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Research brief

Toxoplasma gondii: DNA vaccination with bradyzoite antigens induces protective immunity in mice against oral infection with parasite cysts

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Abstract

Infection of the host by *Toxoplasma gondii* leads to an acute systemic dissemination of tachyzoites, followed by a chronic phase, in which bradyzoites, enclosed in cysts, persist in the brain, the heart, and other tissues. Among putative vaccine candidates, the bradyzoite antigens BAG1 and MAG1 look promising since they are preferentially expressed during the chronic stage of the parasite. This work focused on studying the immunogenicity of bradyzoite antigens in a mouse model of chronic toxoplasmosis. A mixture of plasmids directing the cytoplasmic expression of MAG1 and BAG1 in mammalian cells was used to immunize mice. We show here that immunized mice developed, preferentially, specific anti-MAG1 and anti-BAG1 IgG2a subclass antibodies, indicating a shift towards a Th1-like response after DNA immunization. We then demonstrated that DNA immunization followed by challenge infection elicited effective protection in mice, suggesting that bradyzoite antigens should be considered in the design of vaccines against toxoplasmosis.

Index Descriptors and Abbreviations: *Toxoplasma gondii*; Bradyzoite antigens; DNA immunization; DNA, deoxyribonucleic acid; ELISA, enzyme-linked immunoassay; FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; GST, glutathione *S*-transferase; HRP, horse radish peroxidase; PBS, phosphate buffered saline; PCR, polymerase chain reaction

1. Introduction

Toxoplasma gondii is an intracellular protozoan parasite that causes significant morbidity and mortality in congenitally infected and immunocompromised individuals (Dunn et al., 1999; Petersen et al., 2001; Remington et al., 2000). In humans, *T. gondii* infection is a relapsing disease, which can activate any time during the life of the infected host (Petersen and Ambroise-Thomas, 2000). Toxoplasmic retinochoroiditis is a major cause of visual impairment worldwide and the rate of ocular lesions in congenitally infected individuals is as high as 82% (Mozatto and Procianoy, 2003). In some agricultural regions in southern Brazil seropositivity is nearly 100% and up to 18% of the population has retinochoroidal scars (Silveira et al., 2001).

Toxoplasma exhibits three morphologically distinct infectious stages: tachyzoites (rapidly multiplying parasite of the acute phase of infection), bradyzoites (in tissue cysts), and sporozoites (in oocysts) (Dubey et al., 1998).

In recent years, efforts have been made to develop an anti-*Toxoplasma* vaccine, whose feasibility was suggested by the long-term immunity induced by the primary infection. In the search of parasite antigens involved in protective immunity, most work focused on parasite molecules which are principally expressed during the acute phase of infection (Beghetto et al., 2005; Fatoohi et al., 2002; Nielsen et al., 1999; Vercammen et al., 2000). The *T. gondii* bradyzoite antigen BAG1 (Bohne et al., 1995; Parmley et al., 1995) and the matrix antigen MAG1 (Parmley et al., 1994) look particularly promising

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since they are exclusively or preferentially expressed during the chronic stage of the parasite, respectively.

The involvement of BAG1 and MAG1 in the induction of the human B and T cell mediated immunity against the parasite in healthy adults with acquired infection has been recently investigated (Di Cristina et al., 2004). In the present work, the immunogenicity of these proteins was further investigated in a mouse model of chronic toxoplasmosis. We show here that DNA immunization with a mixture of BAG1- and MAG1-expressing plasmids elicited protective immunity in mice after oral challenge with a cystogenic *T. gondii* strain.

2. Materials and methods

2.1. Plasmids and recombinant proteins

The DNA encoding for the *T. gondii* matrix antigen MAG1 (excluding its hydrophobic secretion leader; residues 28–453; GenBank Accession No. U09029), and for a fragment of the heat shock protein BAG1 (residues 94–229; GenBank Accession No. X82213) was amplified by PCR from *T. gondii* cDNA (RH strain). Specific primers to introduce the *Hin*dIII restriction site followed by a translation start-codon and a stop-codon followed by the *Xba*I site, at their 5′- and 3′-DNA ends, respectively, were used (BAG-for, 5′-GGCCAAGCTTGAAATGGGTACTAGTCGTG CTGG–3′; BAG-rev, 5′-CCGGTCTAGAGCTAACCAG AAACGCTGATTTGTTG–3′; MAG-for, 5′-GGCCAAG CTTGAAATGGGATTGAGCCAAAGGGTG–3′; MAG -rev, 5′-CCGGTCTAGAGTCAAGCTGCCTGTTCCGC TAAG-3′). To direct the expression of the foreign proteins in the cytoplasm of mammalian cells the amplified DNA fragments were cloned in the *Hin*dIII and *Xba*I sites of the mammalian vector pcDNA3.1 (Invitrogen, USA), under the transcriptional control of the cytomegalovirus early promoter, to give pcDNA-MAG1 and pcDNA-BAG1 vectors, respectively. The plasmids were purified from transformed *Escherichia coli* by using "EndoFree plasmid kit" (Qiagen, Germany), dissolved in endotoxin free PBS and stored at −20 °C.

The construction of plasmids pGEX-MAG1 and pGEX-BAG1, respectively, directing the expression of MAG1 (aminoacids 28–126) and BAG1 (aminoacids 94–229) antigens as glutathione *S*-transferase (GST) fusion proteins in *E. coli* cells was described elsewhere (Di Cristina et al., 2004). The expression and purification of recombinant proteins have been performed essentially as described previously (Beghetto et al., 2003).

2.2. DNA immunization and T. gondii challenge

Seven- to eight-week-old female C3H/HeN mice (purchased from Harlan, Scandinavia) were used for this study. Mice (10 per group) received injections of 50 µl of pcDNA3.1-MAG1 and pcDNA3.1-BAG1 plasmid mixture or empty pcDNA3.1 vector into each anterior tibial muscle (final plasmid concentration, 2 mg/ml). Three and six weeks after vaccination the injections were repeated using the same protocol. Tail bleeds were performed on vaccinated mice 3 weeks after the last DNA injection to ascertain the presence of specific anti-*Toxoplasma* antibodies.

Mice immunized with either pcDNA-MAG1 and pcDNA-BAG1 plasmids or empty pcDNA3.1 vector (the control mice) were orally infected with *T. gondii* (30 parasite cysts per mouse) 4 weeks after the last DNA vaccination. The avirulent *T. gondii* SSI 119 strain (Jensen et al., 1998), maintained at Statens Serum Institut, Copenhagen, Denmark, by continual passage in CD1 mice through oral infection, was used for this study. One month after the oral challenge, BAG1/MAG1 immunized mice were killed and their brains were removed. Cysts were obtained by homogenizing the brains of infected mice. The mean number of cysts per brain was determined microscopically by counting four samples (10 µl each) of each homogenate.

2.3. Analysis of the antibody response of immunized mice

Serum samples from immunized mice were tested, in ELISAs, by coating Maxisorb-plates (Nunc, Denmark) with GST-MAG1 or GST-BAG1 fusion proteins at a concentration of 5 µg/ml in 50 mM $NaHCO_3$, pH 9.6. After incubation overnight at 4 °C, plates were left for 1 h at 37 °C in blocking solution (5% non-fat dry milk, 0.05% Tween 20 in PBS) and subsequently incubated for 1 h at 37 °C with mice sera diluted 1:100 in blocking solution. Plates were then washed and anti-mouse-IgG, or anti-mouse-IgG1, or anti-mouse-IgG2a HRP-conjugated antibodies (Sigma–Aldrich, USA) were added to each well. Tetramethylbenzidine (Sigma–Aldrich, USA) was used to reveal enzymatic activity. The results were recorded as the difference between the absorbance at 450 and 620 nm, detected by an automated ELISA reader (Labsystem Multiskan, Finland). For each serum sample the assay was done in duplicate and average values were calculated.

2.4. Expression of bradyzoite antigens in mammalian cells

COS-7 cells were grown in Dulbecco's modified Eagle's medium (Gibco-Invitrogen, USA) containing 10% fetal bovine serum (FBS) (Gibco-Invitrogen, USA). Transfection of cells was performed in 24-well plates, adsorbed with poly-lysine, using Lipofectamine 2000 (Invitrogen, USA). Forty-eight hours after transfection, cells were fixed with formaldehyde (3.7% in PBS) and permeabilized with Triton X-100 (0.2% in PBS). The plates were incubated with mouse sera (diluted 1:20 with 2% FBS in PBS), then with anti-mouse IgG FITC-conjugated antibodies (BD-Biosciences, USA; diluted 1:200 in 2% FBS/PBS). After being extensively washed with PBS, plates were air-dried, mounted with Vectashield (Vector, USA), and finally observed using an inverted fluorescence microscope.