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Evaluation of protective effect of recombinant dense granule antigens GRA2 and GRA6 formulated in monophosphoryl lipid A (MPL) adjuvant against Toxoplasma chronic infection in mice

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Abstract

To investigate the vaccine potential of both the *Toxoplasma* GRA2 and GRA6 antigens, the full length recombinant proteins were produced in *Escherichia coli*, formulated in MPL adjuvant, and used alone and in combination ("mix"), to immunize CBA/J mice. Although high ratios of specific IgG2a/IgG1 were measured against both proteins, only spleen cells from GRA2-immunized mice and mix-immunized mice produced high amounts of both IFN-γ and IL-2 upon induction with *Toxoplasma gondii* Excretory-Secretory Antigens. Intra peritoneal challenge with *Toxoplasma* cysts resulted in significant reduction of brain cysts in GRA2- and in mix-vaccinated mice only. This study shows the protective efficacy of recombinant GRA2 against chronic infection by *T. gondii* and confirms the utility of MPL adjuvant in enabling a vaccine candidate to induce a protective Th1 immune response.
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1. Introduction

The Apicomplexan protozoa *Toxoplasma gondii* can infect all warm-blooded animals including human beings. Toxoplasmosis is quite harmless in immuno-competent individuals: dissemination of the proliferative tachyzoite stage is rapidly controlled by a potent immune response, leading to encystment of quiescent parasites named bradyzoites in the brain and in muscles. In contrast, toxoplasmosis is a significant cause of morbidity and mortality in immuno-compromised and congenitally-infected individuals. Toxoplasmic encephalitis, a major consequence of reactivation of latent infection, occurs in 25–50% of *T. gondii*-seropositive AIDS patients and can be fatal if not recognized and treated soon [1]. Primary infection with *T. gondii* during pregnancy can produce neurological and ocular complications in the fetus or cause abortion [2]. In livestock, abortion of ewes also causes considerable economic losses [3].

Protective immunity to *T. gondii* has been extensively studied in different animal models. Protection requires cascade activation of the innate immunity system mediated mainly by interleukin-12 (IL-12), relayed by both $CD4^+$ and $CD8^+$ T-cells for the development of a Th1 specific immune response [4]. Whereas Th2 immune response preferentially drives humoral immunity, Th1 immune response is thought to drive induction of cellular immunity, which is required for

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protection against intracellular pathogens such as *T. gondii*. Moreover, interferon-γ (IFN-γ) was shown to be the main mediator of protection during both acute and chronic phases of toxoplasmosis [4,5]. As components of the Th2 response, both $CD4^+$ T and B cells, which are involved in production of isotype-switched antibodies, would also contribute to protection against *T. gondii* [6,7].

Since primary infection with *T. gondii* results in a long-lasting protection against future infection, making an effective vaccine against this pathogen should be an achievable goal. Immunization with stage-specific antigens usually leads to stage-limited protection [8]. Transmission of infection by ingestion of cat oocysts has been underestimated for a long time and is now under closer investigation [9]. However, it is likely that the majority of *T. gondii* infections in humans are due to ingestion of tissue cysts present in undercooked meats [10]. Candidate antigens for the design of a vaccine against human infection should thus be expressed in both the bradyzoite and tachyzoite life stages and should be capable of stimulating a strong Th1 immune response. A live vaccine based on an attenuated strain of *T. gondii* is being used in farm animals [11]. However, such a vaccine is not suitable for humans because of the risk of potential reactivation. Current studies are thus exploiting recombinant vaccines which are safer, easier to standardize, cost-effective to produce and importantly, applicable to humans. The most promising vaccine candidates of *T. gondii* include surface antigens (SAG proteins) and Apicomplexa-specific secreted dense granule, rhoptry and microneme proteins. Indeed, several studies have demonstrated the protective immune response induced by native, recombinant proteins as well as DNA vaccine versions of SAG, rhoptry or micronemal proteins (for the most recent references: [12–17]).

Extensive studies have shown that the dense granule antigens (the GRA antigens) are involved in parasite survival and virulence (reviewed in [18]). These proteins are secreted in abundance and constitute an important fraction of the antigens which circulate in the blood stream during the first hours following infection [19] and which peak between days 2 and 4–5 during mice infection by the virulent strain RH [20–21]. They are also major components of both the parasitophorous vacuole in which tachyzoites multiply and the cyst wall surrounding the more slowly dividing bradyzoites (reviewed in [22]). The GRA proteins have thus been considered as attractive vaccine candidates for the prevention and control of toxoplasmosis (for the most recent references: [15,16,23,24]).

We have previously shown that among these antigens, GRA2 is particularly immunogenic during infections in both humans and experimental models [25–27]. Prigione et al. [28] showed that GRA2 induces a long term activation of *T. gondii*–specific helper T cells in humans, which is an essential feature for a candidate vaccine antigen. Furthermore, immunization of mice or rats with native GRA2 formulated in Freund's adjuvant protected against acute [29] and congenital infections [30] while immunization of mice with chimeric SAG1-truncated GRA2 protein delayed the death of mice challenged with a *Toxoplasma* virulent strain [31]. GRA6 was also shown to be the target of humoral immune response in both humans and mice (for the most recent references: [32–34].

The success of any vaccination protocol invariably depends on several parameters, such as adjuvanticity of the formulation component(s), excipients, vaccine carriers, not to mention the route of administration, in addition to the active antigen itself. Among the formulation variables, adjuvant is primarily used to enhance the immune response. The 3-deacylated monophosphoryl lipid A (MPL) adjuvant is a detoxified derivative of the lipopolysaccharide (LPS) from *Salmonella minessota* R595. As such, MPL is a Toll-like Receptor 4 (TLR4) agonist and thus, a potent activator of the Th1 response. In addition, it has been used extensively as an adjuvant in human vaccine trials for several infectious diseases and cancer indications [35].

In the present study, we expressed separately the full length GRA2 and GRA6 cDNAs in bacteria, as recombinant proteins fused to histidine tags, using the pUET expression vector system and purified each protein by immobilized metal ion affinity chromatography. The immunogenicity of these antigens, used alone or in "mix" with the MPL adjuvant, was examined in CBA/J mice, and evaluated for their *de novo* induction of several immunological biomarkers, including cytokines and interleukins, as well as for subclasses of the specific antibody response. Importantly, the protective efficacies of these antigens were evaluated simultaneously against brain cyst production in mice challenged with *T. gondii*.

2. Materials and methods

2.1. Parasites

Tachyzoites of the virulent wild-type strain RH (ATCC 50174) were propagated in human foreskin fibroblasts (HFF) using D10 medium (Dulbecco's Modified Eagle Medium (DMEM) supplemented with 100 U/mL penicillin, 100 μg/ml streptomycin, 2 mM Glutamine and 10% fetal calf serum (FCS) (Gibco, USA). Parasites were harvested after complete lysis of the monolayer, purified through 3.0 μm filters, and washed in phosphate-buffered saline (PBS) (Gibco).

Pru-βgal is the nonvirulent Prugniaud strain, which was genetically modified to express β-galactosidase [36], thus facilitating counting mouse brain cysts through their staining with 5-bromo-4-chloro-3-indolyl-β-D-galactose (X-gal). The Pru-βgal strain was maintained in CBA/J mice as follows: brains were removed one month after infection, homogenized, diluted with PBS in order to adjust the cyst concentration to 100 cysts per ml and 200 μl of the brain homogenate was injected intra-peritoneally (i.p.) into a new lot of CBA/J mice.

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