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Vaccine 23 (2005) 4589–4597
Vaccine
www.elsevier.com/locate/vaccine

Identification of dominant epitopes of synthetic immunocontraceptive vaccines that induce antibodies in dogs

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Received 5 November 2004; received in revised form 5 March 2005; accepted 22 April 2005
Available online 17 May 2005

Abstract

The specificities of immunoglobulin G antibodies obtained from the sera of dogs inoculated with totally synthetic immunocontraceptive vaccine candidates based on luteinising hormone releasing hormone (LHRH: amino acid sequence HWSYGLRPG) were examined using peptides expressed in a phage display library. The three vaccine candidates each contained a different T helper-cell epitope chemically linked with the same LHRH amino acid sequence HWSYGLRPG and all of them elicited high antibody titres against the hormone. Delineation of epitopes recognised by sera from vaccinated dogs using a phage display library indicated that two of the three vaccine candidates induced antibody directed to the consensus sequence xHWSxxLxxx whereas the third vaccine candidate induced antibody against the consensus sequence xxxxxxxRPx. Two of the three vaccine candidates elicited antibodies against B cell epitopes present within the helper T-cell epitope component of the vaccine whereas the third vaccine did not. The occurrence of anti-T helper cell epitope antibodies appeared to have little or no effect on the generation of the anti-LHRH responses indicating that carrier-induced epitope suppression was not operating here. Our results also demonstrated that with animal sera of high quality, it is possible to delineate immunodominant epitopes recognised by polyclonal antibodies with high efficiency using phage display library. The approach has utility in the definition of immunodominant epitopes, which may "decoy" antibody responses away from other epitopes, which may be more useful in prophylaxis or therapy.

Keywords: LHRH; Peptide vaccine; Phage display library; Mimotope

1. Introduction

In the field of vaccine design, the use of peptide or epitope-based vaccines finds particular use where the epitope of interest is well defined. In such cases, the ensuing antibody response is expected to be directed towards a single epitope of interest, thereby resulting in a mono-specific and if not monoclonal then oligoclonal rather than polyclonal response. An example of a defined B-cell epitope against which antibodies can be elicited is the 10 amino acid long hormone luteinising hormone releasing hormone (LHRH), also known as gonadotrophin releasing hormone (GnRH), and has the sequence EHWSYGLRPG. Because LHRH is the hormone that initiates the endocrine cascade, which controls sex hormone production, it is an attractive candidate against which to direct an immune response and consequently control those physiological and pathological processes, which are dependent on sex hormones.

Anti-LHRH vaccines have potential application in a variety of conditions including reproductive control [1–5] and

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doi:10.1016/j.vaccine.2005.04.030

cancer therapies including breast [6] and prostate cancers [1,7]. It is also possible that other conditions such as endometriosis, which can be controlled by analogues of LHRH [8,9], could also be ameliorated using anti-LHRH vaccines. Finally, anti-LHRH vaccines find application in animal husbandry [3,10] and the meat production industry where they are used to decrease the accumulation of skatole and androsterones in the fatty tissue of pigs, which leads to boar taint in pork meat [5,11].

LHRH has attracted a great deal of attention as a vaccine candidate and a variety of strategies have been developed in an attempt to generate a safe and efficacious vaccine. These strategies range from coupling the hormone to protein carriers as a source of epitopes recognised by T helper (Th) cells to the assembly of semisynthetic [12] or totally synthetic epitope-based vaccines that contain a defined and usually promiscuous Th-cell epitope. Our own strategy is to design a totally synthetic LHRH-based vaccine [13], which is self-adjuvanting [14] and incorporates T helper-cell epitopes from the fusion protein (F) of the morbillivirus canine distemper virus or CDV [15]. Our choice of synthetic helper T-cell epitopes derived from a canine virus was to take advantage of any existing T cell help that may exist in dogs that had received CDV vaccine.

Even though LHRH is only 10 amino acid residues in length, there have been a number of reports that antibodies directed to a particular region are important for biological activity. Our purpose in the present study then was to identify those regions of the hormone against which antibodies are directed when dogs were vaccinated with synthetic vaccine candidates. To do this, we adopted a phage display approach in which a total of approximately 1 billion (10^9) different 12-aa peptide sequences was examined for binding to antibodies that were induced by three synthetic vaccines, each of which contained a different T helper-cell epitope from the F protein of CDV assembled in tandem with the LHRH sequence HWSYGLRPG. The approach allows for very large numbers of different epitope sequences to be examined far more cheaply than an equivalent number of chemically synthesised peptide sequences. Furthermore, because the approach is not limited to homologs of the epitope under investigation, data concerning the recognition of non-homologous sequences are also generated.

2. Materials and methods

2.1. Peptide synthesis

The immunogens, P25-LHRH, P27-LHRH and P35-LHRH in which the Th-cell epitopes are assembled N-terminal to LHRH were assembled using Fmoc chemistry throughout either manually or in a Milligen 9050 Plus automatic peptide synthesiser as described previously [16]. The sequences of the immunogens, therefore, correspond to the individual helper T-cell epitope sequences followed by LHRH. As an example the full sequence of P25-LHRH is KLIPNASLIENCTKAEL-HWSYGLRPG. The sequences of each of the peptides and epitope-based vaccines used in this study are shown in Table 1. Peptides were purified by reversed-phase HPLC as described elsewhere [13,14] and analysed using an Agilent 1100 series ion-trap mass spectrometer or a Bruker BIFLEX instrument equipped with delayed ion extraction. All peptides were greater than 90% pure and had the expected mass.

Table 1
Sequences and acronyms used for the peptides described in this study

Peptide sequence	Acronym
KLIPNASLIENCTKAEL-EHWSYGLRPG	P25-LHRH
AELGEYEKLLNSVLEPI-EHWSYGLRPG	P27-LHRH
TAAQITAGIALHQSNLN-EHWSYGLRPG	P35-LHRH
EHWSYGLRPG	LHRH

2.2. Vaccination of dogs with peptide immunogens

Beagle/foxhound dogs, bred and maintained at CSL Ltd., Parkville, Victoria, Australia, were used for the study. All animals were vaccinated with Canvac 3 in 1 (CSL Ltd.), which contains live CDV, canine parvovirus and canine adenovirus. As a consequence these animals possess T helper cells, which are capable of recognising the F protein of CDV [15]. All experimental work on dogs was carried out in accordance with the CSL Animal Ethics and Experimentation Committee. Dogs were inoculated with 40 nmol of the peptide antigen in the presence of 150 μg Iscomatrix® as adjuvant. A similar dose of peptide antigen in Iscomatrix® was administered 4 weeks following the first inoculation. In some experiments dogs received a third dose in week 22. Control dogs received no peptide antigen.

2.3. Antibodies and IgG

Dogs were bled by venepuncture from the front paw and serum collected from the retracted blood clot the following day. Canine IgG was purified from serum by affinity chromatography using a column (1 cm × 5 cm) of Protein G-Sepharose Fast Flow (Amersham-Pharmacia Biotech, Uppsala) following the manufacturer's instructions.

Mouse anti-M13 monoclonal antibody conjugated to horseradish peroxidase (HRP) was purchased from Amersham-Pharmacia Biotech. Sheep anti-mouse IgG, alkaline phosphatase-conjugated, was from Chemicon Australia (Boronia, Victoria). Dynabeads-Protein A was purchased from Dynal Simply Magnetic (Dynal Pty Ltd., Australia).

2.4. Media and solutions

LB Medium (10 g/l Bacto-Tryptone, 5 g/l Nacl) was used throughout this study for all *Escherichia coli* culture requirement. LB/IPTG/Xgal plates were made from LB medium containing 15 g/l agar plus isopropyl β-D-thiogalactoside