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GRA1 protein vaccine confers better immune response compared to codon-optimized GRA1 DNA vaccine

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Abstract

The present study evaluates immunogenicity and protection potency of a codon-optimized GRA1 DNA vaccine, wild type GRA1 DNA vaccine and an adjuvanted recombinant GRA1 protein vaccine candidate in BALB/c mice against lethal toxoplasmosis. Of the three GRA1 vaccines tested, the recombinant GRA1 protein vaccine results reveal significant increase in immune response and prolonged survival against acute toxoplasmosis compared to DNA vaccinations. Immune response and protection conferred by codon-optimized GRA1 DNA vaccine was slightly better than wild type GRA1 DNA vaccine.
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1. Introduction

Toxoplasma gondii is one of the most successful protozoan parasites because it has a very broad host range, infecting all warm-blooded animals, including humans [1] and causes serious clinical presentations. There is no 100% effective drug to treat all clinical presentations of *T. gondii*. Available drugs have many side effects and reactivation may occur any time. The development of a vaccine, which can prevent the consequences of acute infection, is therefore, an attractive alternative. After classification in category B bioterrorism agents as a food and water safety threat, the demand for a protective vaccine has increased [2].

Vaccine strategies against toxoplasmosis aim to induce Th1 response and IFN-γ production because immune protection against *T. gondii* in mice is primarily correlated with Th1 cell mediated immunity [3,4] and IFN-γ secretion [5]. Many attempts have been made to create an immunogenic protective vaccine against toxoplasmosis using mutant *T. gondii* strains, purified proteins, and DNA vaccines since 1990s [6]. The accumulated results suggest that a protective vaccine against *T. gondii* infection is feasible. Recombinant protein vaccines and DNA vaccines using Surface Antigen 1 (SAG1), SAG2, Dense Granule Antigen 1 (GRA1), GRA4, GRA7, Rhoptry Protein 2 (ROP2), Microneme Protein 3 (MIC3) and Heat

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Shock Protein 70 (HSP70) elicit partial, full, or occasionally no protection in animals depending on the virulence of the *T. gondii* strains [7–30]. Of all the vaccine candidates against toxoplasmosis, GRA1 appears to be an immunogenic promising vaccine candidate [8,26–28,31]. Existing GRA1 vaccination trials used recombinant protein or DNA vaccines. Although immune response and protection potency have been investigated in detail about GRA1 DNA vaccination [26,28], the cellular immune response and protection potency of an adjuvanted recombinant GRA1 protein vaccine haven't been evaluated in mice yet [8,27,31]. Taken together, the experimental studies using various GRA1-derived vaccines are encouraging, however much work remains to put together the pieces of the puzzle, i.e. highlight the complexity of the immune response and the unpredictable relationships among various parameters such as vaccine strategy to be used against toxoplasmosis, protection and cellular immune response elicited by recombinant protein vaccine or parasite strain used to challenge the mice. In order to comprehensively evaluate these variables, the present study compares cellular immune response and protection potency of a recombinant GRA1 protein vaccine adjuvanted with a strong Th1 inducer Provax [32,33], a wild type GRA1 DNA vaccine and a GRA1 DNA vaccine codon-optimized for protein expression in mammalian cells. Codon usage optimization is widely accepted as a means of increasing the level of *in vivo* antigen expression from DNA vaccines and improving the cellular and humoral immune responses against the expressed antigens from diverse microorganisms [34–47]. The codon-optimized GRA1 DNA vaccine used in this study is the first example of a codon-optimized gene being used as DNA vaccine candidate against *T. gondii*.

2. Materials and methods

Unless noted otherwise, all reagents were purchased from Sigma–Aldrich Corporation (St. Louis, MO, USA).

2.1. Mice

Female 6 week-old BALB/c mice were purchased from Charles River Laboratories Inc. (Wilmington, MA). Mice were housed and fed under standard, suitable conformist conditions. The experimental plan was performed under the instructions and approval of the Institutional Animal Care and Use Committee of UCI for animal ethics.

2.2. Parasites and preparation of T. gondii antigen

T. gondii RH strain tachyzoites were used to challenge the immunized mice and to prepare *T. gondii* antigen. RH strain tachyzoites were maintained in confluent monolayers of human foreskin fibroblasts (HFF) cell culture grown in Dulbeccos modified Eagle medium (DMEM) supplemented with 10% (v/v) heat inactivated fetal bovine serum (Hyclone, Logan, UT), 2 mM L-glutamine and 50 mg gentamycin per ml (Invitrogen-Gibco, Carlsbad, CA). *T. gondii* antigen (TAg) was prepared with the RH strain tachyzoites obtained from HFF cell culture as described [12].

2.3. Computationally optimized DNA assembly (CODA) of the GRA1 gene

The GRA1 protein sequence from amino acid positions 25–190, excluding the signal peptide, was obtained from GENBANK database (accession number M26007), and submitted to CODA at the Computational Biology Research (CBR) Laboratory of the UC Irvine Institute for Genomics and Bioinformatics. The CODA algorithm generated a list of 36 linearly overlapping and abutting oligonucleotides, 39–64 nucleotides (nts) in length, optimized for self-assembly and expression of the codon-optimized GRA1 gene (co-GRA1) in mammalian cells. These oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA, USA).

2.3.1. Assembly of co-GRA1 intermediate DNA fragments

The full-length co-GRA1 DNA sequence was divided into three partially overlapping intermediate DNA fragments, each of which was subdivided into 12 short overlapping and abutting DNA oligonucleotides (intermediate fragment 0:237 bp; intermediate fragment 1:196 bp; intermediate fragment 2:196 bp). The co-GRA1 gene was assembled in two steps in which the DNA oligonucleotides were assembled first to form the three intermediate DNA fragments and then the intermediated fragments were mixed to form the full-length gene. For the assembly of each of the three intermediate DNA fragments of GRA1, the constituent DNA oligonucleotide set was added to a primer extension reaction at a final concentration of 0.1 μM along with an excess (0.5 μM) of leader and trailer primer oligonucleotides (the most 5′- and 3′-distant DNA oligonucleotides). The reactions included 2.5 U of *Pfu*Ultra High-Fidelity DNA polymerase (Stratagene, La Jolla, CA), 300 μM dNTPs, and 1 × *Pfu*Ultra reaction buffer. The primer extension and PCR amplification reactions were performed in an Eppendorf Mastercycler (Hamburg, Germany) using the following calculated-control protocol: 5 min initial denaturation step at 95 °C, followed by 25 cycles of 20 s at 95 °C, 30 s at 62 °C, and 1 min at 72 °C, and an extension of 5 min at 72 °C. The PCR products were visualized by agarose gel electrophoresis.

2.3.2. TOPO PCR cloning

PCR product of each intermediate DNA fragment of the GRA1 gene was cloned into a pCRII-Blunt-TOPO vector according to the manufacturer's recommended protocol using One Shot electrocompetent Top 10 *Escherichia coli* cells (Invitrogen, Carlsbad, CA). The resulting plasmids, which contained intermediate DNA fragments of the correct size were visualized by agarose gel electrophoresis and purified for DNA sequencing using a plasmid mini purification kit

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