called P137 peptide analogue was rapidly discarded from our further studies, while we kept the P140 analogue, which displayed more favourable physicochemical properties. In water or 5.4%-mannitol pH 7.4, the P140 peptide (Mr = 2640) remains highly soluble at least up to 200 μM or 400 μM, respectively, as determined by dynamic light scattering measurements (Figure 1(a)).[16,17] In phosphate-buffered saline (PBS) and fetal calf serum-containing culture medium (both at pH 7.4), its limit of solubility fell to only 50 and ~25 μM, respectively (Figure 1(a)). In routine assays, it is optimally used at <40 μM (and even 20 μM in cell culture) to avoid any alteration of data that might result from invisible aggregates in the solutions. P140 is a stable compound as was shown by high-performance liquid chromatography (HPLC) analyses over time when the peptide was kept either under lyophilized form[16] or in solution at different temperatures for long durations (Figure 1(b), at 37°C).

The importance of the phosphoryl moiety carried on the Ser^{140} residue was clearly shown in vivo, in several biological settings. P140 peptide only, but not the non-phosphorylated peptide, was protective, in terms of both survival and proteinuria, against the lupus-like disease when administrated intravenously into young MRL/lpr mice[11,18,19] and was able to significantly reduce peripheral hypercellularity, which is a typical feature of this mouse strain.[18] In vitro, however, most of the effects observed with the P140 peptide were also noted with the non-phosphorylated form of the peptide, in general to an equal extent. Thus, both peptides are cross-recognized by T cells from non-autoimmune BALB/c mice immunized with the non-phosphorylated form of the P140 peptide,[11] bind to MHCII molecules in a promiscuous manner[11,20] and also bind to HSPA8/HSC70,[18] a chaperone protein that is constitutively expressed at the surface of a variety of cells and which has been identified as central in the mechanism of action of P140 peptide.[16,21] Both peptides affect HSPA8 refolding and ATPase properties in in vitro assays (non-published and Page et al.[21]). Collectively these data suggest that phosphorylation does not properly hamper the binding properties of the modified peptide P140 to possible acceptor or receptor molecules or to ligands but, rather, plays a role in its stability, biodelivery, pharmacokinetics or homing in an integrated cell and organ system in living animals.

Mechanism of action of P140 in MRL/lpr lupus mice

P140 does not behave as an immunosuppressant but rather as an immunomodulator of the cascade of immunological events that maintain and exacerbate autoimmunity by spontaneous flares in lupus

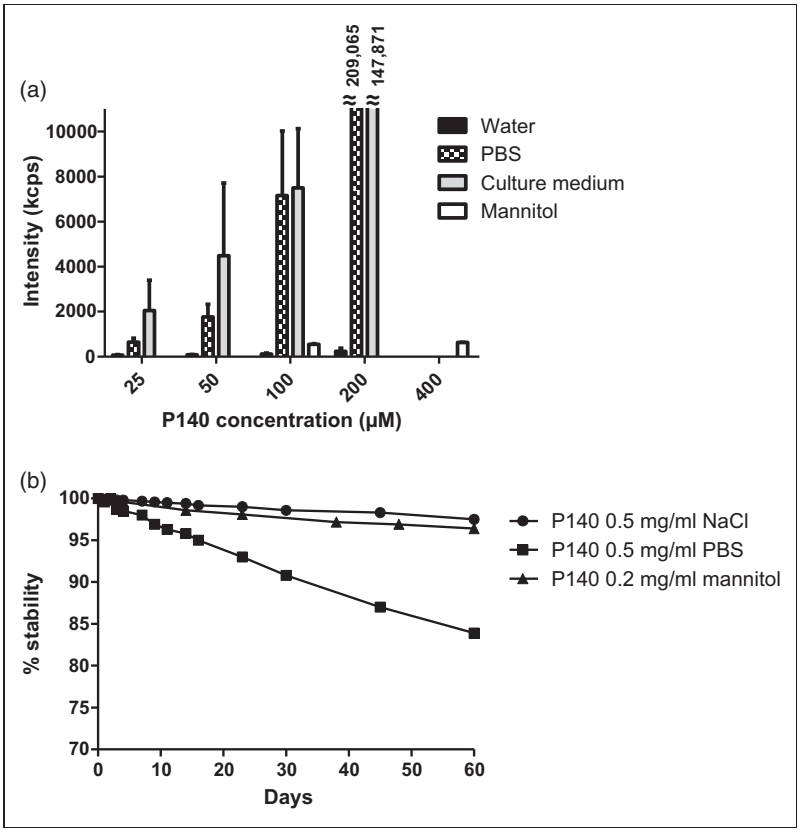

Figure 1 Physicochemical properties of P140 peptide. (a) Determination of the solubility limit of P140 peptide in distilled water, phosphate-buffered saline (PBS), RPMI 1640 culture medium and mannitol 5.4%. The data show the variation of mean intensity of light scattered (expressed as kilocounts (kcps)) that occurs when peptide aggregates are formed (the greater the mean intensity the lower the solubility). (b) Stability over time (expressed as days) and at 37°C of the P140 peptide in NaCl, PBS and mannitol, as measured by high-performance liquid chromatography from the area of the peak corresponding to the intact peptide.

individuals. In MRL/lpr mice, which in contrast to lupus patients show no lupus flares but constant disease progression,[22,23] P140 peptide significantly shifts the appearance of first symptoms and delays mortality.[11,18] Following our findings that P140 readily binds to HSPA8,[16] and knowing the importance of HSPA8 in autophagy processes,[24] we discovered that the underlying mechanism of action of the peptide effectively involves autophagy.[21] This complex process, which plays a central role in cellular homeostasis and adaptation,[24] notably implicates lysosomal-dependent recycling of intracellular components, which has been identified as a route by which cytoplasmic and nuclear antigens are delivered to MHCII molecules for presentation to $CD4^+$ T cells.[25–27] P140 was found to reduce the autophagic flux in antigen-presenting cells (APCs) and the stability of MHCII molecules.[21] These findings, added to the fact that P140-treated mice display very low T cell activity towards several distinct peptides encompassing T cell autoepitopes,[28,29] led us to propose that through its effect on autophagic flux and MHCII stability, P140 provokes a weaker activation of autoreactive T helper cells via a failure to present self-peptides to autoreactive T cells. Without signal from those T cells, autoreactive B cells' differentiation into plasma cells might then be affected, leading thus to a reduction of autoantibody levels observed in P140-treated mice.[19,30]

At this stage, much more work is required to understand how P140 peptide can interfere with the autophagy pathway. Macroautophagy has been found to be altered in both B and T cells in lupus[31–33] and, quite interestingly, several molecules known today to target autophagy components have been used serendipitously or empirically to treat lupus with some success.[34] The molecular and cellular bases for autophagy deregulation are not known in lupus but some *ATG* genes have been identified, which might be at the origin of autophagy dysfunction in this syndrome.[32,33]

It should be emphasized that intravenous P140 peptide administration into young MRL/lpr mice

causes T and B cell egress from peripheral blood but does not affect T cell priming, and does not interfere with the capacity of P140-treated mice to resist an infectious viral challenge.[29] In the peripheral blood, remaining cells respond normally to mitogens, a feature not observed when mice were treated with immunosuppressive agents.[21] The mode of action of P140 peptide seems therefore to be restricted to autoreactive immune response. In our working scheme a crucial point will be to determine how cell selectivity occurs. After treatment, there is an accumulation of autophagy markers p62/SQSTM1 and LC3-II in MRL/lpr B cells (acting here as APCs), perhaps also into other APCs, and abnormally elevated amounts of HSPA8 that appear with age at the surface of various, in general activated, T and B cells subsets in lupus mice decrease.[21] It is worth noting that the simple fact that P140 peptide binds HSPA8 and inhibits some of its functions is not sufficient to explain its remarkable effects. An efficient HSPA8 inhibitor called VER-155008 (5'-*O*-[(4-cyanophenyl)methyl]-8-[[(3,4-dichlorophenyl)-methyl]amino]-adenosine), which interacts with HSPA8 with an IC50 value of 2.6 μM (0.5 μM with HSP70) and exerts different effects on cancer cell lines[35] had no impact on peripheral hypercellularity in MRL/lpr mice (Figure 2).

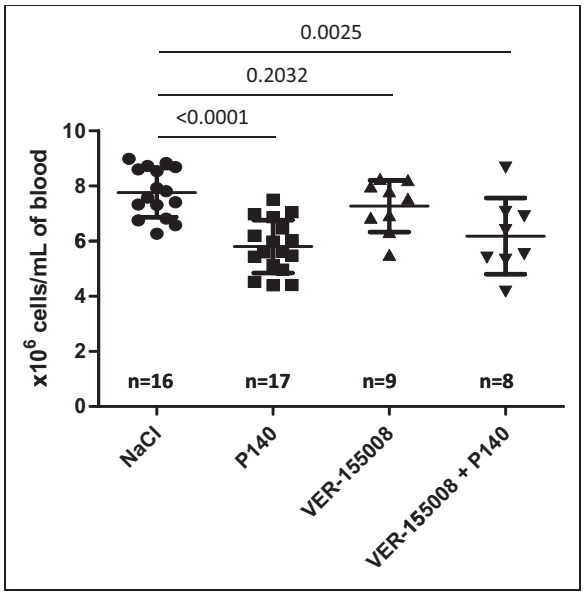

Figure 2 Effect on peripheral hypercellularity in MRL/lpr mice. Female MRL/lpr mice (11–13 weeks old) received intravenously a single administration of either peptide P140 or VER-1550008 or both (100 μg/mouse, of each). The control group received saline only. The number of leukocytes/ml was evaluated by counting cells five days later. Each symbol represents one individual mouse (*n*, number of mice/group). The horizontal bars represent the respective average cell count values. Statistical significance was assessed using Student's *t*-test.

P140/Lupuzor in lupus patients

Following a promising open phase IIa clinical trial in patients with SLE,[36] two multicentre, randomized, placebo-controlled phase IIb clinical trials were undertaken to evaluate the efficacy of the peptide administered either in 5.4% mannitol (Lupuzor) or in 10% trehalose (Forigerimod) as excipient.[30,37] These studies were run separately with similar 'standard' protocols, namely subcutaneous administration of 200 μg P140/individual per month in addition to standard of care (cumulative steroids < 80 mg/week), inclusion of patients with clinical SLE Disease Activity Index (SLEDAI)-2 K scores >6 and no BILAG A score. The demographic characteristics of the study populations were similar in both studies as well as in each treatment group. Drop-out rates were recorded irrespective of their reason and considered as treatment failure. Efficacy was evaluated using the SLE Responder Index (SRI). In the lupuzor study, was +25% when compared with the placebo group (62% versus 37% responders at 12 weeks; $p < 0.025$[37,38]). An interim analysis including 114 patients out of the target population (136 intention-to-treat patients) demonstrated an even better efficacy according to the SLEDAI score. The latter improved in 67.6% of patients under active treatment versus 41.5% of patients under placebo at week 12 ($p < 0.025$) and in 84.2% versus 45.8% of patients, respectively, at week 24 ($p < 0.025$). From this pivotal trial, it was concluded that Lupuzor was safe and had met its primary efficacy end point in lupus patients, thus opening the way towards a phase III clinical trial that should start soon in the USA and Western Europe.

Trehalose, a non-reducing sugar that is used as excipient in several vaccine and therapeutic compositions, is also a known inducer of autophagy.[34] As unfortunately anticipated, it was shown to severely interfere with the beneficial effect of P140. Its use as a filler together with P140 peptide (Forigerimod formulation) was therefore declared to be totally inappropriate in the treatment of lupus patients.[30]

In this second phase IIb clinical trial, immunogenicity of active peptide P140 contained in the Forigerimod preparation was checked. The serum samples of a total of 182 patients were tested by ELISA for the presence of circulating antibodies to P140 peptide, namely 92 patients under placebo

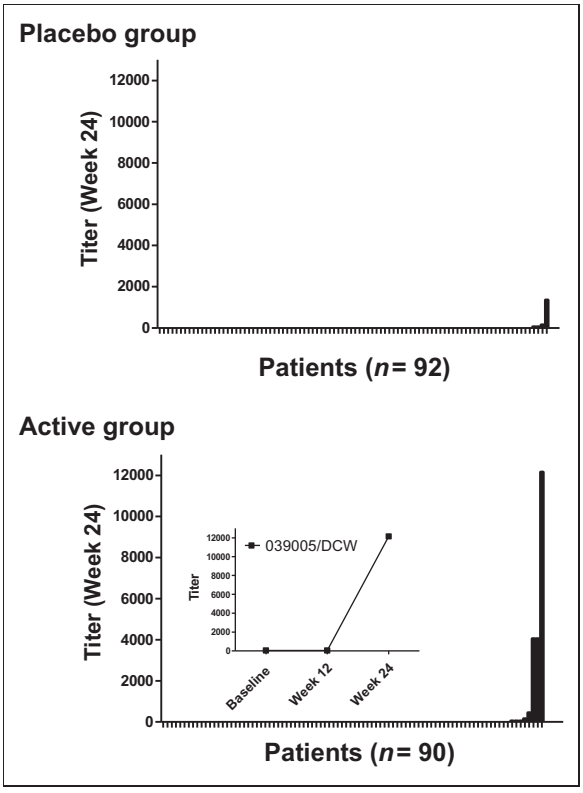

Figure 3 Immunogenicity of P140 peptide in patients with SLE. The presence of P140-reacting antibodies was measured in the serum of 90 lupus patients who received the active peptide drug P140. Sera from the placebo group (92 lupus patients) were tested as control. The data were classified to have the highest titre on the right of the graph. In the insert is shown the sole serum (over 90) that showed an elevated antibody titre at week 24 (titre of 12,150) while the titre was <50 at week 12.

and 90 patients under active product (Figure 3). In the placebo group (non-'reactive' by definition), the samples of five out of 92 patients (5.4%) contained naturally-occurring antibodies reacting with the P140 peptide (titre at least equal to 50 in at least one sample; Table 1). This frequency of occurrence of natural antibodies is classically found in the normal population and, as expected, no progression of their titres was observed with time. In the group of patients who received the active study drug, the samples of eight out of 90 patients (8.9%) were found to contain antibodies reacting with the P140 peptide (titre at least equal to 50 in at least one sample; Table 1). Only two of these eight patients were considered as 'reactive', among which were one at the border of significance (titre = 450 at week 24) and one who showed an elevated anti-P140 titre at week 24 (titre = 12,150 while <50 at week 12) (Figure 3, Table 1). These data allowed us to conclude that at least 97.8% of patients (88/90 patients) did not develop any immune response to

Table 1 Immunogenicity of active study drug P140 peptide in patients with SLE

	Titre/P140 peptide			
Patients[a]	*Baseline*	*Week 12*	*Week 24*	*Result*[b]
Placebo group				
00601/J-A	4050	4050	1350	Non-reactive
008001/BAM	4050	<50	50	Non-reactive
02008/BGC	<50	1350	150	Non-reactive
028001/JSM	450	450	ND	Non-reactive
751007/EVG	1350	<50	ND	Non-reactive
Active group				
001001/T-S	12150	<50	<50	Non-reactive
06002/G-G	150	50	150	Non-reactive
014009/LFN	1350	ND	<50	Non-reactive
018008/B-S	<50	<50	**450**	**Reactive**
019007/HET	1350	<50	<50	Non-reactive
039005/DCW	<50	<50	**12150**	**Reactive**
201001/P-L	4050	4050	4050	Non-reactive
403001/A-A	4050	4050	4050	Non-reactive

[a]Data raised by testing the blood collected from 92 patients under placebo and 90 patients who received the active peptide drug P140. The data of positive sera only are shown in this table.
[b]Considered as 'positive' (bold): all samples giving antibody titres at least equal to 50 in one of the collected samples. Considered as 'reactive' (bold): any patients who showed an elevation of antibody titres in their serum after active peptide drug administration. Patients under placebo were considered as non-reactive by definition since they received no peptide drug.
ND: not determined.

P140 active drug although they received three injections of 200 μg of peptide, and therefore that P140 could be considered as safe and non-immunogenic.

Perspectives

Many questions remain to be solved regarding the mechanism of action of P140. Fortunately, although in many respects the MRL/lpr mouse model differs from patients with SLE (Table 2), this strain has demonstrated its relevance for developing and evaluating an efficient therapeutic strategy that nowadays shows a lot of promise in humans. The MRL/lpr model will thus be further exploited to strengthen our knowledge and fully understand how a single peptide can interfere with such a remarkable efficacy in the complex circuit leading to a so polymorphic disease. Among the diverse questions we raise, that of the entry of P140 within cells and how selectivity occurs according to cell subsets is central. Does P140 use a single, unique receptor, or several, perhaps not especially specific receptors? Where does it specifically traffic within cells? We have shown that within APCs,

Table 2 Conditions and mode of action of therapeutic P140 peptide in mice and humans

Setting	*MRL/MpJ-Fas*^{lpr} *(MRL/*lpr*) mouse model*	*Patients with SLE*
Active dosage	5 mg/kg[a] Four administrations i.v. route (s.c. inefficient) Saline	3 µg/kg[b] Three administrations s.c. route (i.v. not tested) Mannitol
Peripheral cell counts	Hypercellularity due to the *lpr* mutation	Frequent lymphopenia
Main P140 effects (ex vivo)	– Induces T cell proliferation – Induces IL2 and IL10 secretion – Down-regulates MHCII expression – Down-regulates HSPA8 expression – Down-regulates autophagic flux	– No proliferation – IL10 secretion – Down-regulates MHCII expression? – Down-regulates HSPA8 expression? – Down-regulates autophagic flux?
Main P140 effects (in vivo)	– Delays mortality –Significantly decreases: ■ proteinuria ■ anti-dsDNA antibody level ■ white cell counts/peripheral blood ■ inflammation of blood vessels ■ perivascular inflammatory infiltrates ■ dermatitis	– Improves SRI and SLEDAI scores – Significantly decreases: ■ anti-dsDNA antibody levels – Has no effect on: ■ peripheral cell counts
MOA	via autophagy	ND

[a]About 100 µg/ml of blood.
[b]Locally 200 µg/ml.
ds: double-stranded; IL: interleukin; i.v.: intravenous; *lpr*: lymphoproliferation gene; MHC: major histocompatibility complex; MOA: mode of action; MRL: Murphy Roths large; ND: not determined; SLE: systemic lupus erythematosus; s.c.: sub-cutaneous; SRI: SLE Responder Index

P140 seems to interfere with MHC peptide presentation, leading to a weaker delivery to autoreactive T cells. What is the precise mechanism underlying this effect? What are the down-stream consequences? We have demonstrated that P140 acts as a skilled immunomodulator that does not affect the entire immune system, but seems to reprogram the autoimmune cells only. We consider this selective therapeutic approach to be particularly appropriate for treating chronic autoimmune diseases, which are estimated to affect 5% of the general population worldwide.

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Conflict of interest statement

NS is an employee of ImmuPharma; SM is a consultant for ImmuPharma.

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