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The immunostimulatory effect of IL-1β *in vivo* is blocked by antisense peptides complementary to the loop sequence 163–171

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ABSTRACT

Antisense (AS) peptides complementary to the β-bulge surface loop VQGEESNDK (Boraschi loop) of the cytokine interleukin-1β (IL-1β) have been shown to bind IL-1β at the Boraschi loop position, and to inhibit some of the IL-1β-mediated biological effects *in vitro*. Here we show that primary AS peptide FVITFFSLY inhibits IL-1β-mediated immunostimulation *in vivo* in a dose-dependent fashion, while inactive on IL-1β-induced inflammation, an effect that takes place independently of the Boraschi loop. To the best of our knowledge, this is the first time that an AS peptide has been used successfully *in vivo*.

1. Introduction

Interleukin 1β (IL-1β) is a potent inflammatory and immunostimulatory cytokine involved in the first steps of the host response to invasion [1]. Deranged regulation of IL-1 underlies the pathological symptoms of several chronic inflammatory and autoimmune diseases such as rheumatoid arthritis. IL-1β is active through interaction with a specific receptor complex on the cell surface, encompassing the ligand-binding chain IL-1RI and the accessory chain IL-1RAcP. Another receptor (IL-1RII) binds IL-1β with non-signalling decoy function [2].

A nine amino acid-long peptide (VQGEESNDK), corresponding to the exposed β-bulge in position 163–171 (47–55 mature sequence) between the fourth and the fifth β-strand of the IL-1β structure (Boraschi loop), can mimic the immunostimulatory effects of IL-1β *in vivo* being devoid of inflammatory effects [3]. To better understand the role of the Boraschi loop in the IL-1β structure-function relationship, several experimental approaches have been undertaken, involving specific antibodies and synthetic peptides [4–7]. Here we report a new approach that makes use of specific interactions between sense (S) peptides and corresponding antisense (AS) peptides [8–10].

A sense (S) peptide is that encoded by the nucleotide sequence (5′ to 3′) of a given ORF in sense (+) strand DNA. The AS peptide results from translating the complementary nucleotide sequence of the corresponding AS (−) DNA strand. Synthetic AS peptides specifically interact with S peptides, bind to complementary S peptide regions in proteins, and inhibits protein activities [8–10]. To explain this interaction, the theory of inter-peptide pair-wise amino acid interactions (M–I pair theory) gives rise to the concept of the proteomic code, according to which the genetic code also has the embedded capacity to code for through-space interactions between amino acid residues thereby helping to define the three-dimensional structures of polypeptides and protein/protein interactions.

Best characterised among AS peptides are those against IL-1β [11], in particular those designed on the IL-1β Boraschi loop [12–14]. The AS peptides of the Boraschi loop can bind to the corresponding S peptide and to the entire IL-1β and inhibit some of its biological activities. Inhibition was demonstrated only in vitro [12–14]. Here we demonstrate the inhibitory properties of anti-IL-1β AS peptides *in vivo*, and exploit the AS specificity of recognition to examine the relevance of the Boraschi loop for the multifaceted functions of IL-1β.

2. Materials and methods

2.1. Animals and cells

C3H/HeJ female mice (The Jackson Laboratory, Bar Harbor, ME) of 7–11 weeks of age were used. The murine thymoma EL4-6.1 (ATCC; Rockville, MD) and the RAJI clone 1H7 [15] were maintained in

Abbreviations: S, sense; AS, antisense; IL-1β, interleukin-1β; IL-1α, interleukin-1α; IL-1Ra, interleukin-1 receptor antagonist; IL-1R, interleukin-1 receptor.

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culture medium (RPMI-1640; Gibco-Europe, Paisley, Scotland; containing 2 mM L-glutamine, 25 mM HEPES buffer, and 50 μg/ml gentamicin sulfate; Sigma Chemical Co., St. Louis, MO) supplemented with 10% heat-inactivated FBS (HyClone, Sterile Systems, Logan, UT).

2.2. Reagents

Synthetic peptides VQGEESNDK, LKDDKPTLQ, FVITFFSLY, FVVGLLTVD, and FVVGLLTVK were synthesized in solid-phase, purified and prepared as HCl salts as described [3]. Human recombinant IL-1β was expressed in *Escherichia coli* and purified to homogeneity [3]. Sheep red blood cells (SRBC) were obtained from Sclavo (Siena, Italy). Purified pneumococcal polysaccharide type III (SIII) was kindly provided by Phillip J. Baker (National Institute of Allergy and Infectious Diseases, Bethesda, MD). All reagents were endotoxin-free as determined by the LAL assay (QLC-1000; Bio Whittaker, Walkersville, MD).

2.3. In vivo immunostimulation

Determination of specific antibody-producing cells (PFC) in the spleen was performed as previously described [3,16]. Briefly, mice were administered the antigen (SRBC; 1–2 × 10^8/mouse i.v.; or SIII, 2.5 μg/mouse i.p.) and, at the same time, the adjuvants i.p.: IL-1β (150 pg/kg), the Boraschi loop peptide 163–171, or the 189–197 peptide LKDDKPTLQ (both 1 ng/kg) alone or admixed with AS peptides. The number of specific splenic PFC was determined after 4 d (for SRBC) or 5 d (for SIII) by the Cunningham and Szenberg slide technique.

2.4. Neutrophilia

Mice (3/group) received 100 μl endotoxin-free sterile saline i.v. containing 100 ng IL-1β, 10 μg of the Boraschi loop peptide 163–171 or of the primary AS peptide, or IL-1β admixed with peptides. After 90 min, blood was collected and total leukocytes counted on cytocentrifuge smears and cytofluorimetrically (FACScan, Becton-Dickinson, Mountain View, CA).

2.5. Receptor binding assay

Cells expressing IL-1RI (EL4-6.1) or IL-1RII (1H7) were used [15]. Cells (1 × 10^6/tube) were incubated with 0.3 nM ^{125}I-labelled ligands IL-1α (DuPont-NEN, Bad Homburg, Germany), IL-1β, or peptide VQGEESNDK-Y (both radiolabelled as described [4,15]) alone or admixed with AS peptides. Incubations were carried out for 90 min at room temperature for IL-1α and IL-1β, and for 120 min at 37 °C for the peptide 163–171 under gentle agitation. Non-specific binding was determined by adding a 1000-fold molar excess of unlabelled ligand. Cell-bound radioactivity was counted in a gamma counter (Packard, Downers Grove, IL).

2.6. Statistical analysis

Duplicate–quadruplicate determinations were performed for each experimental group in each experiment. Experiments were repeated 2–8 times. Data reported are mean ± S.E.M. of replicate experiments or of replicate determinations within single representative experiments. Statistical significance was assessed by Student's *t*-test.

3. Results

The primary AS peptide sequence was deduced from the Boraschi loop DNA sequence (Table 1). Sequences of two secondary AS peptides were obtained by systematically replacing amino acids with alternative residues according to the M–I pair rules (Fig. 1A). AS peptide optimisation based on the hydropathy–hydrophilicity approach [11] yielded identical results (Fig. 1B). Peptides, synthesized and purified as described [3], are listed in Table 1.

IL-1β and its Boraschi loop peptide are potent adjuvants for *in vivo* T-dependent responses such as that to SRBC [3,16] (Fig. 2A). The synthetic peptide corresponding to the IL-1β loop in positions 189–197 (LKDDKPTLQ) also has adjuvant activity (Fig. 2A). The primary AS peptide inhibited immunostimulation by IL-1β or the Boraschi loop peptide, with 50% inhibition achieved at AS/adjuvant molar ratio 100:1 for IL-1β, and 1:1 for the Boraschi loop peptide (Fig. 2A). By contrast, adjuvanticity of the 189–197 peptide is not affected, consistent with the specificity of the AS peptide for the Boraschi loop S sequence. The AS peptide can also inhibit adjuvanticity of both IL-1β and the Boraschi loop peptide (but not the 189–197 peptide) for the T helper-independent immune response against the pneumococcal SIII polysaccharide (Fig. 2B). In this case, the immunostimulatory effects of both adjuvants were inhibited to the same extent, with 50% inhibition at a molar ratio of about 200:1 (Fig. 2B).

In a preliminary set of experiments, the two secondary AS peptides proved slightly more effective IL-1β inhibitors than the primary AS peptide, as far as the response to SRBC is concerned. For the secondary AS peptide AS-B, inhibition of IL-1β immunostimulation was improved by about 10-fold, as compared to the primary AS peptide, with 50% inhibition achieved at 10:1 ratio (Fig. 2A). For the secondary AS peptide AS-A, inhibition of IL-1β immunostimulation was similar in potency to that obtained with the primary AS peptide, although inhibition of the Boraschi loop peptide effect was increased and comparable to that by the secondary AS-B peptide (data not shown).

To assess the role of the Boraschi loop in the IL-1β inflammatory effects, IL-1β-induced neutrophilia was measured, an early event in the inflammatory response to infection/injury. Neutrophilia is induced in the mouse by IL-1β at doses significantly higher than those required for immunostimulation, whereas the Boraschi loop peptide 163–171 is inactive even at high doses (Fig. 2C). Likewise, the primary AS peptide has no effect in the same assay. Considering the affinity of the primary AS peptide for IL-1β (17 μM) [14] and the higher absolute quantity of cytokine, the AS peptide was admixed with IL-1β at a molar ratio of over 1500:1, largely exceeding that required for inhibiting immunostimulation. Even under such conditions, the primary AS peptide did not show any inhibition of IL-1β-induced neutrophilia (Fig. 2C). As a control, IL-1β was admixed with the Boraschi loop peptide at the same ratio, again without any significant effect on IL-1β-induced neutrophilia.

To evaluate whether the AS peptides inhibited IL-1β activities by interfering with its binding to cognate receptors, two cell lines expressing either IL-1RI or IL-1RII were used (Table 2). Primary and

Table 1
Sequence of primary and secondary Antisense (AS) peptides of the interleukin-1β (IL-1β) β-bulge in position 163–171 (Boraschi loop).

Description	Sequence								
Boraschi loop peptide	Val	Gln	Gly	Glu	Glu	Ser	Asn	Aps	Lys
DNA sequence (5′–3′)	GTA	CAG	GGA	GAA	GAA	AGT	AAT	GAC	AAA
Reverse DNA sequence (3′–5′)	AAA	CAG	TAA	TGA	AAG	AAG	AGG	GAC	ATG
Complementary mRNA (5′–3′)	UUU	GUC	AUU	ACU	UUC	UUC	UCC	CUG	UAC
Primary AS peptide	Phe	Val	Ile	Thr	Phe	Phe	Ser	Leu	Tyr
Secondary AS peptide A	Phe	Val	Val	Gly	Leu	Leu	Thr	Val	Asp
Secondary AS peptide B	Phe	Val	Val	Gly	Leu	Leu	Thr	Val	Lys

Molecular weights of the AS peptides: primary AS peptide FVITFFSLY, 1136.36; secondary AS-A peptide FVVGLLTVD, 962.27; secondary AS-B peptide FVVGLLTVK, 975.36.