Vaccine 28 (2010) 2945–2956

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Retro-inversion enhances the adjuvant and CpG co-adjuvant activity of host defence peptide Bac2A

Erin Scruten[a], Jennifer Kovacs-Nolan[a,1], Philip J. Griebel[a], Laura Latimer[a], Jason Kindrachuk[c], Andy Potter[a], Lorne A. Babiuk[b], Sylvia van Drunen Littel-van den Hurk[a], Scott Napper[a,*]

[a] Vaccine and Infectious Disease Organization, University of Saskatchewan, 120 Veterinary Road, Saskatoon, Saskatchewan S7N 5E3, Canada
[b] University of Alberta, Edmonton, Alberta T6G 2J9, Canada
[c] National Institutes of Health (NIH), Bethesda, MD 20892, USA

ARTICLE INFO

Article history:
Received 14 September 2009
Received in revised form 3 February 2010
Accepted 10 February 2010
Available online 1 March 2010

Keywords:
Host defence peptides
Vaccine adjuvant
Retro-inversion
Inversion
Peptide stabilization
CpG ODN

ABSTRACT

Host defence peptides (HDPs) have a variety of potential therapeutic applications, including as vaccine adjuvants, energizing efforts for modification strategies to address their toxicity and instability. Here we compare L, D and RI-Bac2A as vaccine adjuvants. D and RI-Bac2A are equally resistant to proteolytic degradation with no increases in toxicity, however, only RI-Bac2A maintains adjuvant activity of the natural peptide through conserved induction of a Th2 immune response. As HDPs potentiate the adjuvant activity of CpG ODNs, the isomers were also evaluated as co-adjuvants. L-Bac2A has no significant co-adjuvant activity while CpG/RI-Bac2A induces antibody titres significantly higher than CpG ($P < 0.01$), CpG/L-Bac2A ($P < 0.01$) or CpG/D-Bac2A ($P < 0.01$). None of the isomers influence ODN duration or distribution but L and RI-Bac2A promote ODN uptake into B cells and antigen presenting cells. The enhanced adjuvant and co-adjuvant of RI-Bac2A is hypothesized to result from an undefined combination of increased stability and retained biological activity supporting application of retro-inversion to this, and potentially other HDPs.

1. Introduction

Sub-unit vaccines based on discrete molecules of particular pathogens are advantaged over live or attenuated vaccines in their safety but disadvantaged by their typically weak immunogenicity. Accordingly, the emerging trend towards sub-unit vaccines has been paralleled by efforts for identification of novel adjuvants that promote robust immune responses. Potential sources of novel vaccine adjuvants include natural, endogenous modulators of immunity, such as the host defence peptides, or exogenous ligands of the innate immunity sensory systems, such as CpG ODNs.

Host defence peptides (HDPs) represent a conserved mechanism of innate immune defence found in all complex forms of life [1]. HDPs are small, carry a net positive charge and have a large number of hydrophobic residues [2]. While initially appreciated for their ability to disrupt microbial membranes, it is now apparent that this activity does not encapsulate the full spectrum of their biological actions nor does it reflect the sophistication of their contributions to immune defence. HDPs are now recognized to modulate a variety of host immune functions [3–6]. The ability of HDPs to influence adaptive immune responses, and hence their potential application as vaccine adjuvants, is specifically attributed to their ability to recruit cells to sites of vaccine injection and promote activation and maturation of key immune effector cells [7–9].

The ability of HDPs to regulate host immune responses has a variety of potential immunotherapeutic applications including as antimicrobials, anti-inflammatory agents and vaccine adjuvants [10–12]. There are proof-of-principle demonstrations of HDPs in each of these capacities [13–15]. However, independent of the specific therapeutic objective, many HDPs are limited as systemic agents by their toxicity and biological instability. Identification of peptide modification strategies that capitalize on the biological activity of HDPs, while addressing the limiting characteristics of instability and toxicity, has been identified as a primary obstacle to the use of HDPs as systemic agents [16,17]. A number of peptide modification strategies have been considered including incorporation of D amino acids through either direct substitution or retro-inversion. Retro-inversion involves reversal of the direction of the peptide sequence as well as inversion of the chirality of each amino acid [18]. RI peptides are anticipated to present the same three-dimensional side chain topology as their L-counterparts thus having greater potential for retention of biological activity [19–21].

Inversion and retro-inversion have proven effective for a number of HDPs with respect to direct antimicrobial activity [22–24].

* Corresponding author. Tel.: +1 306 966 1546; fax: +1 306 966 7478.
E-mail address: scott.napper@usask.ca (S. Napper).
[1] Current address: Department of Food Science, University of Guelph, Guelph, Ontario NIG 2W1, Canada.

0264-410X/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2010.02.015

Table 1
Sequences of the Bac2A isomers.

Peptide	Sequence
L-Bac2A	ArgLeuAlaArgIleValValIleArgValAlaArg
D-Bac2A	(D)Arg(D)Leu(D)Ala(D)Arg(D)Ile(D)Val(D)Val(D)Ile(D)Arg(D)Val(D)Ala(D)Arg
RI-Bac2A	(D)Arg(D)Ala(D)Val(D)Arg(D)Ile(D)Val(D)Val(D)Ile(D)Arg(D)Ala(D)Leu(D)Arg

While encouraging, this must be tempered with the appreciation that direct antimicrobial activity is typically mediated through non-specific, non-chiral interactions with microbial membranes and is highly tolerant of structural modification. For example, approximately half of the peptides within a random library of HDP sequence derivatives had maintained or improved direct antimicrobial activity [25]. The higher priority, and more stringent criteria, for evaluation of peptides intended for immunotherapeutic application is their ability to influence immune responses. There is strong evidence that HDPs modulate immune responses through activation of specific host receptors, although different receptors may be employed for different HDPs [26,27]. While some initial reports have indicated the ability for D and RI HDPs to influence host immune responses [28,29] these examples likely reflect indirect mechanisms involving LPS binding [28] or disruption of host cell membranes resulting in cytokine release [29] rather than receptor-mediated effects.

The focus of this investigation is the stability, toxicity and immunomodulatory activities of the D, L and RI isomers of a model HDP Bac2A. Immunomodulatory activity is assessed by the relative abilities to function as stand-alone adjuvants and CpG co-adjuvants. Adjuvant activity was selected as a measure of immune activity since this is a readily quantifiable, complex response which is dependent upon several receptor-mediated peptide effects. Both the D and the RI derivatives resist proteolytic degradation without increasing toxicity but only RI-Bac2A maintains the ability to function as a stand-alone adjuvant. The functional advantages of the retro inversed peptide are even more apparent as a CpG co-adjuvant where CpG/RI-Bac2A induces antibody titres significantly higher than CpG ($P < 0.01$), CpG/L-Bac2A ($P < 0.01$) or CpG/D-Bac2A ($P < 0.01$). Complex formation appears prerequisite as co-injection, as opposed to co-formulation, of RI-Bac2A with CpG eliminates the ability of the peptide to improve on ODN adjuvant activity. Complex formation alone, however, is insufficient for co-adjuvant activity as all the isomers have equal ability to bind the ODN *in vitro*. None of the isomers influence the duration or distribution of the ODN *in vivo*, however, complexes formed with the modified isomers are functionally distinct in that only RI-Bac2A retains the ability of the natural peptide to promote cellular ODN uptake. Enhanced adjuvant and co-adjuvant of RI-Bac2A is believed to result from an undefined combination of increased stability and retained ability to activate conserved host responses as compared to the unsta-

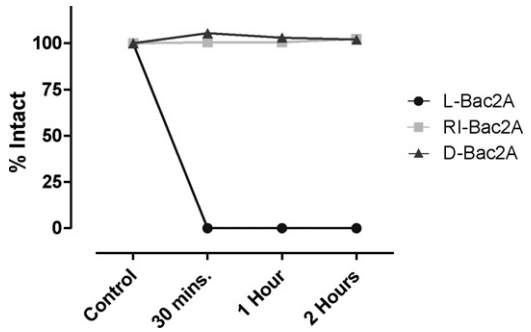

Fig. 1. Resistance to proteolytic degradation. Peptides (1 mg/mL) were digested with trypsin (0.1 mg/mL) in a 50 μl reaction volume (50 mM Tris pH 7.2) at 37 °C. Digestion mixtures were separated via HPLC chromatography and the extent of peptide degradation quantified through comparison of peak areas to that of an undigested sample of the same peptide. Results are the average from two independent experiments.

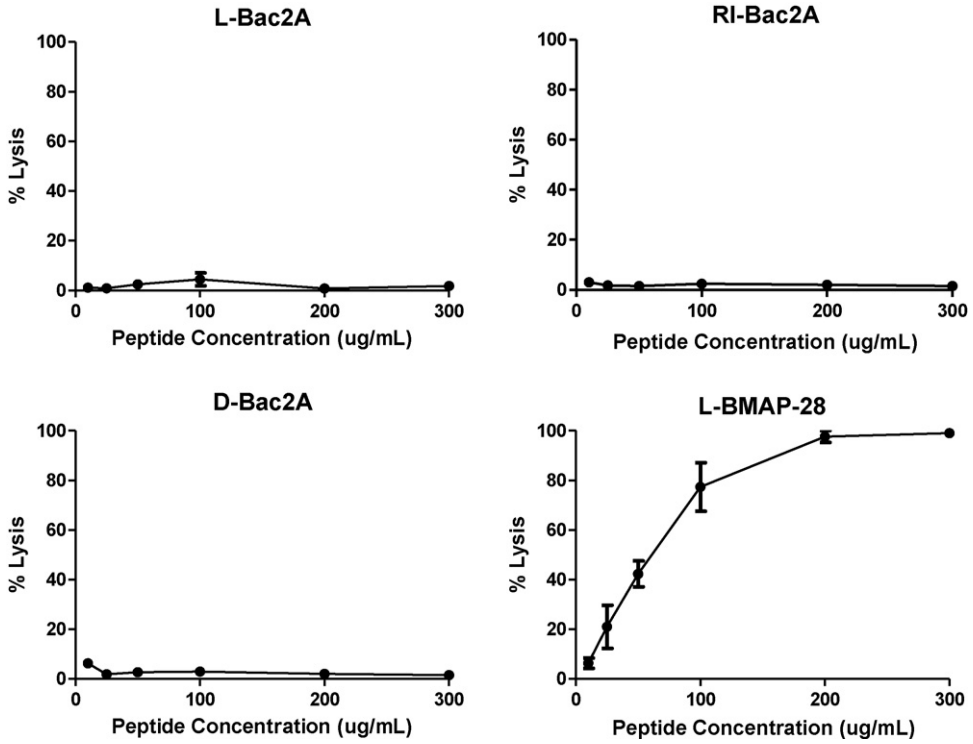

Fig. 2. Toxicity of Bac2A isoforms. Haemolysis was performed by incubating a 1% (v/v) suspension of bovine erythrocytes in phosphate-buffered saline (pH 7.4) with a series of peptide concentrations for 12 h at 37 °C. Samples were then centrifuged and the supernatant absorbance read at 570 nm. Total haemolysis was obtained by re-suspending cells in water rather than PBS. Results are the average from four independent experiments.