Journal of Peptide Science
J. Pept. Sci. 2008; **14**: 1069–1076
Published online 10 July 2008 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.1043

JOURNAL OF PEPTIDE SCIENCE

A complementary La/SSB epitope anchored to Sequential Oligopeptide Carrier regulates the anti-La/SSB response in immunized animals

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Received 8 October 2007; Revised 31 March 2008; Accepted 11 April 2008

Abstract: Complementary peptide epitopes, derived from complementary RNA sequences, have been used for suppressing the autoimmune response in experimental autoimmune diseases as myasthenia gravis, allergic neuritis and allergic encephalomyelitis. Aiming at contributing to the development of a tool that could regulate the autoantibody production against La/SSB, which is the main target of autoantibodies in Sjogren's syndrome (SS) and systemic lupus erythematosus (SLE), the complementary epitope, cpep349–364, of the minor T/major B cell epitope of La/SSB, pep349–364, was utilized for the induction of neutralizing anti-cpep349–364 antibodies in rabbit immunizations. Complementary peptides were coupled to an artificial carrier, developed in our laboratory, in order to enhance the complementary potency of cpep349–364 and its counterpart. This carrier, named Sequential Oligopeptide Carrier, SOC_n, formed by the repeating tripeptide Lys-Aib-Gly, adopts helical conformation, which allows the anchored peptide epitopes to preserve their initial reactivity such as molecular recognition, antigenicity/immunogenicity. Our study provides proof of evidence of specific interactions between idiotypic (Id)/anti-idiotypic (anti-Id) antibodies generated in immunized animals by the sense epitope (conjugate I) of La/SSB and its complementary counterpart (conjugate II). It was also demonstrated that the Id/anti-Id association is specifically disrupted by adding either the sense epitope (conjugate I) or its complementary counterpart (conjugate II). A mutual neutralization of Id/anti-Id antibodies was observed *in vivo*, which implies that generation of anti-Id antibodies by immunization with the complementary La/SSB epitope could scavenge the anti-La/SSB response.

Keywords: complementary La/SSB epitope; Sequential Oligopeptide Carrier; regulation of anti-La/SSB response; rabbit immunizations; immunological assays; ELISA inhibition assays

INTRODUCTION

Complementary RNA sequences encode peptides or proteins defined as complementary or antisense, which interact specifically with each other, apparently as a result of having complementary hydrophobicity [1–4]. This concept derives from the observation that codons for hydrophobic amino acids are complemented in the other DNA strand by codons for hydrophilic amino acids and vice versa [5,6]. It has been shown that complementary peptides (cpep) bind to one another with high specificity and relatively high affinity using synthetic peptide counterparts of corticotropin (ACTH) [1,4], endorphins [1], interleukin 2 (IL-2) [7], calmodulin [8] and tumor necrosis factor (TNFα) [9].

Immobilized synthetic cpep of sense peptides were used for the successful development of affinity purification techniques. The development of anticomplementary polyclonal or monoclonal antibodies, cross-reacting with hormone receptors, is another interesting application. It was found that antibodies against the complementary peptide for ACTH bind to the adrenal hormone receptor, suggesting that they compete for the same adrenal cell binding site [3]. Antibodies to the hormone should have a receptor-like shape and the two types of antibodies, against sense and antisense peptides, would possess an idiotypic (Id)/anti-idiotypic (anti-Id) relationship [10,11].

Experimental evidence has confirmed that immunizations with complementary peptide pairs generate interacting pairs of Id and anti-Id antibodies with complementary combining sites [12,13]. These anti-Id antibodies, reactive with idiotypes of autoantibodies or autoreactive clonotypic T cells, are capable of regulating the autoimmune response and represent ideal therapeutic agents for autoimmune diseases. Application of complementary peptide epitopes in experimental autoimmune myasthenia gravis (EAMG) [14–16], experimental allergic neuritis (EAN) [17] and experimental allergic encephalomyelitis (EAE) [18–20] have been the stimulus of many research efforts.

The humoral autoimmune response, in patients with Sjogren's syndrome (SS) and systemic lupus erythematosus (SLE), is mainly directed against subcellural ribonucleoprotein particles (RNP) called Ro/La RNP complexes (Ro/SSA and La/SSB proteins complexed to RNA) [21]. We have previously mapped the exact location of B and T cell epitopes of the La/SSB autoantigen,

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one major T/minor B cell (pep289–308) and one minor T/major B cell epitope (pep349–364) [22,23]. It was also demonstrated that immunization with a single epitope of La/SSB generates antibodies recognizing the other epitopes, as well as the La/SSB protein, suggesting a molecular spreading of the epitopes [24].

Cpep, deduced from the antisense RNA, corresponding to the epitopes of the La/SSB autoantigen, cpep289–308 and cpep349–364, were tested for their implication in the immune network of pSS and SLE patients [25]. Both cpep289–308 and cpep349–364 acquired inverted hydrophobicity profiles, compared with pep289–308 and pep349–364 respectively, according to the Fauchere/Pliska [26] and Engelman/Steitz/Goldman [27] scales. The most prominent finding emerging from this study was the unmasking of the anti-La/SSB response, in sera from patients with pSS and SLE, by specific blocking of the anti-Id antibodies using the complementary cpep349–364 of the La/SSB epitope pep349–364 [25]. Id/anti-Id circuit, as well as T cell proliferative response was also observed in mice immunized with La/SSB epitopes and their counterparts [28–30].

Aiming at contributing to the development of a tool that could regulate or suppress autoantibodies against La/SSB, the complementary epitope, cpep349–364, of the minor T/major B cell epitope of La/SSB, pep349–364 (Figure 1(A)), was utilized in rabbit immunizations for the induction of neutralizing anti-cpep349–364 antibodies. Cpeps were coupled to an artificial carrier (Figure 1(B)), developed in our laboratory [31,32], in order to enhance the complementary potency of cpep349–364 and its counterpart. The carrier, named Sequential Oligopeptide Carrier, SOC_n, formed by the repeating tripeptide Lys-Aib-Gly, adopts helical conformation, which allows the anchored peptide epitopes to preserve their initial reactivity such as molecular recognition, antigenicity/immunogenicity

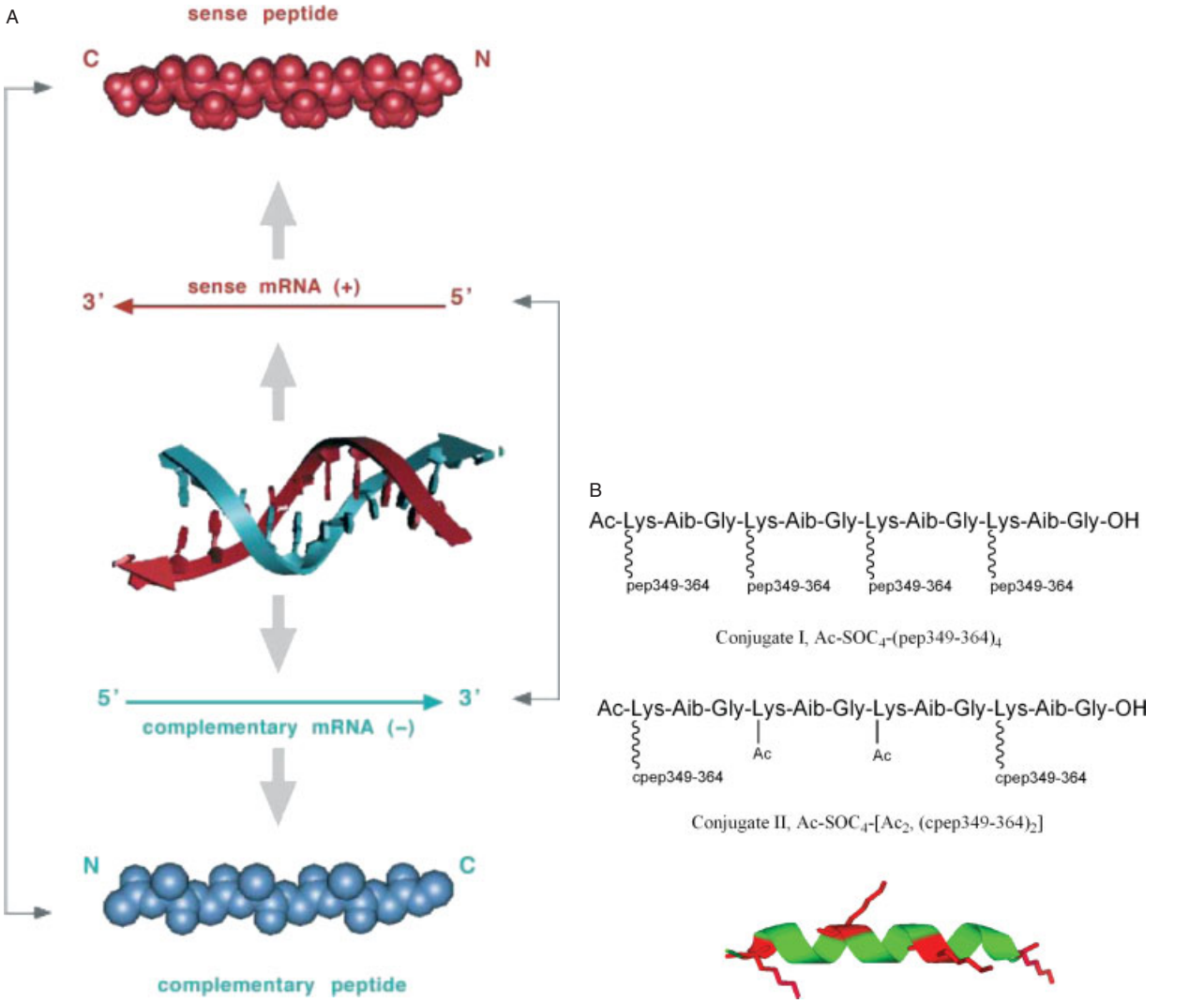

Figure 1 (A) Design of the complementary peptides pep349–364 and cpep349–364 of La/SSB using the antisense approach, where pep349–364 is GSGKGKVQFQGKKTKF and cpep349–364 is KFRFLALKLYFSFTRP. (B) Illustration of conjugate I, Ac-SOC_4-$(pep349–364)_4$, and conjugate II, Ac-SOC_4-$[Ac_2$-$(cpep349–364)_2]$.

DOI: 10.1002/psc

[33,34]. The helical conformation of SOC_n, Ac-(Lys-Aib-Gly)$_n$, confirmed by ^{1}HNMR spectroscopy and molecular dynamic simulations, allows the conjugated peptides to maintain their native conformation [35]. More importantly, the helical structure of the carrier contributes to the precise presentation of the epitopes to the antigen-presenting cells and results in a specific immune response [36].

Our study points out the specific interaction of Id/anti-Id antibodies produced by the complementary conjugates I and II, which is selectively disrupted by adding either the sense epitope (conjugate I) or its complementary counterpart (conjugate II). Also, the mutual neutralization of Id/anti-Id antibodies, observed through *in vivo* experiments, leads to the assumption that immunization with conjugate II could induce anti-Id antibodies capable of trapping the Id anti-La/SSB response.

MATERIALS AND METHODS

Peptide Synthesis

Synthesis of conjugate I, Ac–SOC$_4$-(pep349–364)$_4$. Initially, the protected fragment of the SOC_4 carrier, where SOC_4 is (K-Aib-G)$_4$, was synthesized on a Boc-Gly-OCH$_2$-Pam resin by the stepwise solid phase procedure (SPPS) using the Boc/Bzl methodology [37,38] (Boc: tert-butyloxycarbonyl, Bzl: benzyl, Pam: phenylacetamidomethyl). Glycine and α-amino isobutyric acid were introduced as Boc-Gly-OH and Boc-Aib-OH respectively, while lysine was introduced as Boc-Lys(Fmoc)-OH (Fmoc: 9-fluorenylmethyloxycarbonyl). The protocol of the synthesis was the following: (i) deprotection with 40% TFA/DCM (1×2 min, 1×13 min); (ii) washings with DCM (3×1 min), MeOH (3×1 min), DCM (3×1 min); (iii) neutralization with 7% DIEA (*N*,*N*′-diisopropylethyamine)/DCM (2×2 min); (iv) washings with DCM (3×1 min), MeOH (3×1 min), DCM (3×1 min); (v) couplings using a 3/2.9/3/6/1 molar ratio of amino acid/TBTU/HOBt/DIEA/resin in DCM-DMF mixture depending on the solubility of Boc-amino acid derivatives (2 h); (vi) washings with DCM (3×1 min), MeOH (3×1 min), DCM (3×1 min); (vii) ninhydrin assay. (TBTU: O-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetra-methylluronium tetra fluoroborate, HOBt: 1-hydroxybenzotriazole). A similar protocol was applied for the sequential propagation of the Boc-K(Fmoc)-Aib-G moiety until the formation of the tetrameric SOC_4. The N^{α}-terminal Boc group of Lys of the carrier was cleaved by 40% TFA in DCM and Lys was N^{α}-acetylated with $(CH_3CO)_2O$ in pyridine in molar ratio of $(CH_3CO)_2O$/resin 30/1.

Fmoc-protective groups of Lys-N$^{\varepsilon}$H$_2$ were removed by 20% piperidine in DMF (1×2 min, 1×15 min), and the La/SSB epitope GSGKGKVQFQGKKTKF (pep349–364) was covalently attached in four copies to the LysN$^{\varepsilon}$H$_2$ groups of the SOC_4 carrier by the stepwise solid phase synthesis, following the Boc/Bzl methodology. After completion of the synthesis the peptide conjugate was cleaved from the resin support by liquid hydrogen fluoride (HF) in the presence of phenol and anisol as scavengers (HF : phenol : anisol = 10 ml : 0.5 g : 1 ml) for 30 min at $-8\,^{\circ}$C and 1.5 h at $0\,^{\circ}$C, and extracted with 2 M aqueous acetic acid. The crude peptide (yield 80%) was purified by semipreparative reverse phase high-performance liquid chromatography (RP-HPLC) on a C_{18} column. Appropriate programmed gradient was applied using eluent A (H_2O/0.1%TFA) and B (CH_3CN/0.1%TFA). The purified peptide (yield 30%) was checked by analytical RP-HPLC ($t_R = 23.7$ min) and the correct molecular mass was confirmed by electrospray ionization mass spectrometry (ESI-MS), calculated M$^+$: 7969.41 found M$^+$: 7969.86.

Steps in the synthesis of conjugate I are illustrated in Figure 2, while parameters of synthesis, purification and characterization are summarized in Table 1.

Synthesis of conjugate II, Ac–SOC$_4$-(Ac$_2$, (cpep349–364)$_2$). Synthesis of the protected SOC_4 carrier was performed as for conjugate I, except that lysines were introduced as Boc-Lys(Fmoc)-OH at the 1st and 4th positions of SOC_4, while lysines at the second and third positions were inserted as Boc-Lys(Ac)-OH. After cleavage of the Fmoc-protective groups of lysine side chains the synthesis of the complementary peptide KFRFLALKLYFSFTRP (cpep349–364) was carried out in two copies following the Boc/Bzl methodology. The peptide was cleaved from the resin by liquid HF in the presence of phenol and anisole as described for conjugate I. The crude peptide (yield 90%) was purified by semipreparative RP-HPLC on a C_{18} column. Appropriate programmed gradient was applied using eluent A (H_2O/0.1%TFA) and B (CH_3CN/0.1%TFA). The purified peptide (yield 40%) was checked by analytical RP-HPLC ($t_R = 21.4$ min) and the correct molecular mass was confirmed by ESI-MS, calculated M$^+$: 5260.38 found M$^+$: 5260.35. Table 1 summarizes the parameters of the synthesis, purification and characterization of conjugate II.

Table 1 Parameters of the synthesis, purification and characterization of conjugates I and II

Conjugates	Yield %	RP-HPLC		ESI-MS
		Gradient elution	t_R (min)	
Conjugate I	Crude: 80	A/B 80 : 20	23.7	Calculated M$^+$: 7969,41
	Purified: 30	A/B 30/70		Found M$^+$: 7969,86
Conjugate II	Crude: 90	A/B 90/10	21.4	Calculated M$^+$: 5260,38
	Purified: 40	A/B 40/60		Found M$^+$: 5260,35

A; H_2O/0.1% TFA, B; CH_3CN/0.1% TFA, flow rate of gradients, 4.4 mL/min, elution time 30 min, t_R; retention time.

DOI: 10.1002/psc

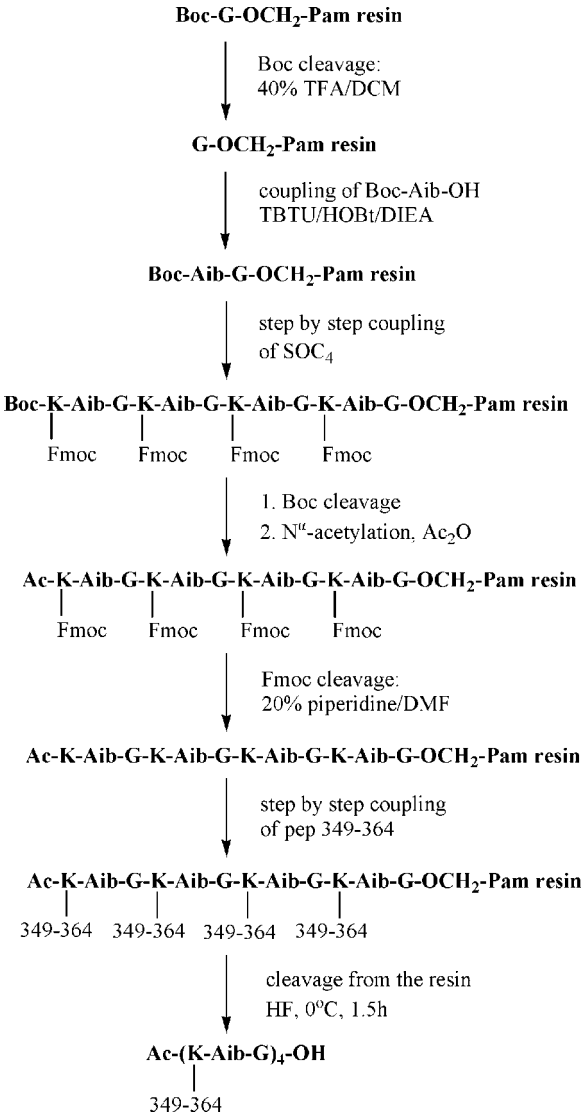

Figure 2 Steps in the synthesis of conjugate I, Ac-SOC_4-(pep349–364)$_4$.

Biological Assays

Rabbit immunizations – ELISA experiments. New Zealand white rabbits were immunized, two for each conjugate, following three different protocols.

Protocol A: One milligram of conjugate I or conjugate II in 500 µl of H_2O, emulsified in 500 µl of complete Freund's adjuvant (CFA) was injected on day 1. Boostings with 0.5 mg/500 µl H_2O, emulsified in 500 µl of incomplete Freund's adjuvant (IFA), were performed on days 15, 36, 79, 99 and 123. Blood was collected from each animal, seven days after each boosting, on days 21, 43, 86, 106 and 130. The presence of anti-conjugate I (anti-pep349–364) and anti-conjugate II (anti-cpep349–364) antibodies were tested by ELISA assays described below.

Protocol B: Animals were preimmunized with 1mg of conjugate II following the protocol A. After the fifth bleeding on day 130, high titer of anti-cpep349–364 antibodies were detected by ELISA. Subsequently, the animals were immunized subcutaneously with conjugate I according to the following schedule. One milligram of conjugate I in 500 µl of H_2O, emulsified in 500 µl IFA, was injected on day 1. Boostings with 0.5 mg/500 µl H_2O, emulsified in 500 µl IFA, were performed on days 14, 28 and 42. Blood was collected from each animal (seven days after each boosting) on days 7, 21, 35 and 49 after the first immunization with conjugate I.

Protocol C: Animals were preimmunized with 1mg of conjugate II following the protocol A. After the fifth bleeding on day 130 and the generation of anti-cpep349–364 antibodies the animals were immunized with conjugate I according to the following schedule. Twenty-five µg in 500 µl of H_2O, emulsified in 500 µl IFA, was injected on day 1. Boostings with 50, 100, 250 and 500 µg of conjugate I, in 500 µl H_2O and 500 µl IFA each, were performed on days 14, 28, 42, 56 and 63. Animals were bled (seven days after each injection) on days 7, 21, 35, 49, 63 and 70 after the first immunization with conjugate I.

The collected sera were tested for the presence of antibodies against conjugates by ELISA [39] according to the following protocol. Conjugates (5 µg/ml) in $Na_2CO_3/NaHCO_3$ buffer, pH = 9.6, were coated on 96-well polystyrene plates (100 µl/well) and incubated overnight at 4 °C. After washings with phosphate buffer saline (PBS), pH = 7.2, the nonspecific binding sites were blocked with 3% skimmed milk in PBS and the plates were incubated for 2 h at room temperature. Serum samples from the preimmunized and postimmunized animals were added (100 µl/well) at dilutions, with blocking buffer, from 1 : 100 to 1 : 51 200, and the plates were incubated overnight at 4 °C. After extensive washings with PBS, the wells were incubated with goat antirabbit IgG conjugated to peroxidase (dilution 1 : 2000 with blocking buffer) for 2 h at 37 °C. Finally the plates were washed with PBS buffer and 50 µl of substrate solution TMB (3,3′,5,5′ tetramethyl-benzidine, 1/1 v/v H_2O) and 50 µl of H_2O_2 (1/1, v/v H_2O) were added to each well and the absorbance was measured at 450 nm. Data, depicted in ELISA experiments, are shown as means of three independent experiments carried out in triplicate, using a pool of two rabbit sera, that gave similar results when used independently.

RESULTS AND DISCUSSION

An ideal treatment for an autoimmune disease such as SS would be one that specifically affects the immunologic reactivity leading to the disorder without compromising the immune system's ability to react to foreign antigens. Anti-Id antibodies represent such specific agents, and harnessing the immune system itself to correct autoimmune disorders would seem to be a very promising approach.

The problem, however, is that for this approach to be effective the disease-causing antibodies or T cells must have a common characteristic such as a shared or cross-reactive Id for antibodies or restricted V gene usage for the T cell antigen receptor. In order to overcome the nonavailability of sequence data from V regions of Id antibodies or clonotypic T cells the complementary peptide process was utilized to investigate the Id/anti-Id network in the anti-La/SSB response and its regulation.

DOI: 10.1002/psc

The sequence of cpep349–364 was derived by 5′ to 3′ assignment of amino acids to the nucleotide sequence complementary to that of La/SSB mRNA encoding amino acids 349–364, a minor T/major B cell epitope (pep349–364). Cpeps were coupled to the SOC_n carrier in order to enhance their complementary potency, and to use them as immunogens. Conjugates I and II were injected in animals for the induction of anti-pep349–364 (Id) and anti-cpep349–364 (anti-Id) antibodies. These antibodies were tested for their specific recognition by the priming conjugates, as well as for their efficacy in neutralizing each other in vitro and *in vivo*.

Autoantigen La/SSB is a molecular target of humoral autoimmunity in patients with SS, and the autoantibody response to Ro/La RNPs is antigen driven. In this regard, regulation of the Id anti-La/SSB response by the anti-Id antibodies, induced by conjugate II, could be proven the method of choice.

In this study, sera obtained by rabbit immunizations were tested for their reactivity in recognizing the priming conjugates I and II (Figures 3 and 4). Inhibition, in vitro, experiments disclosed the specific interaction of anti-pep349–364 and anti-cpep349–364 (Id/anti-Id) antibodies (Figure 5). Furthermore, the ability of the complementary epitope of La/SSB to revoke the inhibitory effect of anti-Id antibodies on the binding of Id antibodies to the sense epitope of La/SSB confirmed the specificity between Id/anti-Id antibodies and complementary epitopes (Figure 6). *In vivo* experiments were also established to test whether the generation of anti-Id antibodies in animals immunized with the complementary epitope of La/SSB could block the development of Id antibodies of the La/SSB epitope. A mutual neutralization of Id/anti-Id antibodies was observed, which implies that generation of anti-Id antibodies by the complementary epitope of La/SSB could capture the anti-La/SSB response (Figures 7–9).

Serological Study

Induction of antibodies against conjugates I and II. Sera from rabbits immunized with each conjugate were collected before and after immunizations and were tested for their reactivity against the priming conjugate. The antibody reactivity was gradually increased and after the fourth boosting remained high until the end of the experiment. ELISA against conjugates I and II at serum dilution 1 : 800 are shown in Figures 3 and 4 respectively.

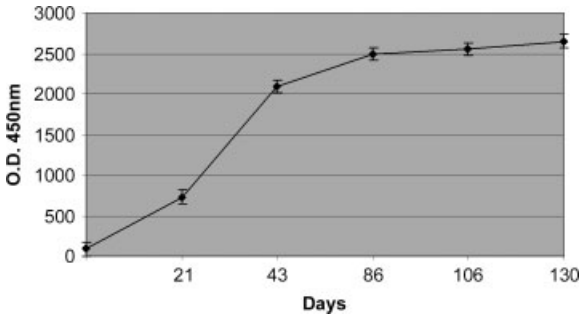

Figure 3 Binding of antisera raised in rabbits immunized with conjugate I. Control sera: preimmune, coating peptide: conjugate I, sera dilution 1/800.

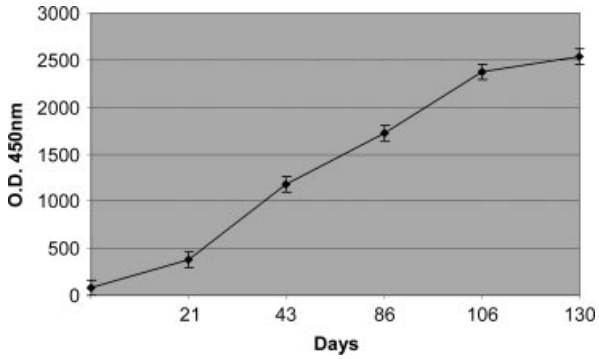

Figure 4 Binding of antisera raised in rabbits immunized with conjugate II. Control sera: preimmune, coating peptide: conjugate II, sera dilution 1/800.

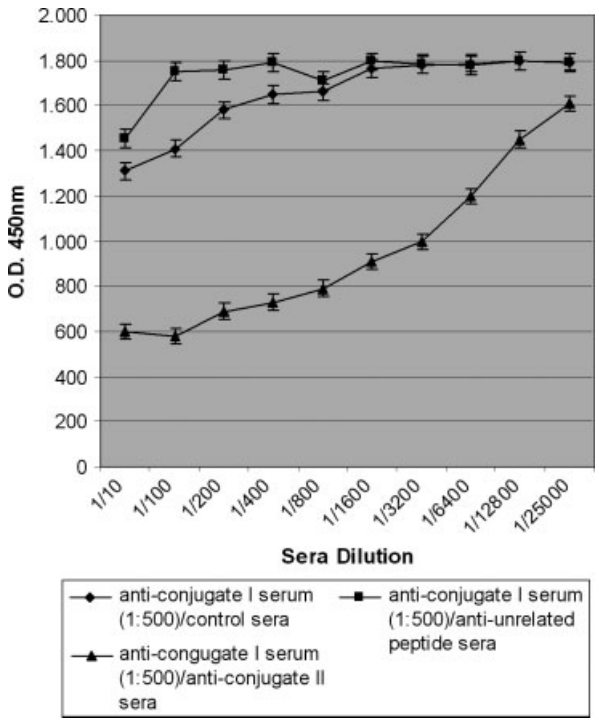

Figure 5 Inhibition ELISA of antisera binding to conjugate I. Fifty microliters of anti-pep349–364 serum (dilution 1/500) was incubated with 50 µl of anti-cpep349–364 serum, or with control serum (preimmune), or with antiunrelated peptide serum, at the indicated dilutions. Coating peptide: conjugate I.

Inhibition Studies

Specific interaction of anti-pep349–364 and anti-cpep349–364 antibodies. The Id/anti-Id relationship of anti-pep349–364 and anti-cpep349–364 antibodies

DOI: 10.1002/psc

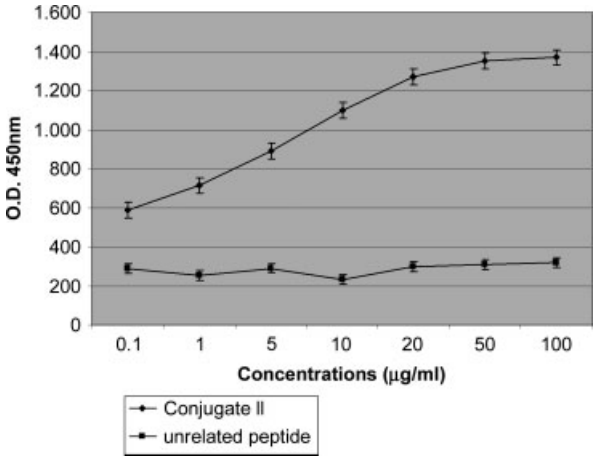

Figure 6 Inhibition ELISA of antisera binding to conjugate I. Fifty microliters of anti-pep349–364 serum (dilution 1/500) was incubated with a mixture of 25 μl of anti-cpep349–364 serum (dilution 1/2) and conjugate II, at the indicated concentrations, or with unrelated peptide. Coating peptide: conjugate I.

was evaluated by inhibition ELISA (Figure 5). Mixtures of anti-pep349–364 (50 μl, dilution 1/500) and anti-cpep349–364 sera (50 μl, dilutions ranging from 1/100 to 1/25 600), were added to ELISA plates coated with conjugate I. The presence of anti-cpep349–364 antibodies inhibited the anti-pep349–364 antibody binding to the immunizing peptide (conjugate I) in a dilution-dependent manner. On the contrary, control sera (preimmune) and antisera raised in rabbits immunized with unrelated peptide were unable to interrupt the anti-pep349–364 binding to the priming conjugate I. These data suggest the specific recognition of anti-pep349–364 and anti-cpep349–364 antibodies, which are Id/anti-Id antibodies derived from the complementary epitopes of the La/SSB autoantigen.

Specific inhibition of the anti-pep349–364/anti-cpep349–364 interaction by conjugate II. Figure 6 illustrates an anti-conjugate I ELISA in which increasing quantities of conjugate II (from 0.1 to 100 μg/ml) were added to a mixture of anti-pep349–364 (50 μg, dilution 1/500) and anti-cpep349–364 (25 μg, dilution 1/2) antibodies. Anti-pep349–364 antibodies were released from the Id/anti-Id complex upon addition of the complementary conjugate, which is specifically recognized by the anti-cpep349–364 antibodies. The ability of conjugate II, but not the unrelated peptide, to abrogate the inhibitory effect of anti-cpep349–364 antibodies on the binding of anti-pep349–364 antibodies to conjugate I confirms the specificity of the inhibition.

These results (Figures 5 and 6) strongly suggest that immunization with conjugate II induced an anti-Id antibody response against Id-bearing antibodies specific for the La/SSB epitope pep349–364. Furthermore, blocking of recognition of conjugate I proves that the anti-Id antibodies are apparently directed against the paratope, or combining site, of the Id-bearing anti-pep349–364, presumably against La/SSB.

Neutralization of anti-pep349–364 antibodies by anti-cpep349–364 antibodies in vivo. To test whether the generation of anti-Id antibodies by the complementary epitope of La/SSB would block the development of anti-pep349–364 antibodies, animals were preimmunized with conjugate II and were then challenged with the sense epitope of La/SSB (conjugate I).

One week after the sixth injection the anti-cpep349–364 response was fully expanded and high anti-Id titers were generated. One milligram of conjugate I was then injected followed by boostings with 0.5 mg of the same conjugate (protocol B). Blocking of the anti-cpep349–364 response by the production of anti-pep349-364 antibodies revealed the *in vivo* neutralization of the Id/anti-Id network (Figure 7). In another *in vivo* experiment, an increasing dose of conjugate I (from 25 to 500 μg) was applied (protocol C) to follow up the kinetics of the anti-pep349–364 Id antibodies. A progressive neutralization of the anti-Id antibodies by the Id antibodies was observed confirming the previous experiment (Figure 8). The gradual neutralization, *in vivo*, of the anti-Id antibodies by the Id antibodies was also demonstrated by the anti-conjugate II ELISA, depicted in Figure 9.

The reported *in vivo* experiments (Figures 7–9) point out, in agreement with the inhibition experiments in vitro (Figures 5 and 6), the potential of the complementary to the La/SSB epitope, cpep349–364, in lowering the anti-pep349–364 response. Previous studies have shown molecular spreading of epitopes to La/Ro RNP after immunization with a single epitope. In fact, immunization with pep349–364 generates antibodies recognizing the other La/SSB epitopes, as well as the La/SSB protein [23,24]. Eventually, decrease of the anti-pep349–364 response by the complementary cpep349–364 anti-Id antibodies might reflect a possible

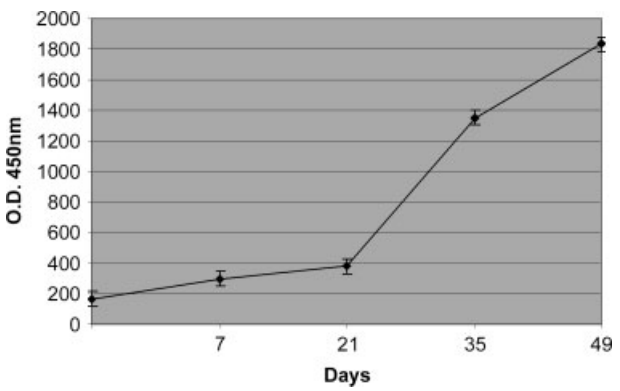

Figure 7 Anti-conjugate I ELISA. Effect of 0.5 mg immunization dose of conjugate I on the development of anti-pep349–364 antibodies in rabbits preimmunized with conjugate II. Control sera: preimmune. Coating peptide: conjugate I, sera dilution 1/400.

DOI: 10.1002/psc

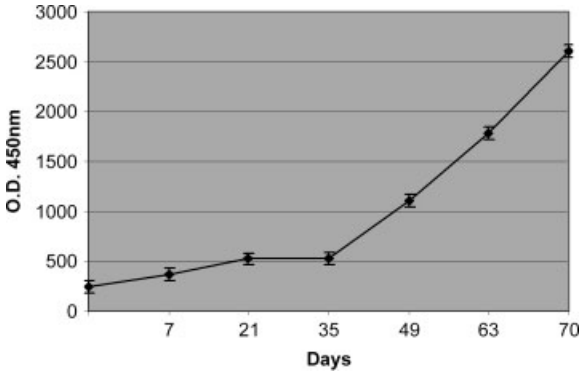

Figure 8 Anti-conjugate I ELISA. Effect of increasing immunization doses of conjugate I (from 25 to 500 μg/500 μl of H_2O) on the development of anti-pep349–364 antibodies in rabbits preimmunized with conjugate II. Control sera: preimmune. Coating peptide: conjugate I, sera dilution 1/400.

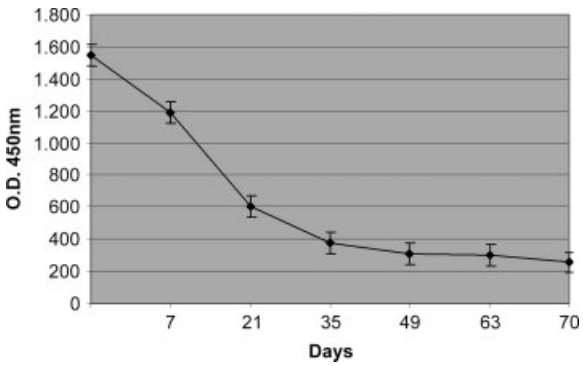

Figure 9 Anti-conjugate II ELISA. Effect of increasing immunization doses of conjugate I (from 25 to 500 μg/500 μl of H_2O) on the development of anti-pep349–364 antibodies in rabbits preimmunized with conjugate II. Control sera: preimmune. Coating peptide: conjugate II, sera dilution 1/800.

regulation of the anti-La/SSB autoimmune response in SS and SLE patients.

On the other hand, the presence of both anti-pep349–364 and anti-cpep349–364 antibodies in the sera of autoimmune patients with SS and SLE [25] raises the question for the utility of anti-Id antibodies in regulating the autoimmune response, since it is still not clear whether the anti-cpep349–364 response is the initiator for the formation of anti-pep349–364 antibodies or it is a consequence of them [25]. However, recent studies suggest that the activation of the Id/anti-Id circuit is depended on the mutual interaction of Id and anti-Id B cells, presenting continuously idiopeptides derived from the V-region to specific T lymphocytes [40–42]. In this regard one might hypothesize that the complementary epitope cpep349–364 of La/SSB could intervene in the mutual interaction of Id/anti-Id B cells by generating anti-Id antibodies, which could scavenge the Id anti-La/SSB autoantibodies.

CONCLUSIONS

In this study, the complementary La/SSB epitope K^{364}FRFLALKLYFSFTRP^{349} (cpep) and the sense minor T/major B cell epitope G^{349}SGKGKVQFQGKKTKF^{364} (pep), coupled to the SOC_4 carrier to enhance their complementary potency, were utilized in rabbit immunization experiments as an alternative modality for regulating the anti-La/SSB immune response.

Anti-cpep349–364 antibodies inhibited the anti-pep349–364 antibody binding to the immunizing peptide (conjugate I) in a dilution-dependent manner, while increasing amounts of the complementary conjugate II added to a mixture of Id/anti-Id antibodies released anti-pep349–364 antibodies. Our data provide proof of evidence for specific recognition between Id/anti-Id antibodies, and substantiate the specific dissociation of the Id/anti-Id heterodimeric complex by adding either the sense epitope (conjugate I) of La/SSB or its complementary counterpart (conjugate II).

On the basis of the reported in vitro results, rabbit immunization experiments were established suggesting that the produced *in vivo* anti-Id antibodies neutralize the generated Id antibodies. Taking into account the presence of both Id/anti-Id antibodies in sera of autoimmune patients with SS and SLE and the mutual interaction of Id and anti-Id B cells in activating the Id/anti-Id circuit, one might assume that the complementary epitope of La/SSB, cpep349–364, could intervene in the Id/anti-Id B cell interaction by generating anti-Id antibodies, which could trap the Id anti-La/SSB response.

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