Routsias JG, Dotsika E, Touloupi E, Papamattheou M, Sakarellos C, Sakarellos-Daitsiotis M, Moutsopoulos HM, Tzioufas AG. Ιδιοτυπικό – αντιιδιοτυπικό δίκτυο ***δίκτυο σε μη αυτοάνοσα ποντίκια έπειτα από ανοσοποίηση με τον επίτοπο και τον συμπληρωματικό επίτοπο 289-308αα του La/SSB. J Autoimmun. 2003*** Aug;21(1):17-26.

Με βάση τα δεδομένα πρόσφατων εργασιών του εργαστηρίου μας, εκτός από την παρουσία αντισωμάτων έναντι του La/SSB στον ορό ασθενών με ΣΕΛ και Σύνδρομο Sjogren, αποκαλύψαμε την παρουσία ενός ενεργού ιδιοτυπικού- αντι-ιδιοτυπικού δικτύου έναντι των επιτόπων του La/SSB, μετά από ανίχνευση με τη χρήση συμπληρωματικών πεπτιδίων έναντι των Β-κυτταρικών επιτόπων του αυτοαντιγόνου. Σκοπός της παρούσας εργασίας ήταν η μελέτη της παραγωγής αντισωμάτων και των Τ-κυτταρικών απαντήσεων μετά από ανοσοποίηση μη επιρρεπών στην εμφάνιση αυτοανόσου νοσήματος ποντικών (Balb/c, πέντε πειραματόζωα/ ομάδα) με τον επίτοπο La/SSB 289-308 aa, καθώς και με τον συμπληρωματικό του. Η ανοσοποίηση τόσο με το ανοσογόνο, όσο και με το συμπληρωματικό πεπτίδιο οδήγησε στην παραγωγή αντισωμάτων, ενώ σε δύο από τα πέντε ποντίκια παρατηρήθηκε εξάπλωση της ανοσολογικής απόκρισης και στον κύριο Β-κυτταρικό επίτοπο του La/SSB 349-364 aa. Στα υπόλοιπα τρία ποντίκια, μετά από κατεργασία των ορών αποκαλύφθηκε το ισχυρό αντι-ιδιοτυπικό δίκτυο που κάλυπτε την παρουσία των αντισωμάτων έναντι του 349-364 aa, ενώ ισχυρές Τ-κυτταρικές απαντήσεις ανιχνεύθηκαν και για τα δύο πεπτίδια, δεδομένα που μπορεί να μας βοηθήσουν στην περαιτέρω κατανόηση της έναρξης και της παραμονής της αυτοάνοσης απάντησης.

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Idiotype–anti-idiotype circuit in non-autoimmune mice after immunization with the epitope and complementary epitope 289–308aa of La/SSB: implications for the maintenance and perpetuation of the anti-La/SSB response

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Abstract

Background: Antibodies to La/SSB are usually found in sera of patients with Sjogren's Syndrome (SS) and Systemic Lupus Erythematosus (SLE). Recent work from our laboratory (Mol Med 2002;8:293–305) revealed that an active idiotypic network involving antibodies to epitopes of La/SSB and their anti-idiotypes exist in human sera. The anti-idiotypic antibodies were detected using complementary peptides to B-cell epitopes of the autoantigen. The principle of the complementary peptides is based on the 'molecular recognition' theory. According to this theory, translation of two complementary RNA strands (coding and non-coding strand) into protein, generate a pair of peptides, which bind each other with specificity and high affinity.

Aim: To investigate antibody production and T-cell responses in non-autoimmune-susceptible animal strains which were immunized with the epitope 289–308aa of La/SSB as well as its complementary epitope.

Materials and Methods: Balb/c mice were immunized with a peptide corresponding to epitope 289–308aa (pep) or its complementary (cpep) peptide (5 animals/group). The sera were tested for the presence of antibodies to pep and cpep as well as for epitope spreading to recombinant human La/SSB and a major B-cell epitope of La/SSB spanning the region 349–364aa. Another group of animals was sacrificed on day 10 and T-cell responses against pep and cpep were evaluated in cells from lymph nodes and spleen.

Results: Immunizations with either pep or cpep led to the appearance of antibodies against the immunogen peptide by day 31 which subsequently was followed by antibody production to its complementary peptide by day 55. In two out of five animals immunized with the epitope 289–308aa, a spreading of the immune response to epitope 349–364aa was observed. In the remaining three animals, negative for antibodies to pep349–364, a specific treatment of sera, using cpep349–364 revealed that anti-idiotypic antibodies masked antibodies to pep349–364. In all immunization experiments high T-cell proliferative responses to both pep and cpep peptides were detected.

Conclusions: Complementary peptides to epitopes of La/SSB can be utilized as probes to study the development of an idiotypic–anti-idiotypic network towards the major autoantigen. The ability of pep and cpep peptides to induce both B-cell and T-cell responses may provide useful insights into understanding further the initiation and maintenance of autoimmune response and create new tools for therapeutic intervention.

Keywords: La/SSB; Anti-idiotypic antibodies; Complementary peptides; Sjogren's syndrome; Lupus

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1. Introduction

La/SSB autoantigen is a highly conserved protein, originally defined as a major antigenic target in sera of patients with Sjogren's Syndrome (SS) and Systemic Lupus Erythematosus (SLE) [1]. Despite the extensive investigations into the mechanisms involved to breakdown the immunological tolerance and the establishment of an autoimmune response against La/SSB in these diseases, yet little is known [2]. Considerable evidence, however, suggests that autoreactive La/SSB specific B-cells exist in normal population and their activation depends upon T-cell assistance [3]. T-cell help could be achieved either utilizing tolerance—escaped cryptic/non-dominant T-cell determinants within the La/SSB autoantigen [4–6] or through activation of an idiotypic–anti-idiotypic T-helper circuit that involves tolerance—devoid peptides, located within the idiotypes of the autoantibodies (idiopeptides) [7–9]. The latter mechanism might be implicated for previously published results, reporting the establishment of an anti-La/SSB response after immunization with a monoclonal anti-DNA antibody [10], as well as the induction of anti-DNA response in mice after immunization with a monoclonal anti-La/SSB antibody [11].

In our recent work, we employed complementary peptides to the epitopes of La/SSB, in order to study the idiotypic–anti-idiotypic network of anti-La/SSB response in human autoimmune diseases [12]. Complementary peptides are artificially made and specified by complementary nucleotide sequences of the RNA encoding the antigenic epitopes. These peptides have the ability to recognize the epitope peptides with high affinity and specificity like the antigen binding site (paratope) of an antibody does and have been previously described as suitable substitutes of idiopeptides in generation of anti-idiotypic antibodies [13,14]. Using these complementary epitopes for one immunodominant T-cell/minor B-cell epitope (La/SSB: 289–308aa) [6,15] and one subdominant T-cell/major B-cell epitope (La/SSB: 349–364aa) [15,16], we have uncovered the existence of an active anti-Id response that targets and mask anti-La/SSB antibodies in human autoimmune sera [12].

The present study extends our previous work, demonstrating the potential of the complementary epitope 289–308aa to induce the generation of anti-Id antibodies against La/SSB followed by the development of a full blown anti-La/SSB response, targeting both minor and major B-cell epitopes of La/SSB, in non-autoimmune susceptible background. Immunization of BALB/c mice with either epitope or complementary epitope resulted in both humoral (B-cell) response and strong T-cell proliferative response against both peptides. The quantitative analysis of antibody production over time allowed us to define the development of the Id–anti-Id network in vivo in mice and to gain insight into the development of similar networks in humans associated with pathogenesis and maintenance of the autoimmune disease.

2. Materials and methods

2.1. Peptide synthesis and purification

Complementary epitopes, were in silico—designed by 5′ to 3′ assignment of amino acids to nucleotide sequence complementary to the regions of La/SSB mRNA encoding the antigenic determinants 289–308aa and 349–364aa. The epitopes ^{289}NNGNLQLRKEVTWEV LEG^{308} (pep289–308) and G^{349}SGKGKVQFQGKK TKF^{364} (pep349–364) as well as the corresponding complementary epitopes S^{308}FEYFPSHFFVPELEVTIIC^{289} (cpep289–308) and K^{364}FRFLALKLYFSFTRP^{349} (cpep349–364) were synthesized anchored to the Lys residues of the helicoid backbone Ac-(Lys-Aib-Gly)$_4$, (SOC_4) by standard solid phase peptide synthesis [17].

An irrelevant SOC_4-peptide sequence [(IASRYDQL)$_4$-SOC, corresponding to the sequence 250–257aa of Leismania gp63] was also constructed and used as a control peptide.

2.2. La/SSB recombinant protein

La/SSB recombinant protein (hLa/SSB) was prepared from a human La/SSB cDNA as previously described [18] and purified by poly(U)-Sepharose affinity chromatography [19].

2.3. Animals and immunizations

Eight week old female BALB/c mice were obtained from the Animal Breeding Unit, Hellenic Pasteur Institute. All mice were maintained under specific pathogen free conditions. Groups of 6–8 mice were immunized subcutaneously with 50 μg of pep289–308 or cpep289–308 conjugated to the new carrier SOC_4 emulsified in Complete Freud's Adjuvant (Difco, Detroit Michigan, USA) followed by three boostings of the same dose of conjugated peptides in Incomplete Freud's Adjuvant (Difco, Detroit Michigan, USA) on days 15, 30 and 150. Controls were immunized with the adjuvant or the SOC_4 carrier alone in similar conditions. Sera obtained from mice were collected at different postimmunization time points and stored at −20 °C until testing for specific antibodies.

2.4. Assays for anti-peptide and anti-La/SSB antibody detection

Antibody responses against epitope, complementary epitopes and recombinant hLa/SSB antigen were monitored by solid phase assay as previously described [12]. Briefly, ELISA plates (Corning-Costar, NY, USA) were

coated overnight at 4 °C with the appropriate coating solution: (i) for pep289–308 or cpep289–308, 10 μg/ml in carbonate/bicarbonate buffer pH=9.1; (ii) pep349–364 or cpep349–364, 10 μg/ml in phosphate buffer pH=7.1; and (iii) recLa/SSB 3 μg/ml in phosphate buffer pH=7.1. Non specific binding was eliminated using blocking buffer consisted of 2% bovine serum albumin in phosphate buffer pH=7.1). Subsequently, the plates were incubated overnight at 4 °C with mice sera, diluted 1:400 in blocking buffer. Antibodies bound onto the solid phase were detected using an alkaline phosphatase conjugated anti-mouse IgG antibody (Jackson Immuno-Research, Baltimore, USA). Afterwards, the substrate paranitrophenylphosphate was added and the absorbance was measured at 405 nm.

2.5. Inhibition assays

(i) Homologous inhibition of anti-peptide antibodies: The specificity of the binding of immunized mice sera to pep289–308 and cpep289–308, was evaluated by homologous inhibition using known amounts of pep289–308, cpep289–308 or SOC carrier as inhibitor. Inhibitors were added in the diluted mouse sera at increasing concentrations ranging from 10 ng/ml to 10 μg/ml and the mixtures were incubated for 2 h at room temperature before the application in peptide coated ELISA wells. Inhibition was expressed as a percentile reduction of antibody binding of the uninhibited positive mouse serum.

(ii) Inhibition of antibody binding to recombinant hLa/SSB: The ability of the hLa/SSB epitope analogue pep289–308 to abrogate the binding of mouse specific anti-pep289–308 antibodies to recombinant hLa/SSB was sought by an ELISA inhibition assay. Microtiter plates were coated with recombinant La/SSB and blocked as described previously. Subsequently, pre-incubated (for 2 h at room temperature) mixtures of diluted mouse sera (1:400 in blocking buffer) with different concentrations of pep289–308 (varying from 10 ng/ml to 10 μg/ml) were added to the wells. The procedure was then continued as described for anti-La/SSB ELISA assay.

2.6. Heat and complementary peptide treatment of immune sera for deactivation of anti-Id antibodies

As demonstrated previously in human autoimmune sera [12], antibodies to complementary epitopes anti-cpep289–308 or anti-cpep349–364 compete with the La/SSB antigen for binding to the paratope of anti-pep289–308 or anti-pep349–364 antibodies, respectively. In order to overcome possible anti-Id interference in the detection of anti-peptide or anti-La/SSB antibodies, we used a procedure where, sera from immunized mice were heated at 55 °C for the dissociation of Id–anti-Id complexes in the presence of 30 μg/ml cpep289–308 or cep349–364 [12]. Afterwards, the mixture was cooled slowly from 55 to 25 °C over a period of 3 h, allowing the complementary peptide which had been added in molar excess to block the anti-complementary peptide antibodies, releasing eventually a portion of anti-La/SSB antibodies. These antibodies were measured afterwards, using an ordinary anti-hLa/SSB or anti-pep349–364 ELISA.

2.7. T-cell proliferation assays

Eight-week old female BALB/c mice, were immunized with 100 μg of either pep289–308 or cpep289–308 emulsified in complete Freund's adjuvant (Difco, Detroit, Michigan, USA) subcutaneously in one hind footpad. Ten days after immunization, draining inguinal and popliteal lymph nodes were removed and single-cell suspensions (1×10^6 cells/ml) were prepared. Microcultures were established in 96-well flat-bottom microtiter plates with RPMI-1640 (Seromed) culture medium supplemented with 10 mM Hepes, 5×10^5 M 2-Me, 2 mM L-glutamine, 24 mM NaHCO3, 100 Units/ml penicillin, 100 μg/ml streptomycin and 5% heat inactivated fetal calf serum. Cells were cultured with various concentrations (ranging from 25 to 800 μg) of peptides pep289–308 and cpep289–308. Plates were incubated for 4 days at 37 °C in 5% CO_2 with 1 μCi/well of [^{3}H]-thymidine added during the final 18 h of the culture. Cells were harvested onto glass-fiber filters and the radioactivity was determined using a liquid scintillation counter. All cultures were performed in triplicate and the results were expressed as stimulation index (SI).

3. Results

3.1. Immunization with either epitope or complementary epitope of human La/SSB induces antibody production to both peptides in non-autoimmune susceptible mice

To establish the etiologic relation of anti-cpep and anti-pep antibodies, animal immunizations with pep289–308 and cpep289–308 were performed. Immunization of BALB/c mice with cpep289–308 led to the development of anti-cpep289–308 antibodies by day 31 followed by the production of anti-pep289–308 antibodies by day 55 (Fig. 1A). When pep289–308 was used as immunogen anti-pep289–308 antibodies was first detected at day 31 while anti-cpep289-308 antibodies followed later by day 55. The delayed appearance of antibodies to the complementary epitopes suggests that they are generated in response to antibodies against the epitopes and not to the initial antigenic stimulus. The same phenomenon was also observed in

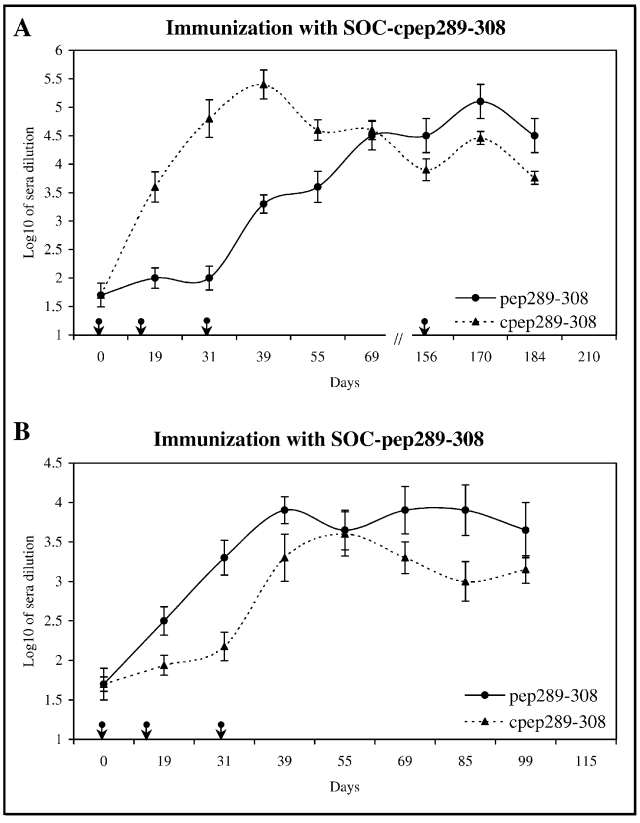

Fig. 1. Immunization with either epitope or complementary epitope of human La/SSB induces antibody production to both peptides. Kinetics of antibody production against pep289–308 and cpep289–308 peptides after immunization with either cpep289–308 (A) or pep289–308 (B) are depicted. Arrows indicate the day of antigen administration (immunization+3 boostings).

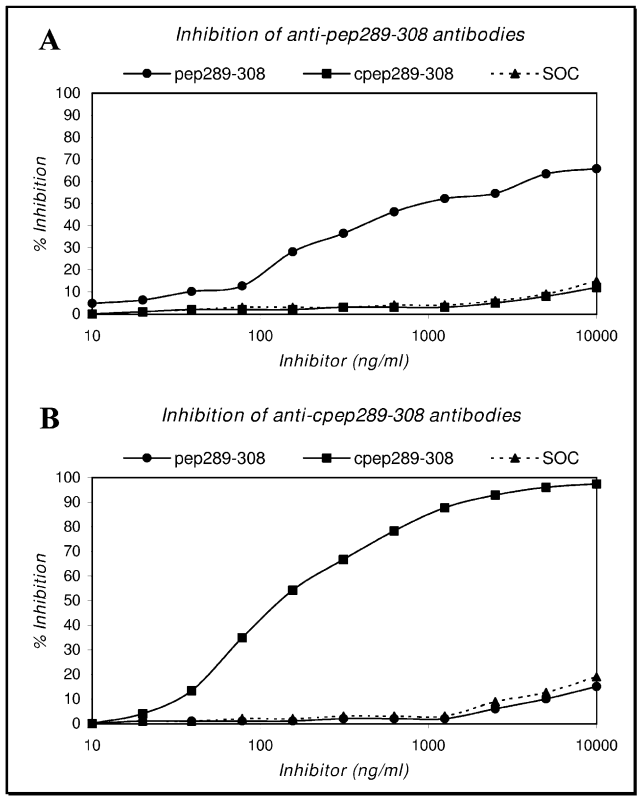

Fig. 2. Homologous inhibition of antibodies against pep289–308 and cpep289–308 from sera drawn from mice immunized with cpep289–308. Soluble pep289–308 inhibited the binding to pep289–308 by 62% (A) while cpep289–308 produced 97% inhibition of the binding to cpep289–308 (B). Control peptides caused minimal or no inhibition.

the case of animals immunized with the epitope 289–308.

Interestingly, mice immunized with the complementary epitope cpep289–308 gradually developed increasing levels of antibodies to epitope pep289–308 anti-pep, which rose to levels close to those of anti-cpep289–308 by 69 days after immunization (Fig. 1A). This finding prompted us to examine the kinetics of antibody production for a longer period of time. Thus, mice immunized with cpep289–308 exhibited antibody titers of 1/32,000 (OD≈2.300 at 1/300 serum dilution) for anti-cpep289–308 and 1/4000 (OD≈0.900 at 1/300 serum dilution) for anti-pep289–308 by day 55, which was reversed to 1/4000 (OD≈0.900 at 1/300 serum dilution) for anti-cpep289–308 and 1/32,000 (OD≈2.300 at 1/300 serum dilution) for anti-pep289–308 at day 184 (Fig. 1A).

The anti-pep/anti-cpep antibody production, in mice immunized with the epitope 289–308, was quantified over a period of 99 days. In these mice the emergence of high titer (OD≈0.900 at 1/300 serum dilution) anti-cpep289–308 antibodies was accompanied by a simultaneous decrement of anti-pep289–308 titer (from OD≈1.100 to OD≈0.900 at 1/300 serum dilution) at day 55. Thirty days later, the reduction of anti-cpep289–308 antibody levels (OD≈0.700 at 1/300 serum dilution) was followed by an increase of anti-pep289–308 antibody titer (OD≈1.100 at 1/300 serum dilution) (Fig. 1B). Such fluctuation in anti-pep and anti-cpep antibody levels was not observed in mice immunized with cpep289–308.

The specificity of anti-pep289–308 and anti-cpep289–308 assays was confirmed by homologous inhibition experiments using increasing quantities of soluble pep289–308, cpep289–308 and SOC_4 carrier peptides as inhibitors. The binding of sera, drawn from mice immunized with cpep289–308, to pep289–308 and cpep289–308 was inhibited by 62 and 97% after prior incubation with peptides pep289–308 and cpep289–308, respectively (Fig. 2). In both assays, the control peptides or the SOC_4 peptide carrier alone, when used as inhibitors did not affect the antibody binding (Fig. 2). In addition, the reaction of immobilized pep289–308 and cpep289–308 with sera from mice immunized with pep289–308, was inhibited by 72 and 83% after prior incubation with

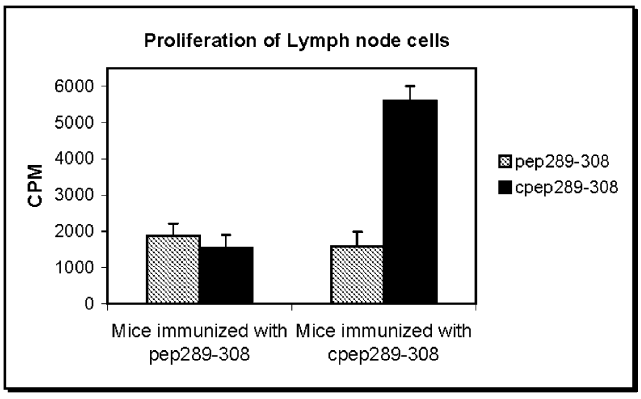

Fig. 3. Proliferative responses of lymph node cells to pep289–308 and cpep289–308 after immunization with either pep289–308 or cpep289–308 peptides.

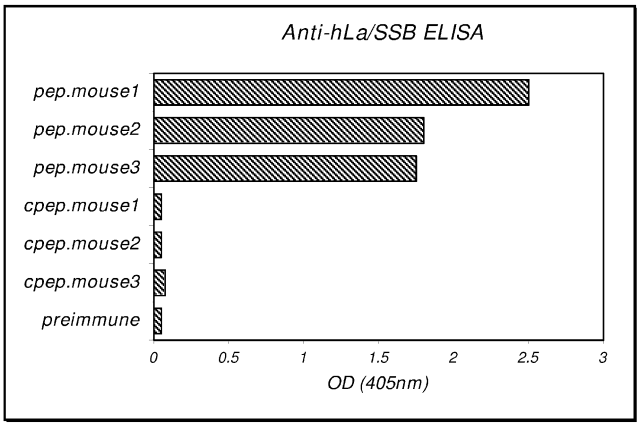

Fig. 4. Recognition of hLa/SSB autoantigen by sera from three representative mice immunized with either the La/SSB epitope pep289–308 or the complementary epitope cpep289–308.

soluble pep289–308 and cpep289–308, respectively (data not shown).

3.2. Immunization with either epitope or complementary epitope of La/SSB, induces strong proliferative T-cell responses showing specificity to both pep and cpep peptides

In order to determine if the Id and anti-Id antibodies, generated upon immunization with either epitopes or complementary epitopes, involve the presentation of the same peptides to specific T-lymphocytes, lymph node T-cells were tested for their specificity in vitro. Animals immunized with either pep289–308 or cpep289–308 peptide showed substantially increased levels of T-cells responding to both pep289–308 and cpep289–308 peptides (Fig. 3). The ability of pep289–308 and cpep289–308 peptides to induce strong T-cell proliferative responses, independently of the peptide used for immunization, indicates that both peptides can serve as T-cell epitopes. The proliferative response of T-cells taken from mice immunized with cpep289–308 against the complementary epitope cpep289–308 was stronger compared to other responses (Fig. 3), indicating most probably the dominance of the T-cell epitope located within cpep289–308, in the context of the specific genetic background of BALB/c (H-2d) mice. If the later is true, then different proliferative responses could be expected in animals with different genetic background.

3.3. Specific recognition of hLa/SSB autoantigen by sera from mice immunized with the La/SSB epitope 289–308

As shown above, BALB/c mice immunized with the epitope pep289–308 of hLa/SSB or its corresponding complementary epitope, produced anti-pep289–308 antibodies after 31 or 55 days, respectively. To test whether immunized mice contained also antibodies directed towards the whole hLa/SSB protein, sera taken 55 days after the first immunization were evaluated in a recombinant hLa/SSB ELISA assay. All sera from pep289–308 immunized mice recognized the hLa/SSB, while sera from mice immunized with cpep289–308 did not react with the recombinant hLa/SSB (Fig. 4).

3.4. Unmasking the anti-hLa/SSB response in mice immunized with the complementary epitope cpep289–308

The failure of sera from mice immunized with cpep289–308 to recognize hLa/SSB protein was unexpected, since the same sera exhibited high reactivity against the epitope pep289–308 of La/SSB. This observation prompted us to examine whether anti-cpep289–308 antibodies interfere in hLa/SSB ELISA assay, blocking with high affinity bivalent binding the anti-pep289–308 antibodies. If this is the case, monovalent binding of antibodies to epitope 289–308 onto recombinant hLa/SSB is not sufficient to release them from their soluble inhibitor (anti-cpep289–308) and bivalent binding to the more flexible, absorbed in high density, multimeric peptide pep289–308 is required. In this regard, we applied heat and complementary peptide treatment procedure for inactivation of anti-cpep antibodies (Fig. 5A and B). Blocking the anti-cpep antibodies with cpep289–308 peptide, restored a part of the anti-hLa/SSB reactivity in all sera from mice immunized with cpep289–308, but not in normal mouse sera, pointing the critical role of Id–anti-Id network in the detection of anti-La/SSB antibodies (Fig. 5C). The possibility that heating at 55 °C caused a non-specific enhancement of antibody binding to hLa/SSB in mice sera must be excluded, since neither thermal treatment alone or

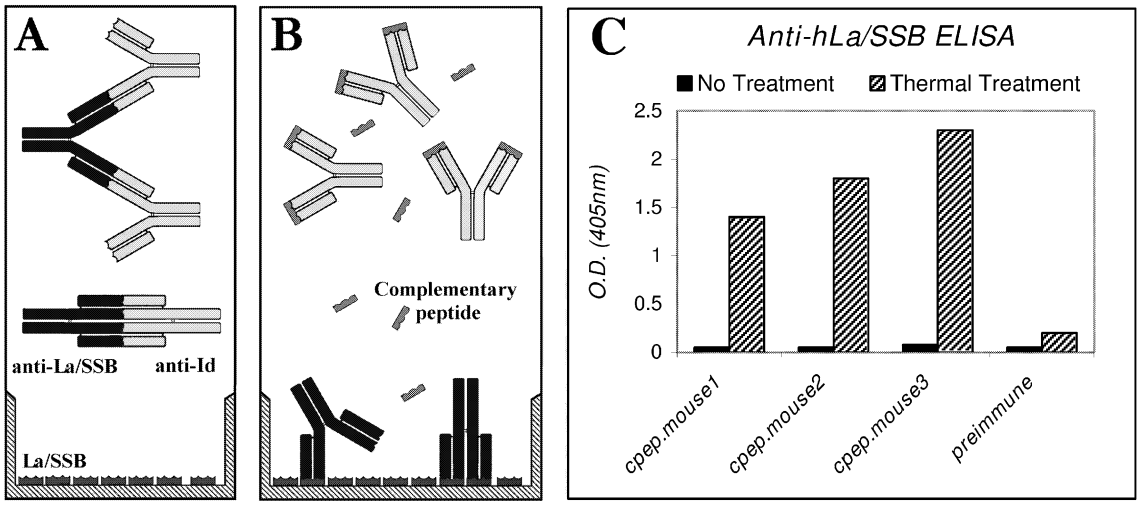

Fig. 5. Recovery of masked anti-hLa/SSB reactivity in mice immunized with cpep289–308. In these sera, anti-cpep289–308 antibodies (shown in gray) bind and mask anti-La/SSB (anti-pep289–308) antibodies (shown in black) (A). After the complementary peptide treatment procedure, the anti-pep289–308 antibodies unmasked and detected by an anti-hLa/SSB ELISA (B). The reactivity of sera against hLa/SSB, before and after application of the complementary peptide treatment procedure, is depicted in panel C for three representative animals immunized with cpep289–308 peptide and one preimmune serum.

Table 1
Recognition of hLa/SSB by mouse sera

	Immune Serum	Preimmune serum
No treatment	0.23	0.06
Thermal treatment+cpep289–308	2.50	0.21
Thermal treatment without peptide	0.13	0.03
Thermal treatment+control peptide	0.08	0.05
Addition of control peptide	0.08	0.05

thermal treatment with control peptide affected the recognition of hLa/SSB by mouse sera (Table 1).

3.5. Blocking of anti-Id antibodies unveils epitope spreading to La/SSB epitope 349–364

To assess the possibility that anti-La/SSB response in mice immunized with cpep289–308 was also diversificated beyond pep289–308 to other determinants of La/SSB, sera from mice immunized with cpep289–308 were tested for their reactivity against the epitope pep349–364 (a major B-cell epitope of La/SSB) and its complementary peptide cpep349–364. All five mice sera tested exhibited high reactivity against cpep349–364 while only two out of five of the same sera recognized the La/SSB epitope pep349–364. It is difficult to explain the anti-cpep349–364 reactivity without the presence of anti-pep349–364 antibodies at the same time, since the generation of anti-cpep349–364 is supposed to occur as an anti-idiotypic response to anti-pep349–364 antibodies. One interpretation of these results is that anti-cpep349–364 antibodies block and mask anti-pep349–364 antibodies, in a way similar to that observed for the anti-cpep289–308 and anti-pep289–308 antibodies, resulting in the abolishment of anti-pep349–364 reactivity in three out of five sera tested. If the latter is true, then specific inactivation of anti-cpep349–364 antibodies, using increased temperature for the dissociation of Id–anti-Id immune complexes, followed by the addition of cpep349–364 in order to inhibit the anti-Id (anti-cpep) antibodies, could be expected to restore anti-pep349–364 reactivity in these sera. Indeed, after this specific treatment all immunized sera exhibited high reactivity with the epitope pep349–364, suggesting that true intramolecular epitope spreading had occurred but it was hidden by anti-idiotypic antibodies (Fig. 6). We examined next the kinetics of the appearance of masked anti-pep349–364 antibodies. We detected anti-pep349–364 antibodies 20 days before the appearance of anti-cpep349–364 antibodies (at day 60), suggesting that anti-cpep349–364 antibodies occur as anti-Id response to anti-pep349–364 antibodies (Fig. 6).

4. Discussion

Many studies have emphasized the importance of idiotypic–anti-idiotypic network in the initiation, maintenance and regulation of the autoimmune response [8,10,11,20,21]. In our recent work we sought to study Id–anti-Id regulation of anti-La/SSB response in patients with SLE and SS by employing complementary epitopes (cpep289–308 and cpep349–364) to linear epitopes pep289–308 and pep349–364 of La/SSB, as immunoglobulin V-region peptide (idiopeptide) mimetics [12]. The pairs of epitopes and complementary epitopes were found to interact with each other by

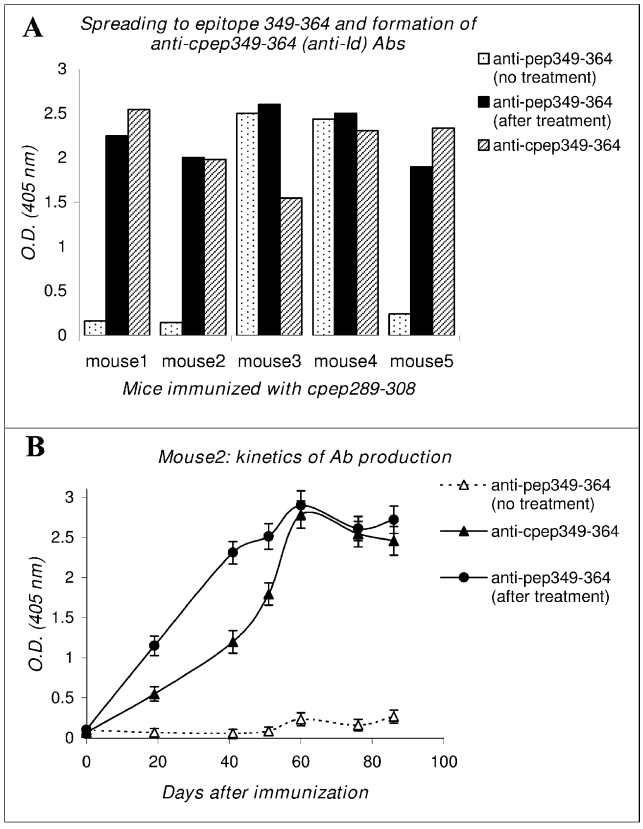

Fig. 6. Blocking of anti-cpep349–364 antibodies (after treatment with cpep349–364 peptide) unveils epitope spreading to La/SSB epitope 349–364 (A). Kinetics of antibody production against pep349–364 and cpep349–364 are presented in panel B for cpep289–308 immunized animals.

means of electrospray ionization mass spectroscopy and competition ELISA assay [12]. Anti-cpep antibodies, isolated from autoimmune sera, were found to possess anti-idiotypic activity and to compete with pep- or La/SSB antigen for binding to the paratopic site of purified anti-pep antibodies. In the present work, we have explored the mechanisms involved in the genesis of these Id–anti-Id (anti-pep–anti-cpep) antibodies by performing animal immunizations with La/SSB epitope pep289–308 and its complementary idiopeptide-mimetic cpep289–308.

The etiologic relation of Id and anti-Id antibodies was clearly demonstrated in BALB/c mice immunized with either the epitope pep289–308 or the complementary epitope cpep289–308. In both immunization experiments, the appearance of idiotypic antibodies (antibodies against the immunizing peptide), was established by day 31, followed later, by day 55, by anti-idiotypic antibody production (antibodies against the complementary form of the immunizing peptide). Therefore, immunization with either pep289–308 or cpep289–308 led to the generation of both anti-pep289–308 and anti-cpep289–308 antibodies within a period of 55 days, suggesting their Id–anti-Id relationship.

The quantitative analysis of the Id and anti-Id antibody production revealed that immunization with cpep289–308 resulted initially in high levels of anti-cpep289–308 IgG (1:32,000 at day 55), which after 130 days dropped to lower levels (1:5000 at day 184) with a simultaneous increase of anti-pep289–308 IgG titer (from 1:3200 at day 55 to 1:32,000 at day 184). One interpretation of this finding is that in the absence of cpep stimulus, the production of antibodies to complementary epitopes fell to lower levels, while the immune response against the epitopes was maintained and enhanced by the endogenous mouse La/SSB processing and presentation which is highly homologous with human La/SSB (95% similarity). Therefore, the perturbation of the Id–anti-Id network by the endogenous La/SSB processing could be responsible for the dominance of anti-pep289–308 antibodies in both pep289–308 and cpep289–308 immunized mice after a long period of time. Data to substantiate the continuous processing and presentation of endogenous La/SSB to autoreactive B-cells has been obtained recently by Keech et al., using transgenic mice expressing La/SSB [3]. Regardless of the potential interference of endogenous La/SSB in anti-idiotypic antibody production, the data presented in this report, suggest that anti-La/SSB antibodies can occur as a response to cpep289–308 in a way similar to that reported recently for the induction of anti-DNA response upon immunization with pCDR1 and pCDR3 idiopeptides of an anti-DNA antibody [8]. Hence, cpep289–308 (or antigenic structures resembling cpep289–308) could be the initiating agent for the formation of antibodies against La/SSB. From this point of view, molecular mimicry of cpep289–308 with an infection agent might be considered as a potential triggering factor of autoimmune response against La/SSB. The most prominent sequence similarities of cpep289–308 are summarized in Table 2.

Another finding, emerged from the analysis of the antibody specificity in immunized animals, is that sera from mice immunized with the epitope 289–308 contain antibodies that could readily bind to recombinant hLa/SSB, in contrast to sera from mice immunized with complementary epitope (cpep289–308) which reacted with hLa/SSB protein only after the inhibition of anti-cpep289–308 antibodies. These findings suggest that the antibodies to complementary epitope, overshadow and inhibit the anti-pep289–308 antibody binding onto hLa/SSB, most probably due to a higher affinity of anti-pep289–308 antibodies for anti-cpep289–308 (against which originally rose as anti-idiotypic response), than for the hLa/SSB protein which possess complementary structures to cpep289–308 (in its region spanning the sequence 289–308aa).

Table 2
Sequence similarities of cpep289–308 with xenoantigens

Sequence	Protein	Species	Positives (%)	Identities (%)
SFEYFPSHFFVPELEVTIIC	La/SSB complementary peptide			
YYPHDFFVPNSE	Rv2067c Protein	*Mycobacterium tuberculosis*	66	58
YFPAHFISEGLDQT	Isoleucyl–tRNA synthetase	*Treponema pallidum*	64	50
HFFVPSTEKT	Protease ydcP precursor	*Escherichia coli*	70	70
SDEYFKKYFKALEEEITV	V-ATPase A-subunit	*Plasmodium falciparum*	60	44
PSNFFIPDPE HAFIPDADVRLI	Tryptophan synthase beta chain	*Streptomyces coelicolor*	90	60
FKSHAYLKELEKTL	Triosephosphate isomerase	*Helicobacter pylori*	71	50

We have previously shown that immunization of young New Zealand white rabbits with the La/SSB epitope pep289–308 led to intramolecular spreading of anti-La/SSB response to other previously defined antigenic regions of La/SSB antigen, such as the epitope 349–364aa [16]. The epitope 349–364 serves as major B-cell epitope in La/SSB, since it is recognized by the majority of anti-La/SSB positive autoimmune sera [15]. In the present study, it was found that immunization of BALB/c mice with the complementary epitope 289–308 induced not only the generation of antibodies to the epitope pep 289–308 of hLa/SSB, as an anti-idiotypic response, but also to the major antigenic determinant 349–364aa of La/SSB. This finding indicates that the anti-idiotypic response to the complementary epitope 289–308 of La/SSB, is a full-blown anti-La/SSB response which exhibits the immunologic characteristics of the anti-La/SSB response of human autoimmune diseases i.e. the isotype switching and epitope spreading. These observations argue in favor of the assumption that cpep289–308 could serve under certain, still unidentified, circumstances as a triggering agent of the anti-La/SSB response.

The proliferation experiments with T-cells harvested from lymph nodes of either pep289–308 or cpep289–308 immunized animals, disclosed that both peptides were capable of inducing substantial proliferative T-cell responses, implying that both peptides can also serve as T-cell epitopes. This finding is consistent with recent studies which suggest that the activation of Id–anti-Id circuits is dependent on the mutual interaction of idiotypic and anti-idiotypic B-cells, presenting continuously idiopeptides derived from the V-region to specific T-lymphocytes [9,22,23]. The structure of pep289–308 and cpep289–308 peptides, which resemble idiopeptides of anti-Id or Id La/SSB antibodies, respectively [12], in conjunction with their ability to induce both B-cell and T-cell responses, might have potential implications for the maintenance and regulation of autoimmune response against La/SSB. According to this scenario (Fig. 7), the initial antigenic stimulus is followed by the production of idiotypic antibodies (Ab1: anti-pep antibodies) by antigen specific plasma cells (B^{Id} plasma cells in Fig. 7). In the process of the immune response and after the elimination of the antigen from the system, lymphocytes with anti-idiotypic specificity, which recognize idiopeptides (or idiopeptide mimetics like cpep) in Ab1 antibodies, are selected from the naïve pool. Consequently, these B-cells (B′ cells in Fig. 7) may internalize the idiotypic antibodies (Ab1) and present selected idiotypic fragments, in the context of MHC class II molecules, to idiopeptide-specific Th cells, in order to receive help for clonal expansion and differentiation to plasma cells [7]. The B′ plasma cells, in turn, secrete anti-idiotypic antibodies (Ab2: anti-cpep antibodies), which carry the antigen mimics in their V-region and can be internalized by the surface Ab1 antibody on B^{Id} cells. The B^{Id} cells present the antigen mimics to antigen specific Th cells, resulting also in clonal expansion and differentiation to plasma cells. The system now enters into a vicious circle with B^{Id} and B′ cells to receive cognate T-cell help and thereof activate each other, producing both idiotypic (Ab1) and anti-idiotypic (Ab2) antibodies, in the absence of antigen. The proposed scenario of mutual Id–anti-Id B-cell activation, is also applicable in the case of complementary peptide immunizations with the difference that the system enters the vicious Id–anti-Id circle by stimulation of B′ lyphocytes with complementary peptide instead of stimulation of B^{Id} cells with epitope peptide.

Evidence that such kind of mechanism could be implicated for the regulation and maintenance of autoimmune response has been recently provided by Nayak et al. [9,22]. These investigators reported that upon immunization with either antigen or idiotype, specific T- and B-cell responses are mounted against both elements and proposed a mechanism for the perpetuation of the immunological memory, based on continuous Id–anti-Id cell activation [9,23]. They have also emphasized the requirement of controlling the continuous cascade of Id–anti-Id cell–cell interaction, activation and proliferation, in order to avoid a malignancy like situation [23]. In the proposed scenario presented here, three

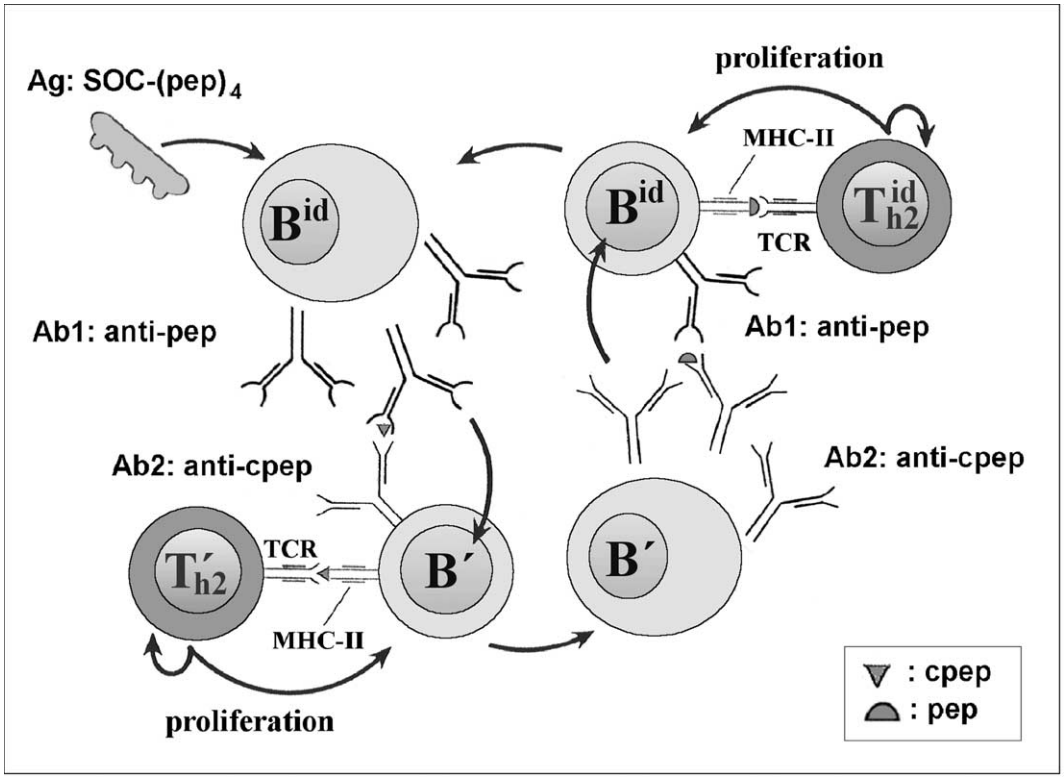

Fig. 7. Proposed model of mutual Id–anti-Id B-cell activation for the maintenance and regulation of autoimmune response against La/SSB. B^{Id}, antigen specific B-cells; $T^{Id}{}_{h2}$, antigen specific T_{h2}-cells; B′, idiopeptide specific B-cells; T'_{h2}, idiopeptide specific T_{h2}-cells.

suppressive mechanisms, which can potentially counterbalance the population of B′ and B^{Id} cells, could be considered: (i) the binding of complement to surface Ig on a portion of B^{Id} and B′ cells [22]; (ii) the presentation of idiopeptides and antigen mimics to cytotoxic T-lymphocytes [24]; and (iii) the potential of some anti-idiotypic antibodies to act as anti-clonotypic antibodies, blocking TCR of Th cells [25,26].

In conclusion, complementary peptides to epitopes of La/SSB can be utilized as probes to study the idiotypic–anti-idiotypic network. The ability of complementary epitopes to induce both T- and B-cell responses to La/SSB has potential implications for the initiation of the autoimmune response. Furthermore, the potential of tolerance-devoid idiopeptides, which belong to idiotypic and anti-idiotypic antibodies, to maintain the anti-La/SSB Id–anti-Id circuit, provides new insights into the mechanisms employed for the perpetuation and regulation of autoimmunity and create a new field for therapeutic intervention.

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