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GAMMA IRRADIATED ANTIGEN EXTRACTS IMPROVES THE IMMUNE RESPONSE AND PROTECTION IN EXPERIMENTAL TOXOPLASMOSIS

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ABSTRACT

We aimed to use ionizing radiation on soluble extracts of *T. gondii* tachyzoites (AgTg) and tested the ability of these extracts to induce immunity in BALB/c mice against a challenge. *T. gondii* RH strain AgTg was irradiated with Co-60 at 0.25 to 4 kGy and were affected after 1 kGy, as evidenced by a progressive high molecular weight protein aggregates and no loss in antigenicity, as detected by immunoblotting, except after 4kGy. BALB/c mice were immunized with biweekly doses of 03 s.c. native or irradiated AgTg without adjuvants; the anti-*T.gondii* IgG production was detected by ELISA, and higher levels and avidity were detected in mice immunized with 1.5 kGy AgTg compared to controls ($p<0.05$). Mice immunized with native AgTg exhibited spleen $CD19^+$ B, $CD3^+CD4^+$ or $CD3^+CD8^+$ T cell proliferation levels of 5%, while 1.5 kGy-immunized mice exhibited spleen cell proliferation levels of 12.2%, primarily for $CD19^+$ or $CD3^+CD8^+$ lymphocytes and less evidently for $CD3^+CD4^+$ (8.8%) helper T lymphocytes. All cells from control mice showed little to no proliferation when stimulated with AgTg. These cells secreted more IFN-γ in the 1.5 kGy AgTg-immunized group ($p<0.05$). BALB/c mice immunized with 1.5 kGy and challenged with different strains of *T. gondii* were partially protected, as evidenced by survival after RH virulent strain challenge ($p<0.0001$) but also after ME-49 strain challenge: the brain cyst numbers ($p<0.05$) and the levels of *T. gondii* DNA measured by real-time PCR ($p<0.05$) decreased compared to non-immunized controls.

1. INTRODUCTION

Vaccines for Apicomplexa infections as malaria are tried for many years, and currently only one vaccine has been approved, with irradiated sporozoites of *Plasmodium falciparum* [1]. Complex life cycle and difficult *in vitro* culture generated research and production of recombinant or synthetic subcomponent vaccines but with poor results [2]. Toxoplasmosis is widely distributed throughout the world, caused by Apicomplexa protozoan *Toxoplasma*

gondii [3], with estimates of one-third of the world population with antibodies and probably tissue cysts [4]. Usually benign, toxoplasmosis can involve the eyes with blindness and cause severe disease, death and sequels in immune-compromised hosts or children with congenital infection [5]. *T. gondii* is an ideal protozoan for study vaccine models due to its significant immunogenicity, despite its complex life cycle and the existence of several forms with different growth rates [3]. Aside to sterilizing effect, gamma radiation acts on antigens resulting in enhanced subcomponent antigen immunity in leprosy [6, 7] or enhanced antisera production against snake venoms [8]. Gamma radiation affects proteins in solution both directly but also indirectly by action of oxidant radicals from water radiolysis [9]. Irradiated proteins have been reported as recognised and internalized by scavenger receptors in macrophages, and possibly other antigen-presenting cells (APC), in the absence of an adjuvant [10]. This fact could result in the enhancement of immune response without adjuvants, which is important in vaccines for human use [11]. Here, we intend to study the immunity and protection induced without adjuvants in mice challenged with gamma irradiated soluble protein extracts of *T.gondii* tachyzoites.

2. MATERIAL AND METHODS

All reagents and conjugates were purchased from commercial sources, mainly of Sigma Co (St. Louis) and solutions were prepared with high quality water (Milli-Q).

2.1. Ethical Committe protocol number

Ethics Commission on the use of animals in research of Instituto de Medicina Tropical de São Paulo, CEUA-IMTUSP nº 2012/115;
Ethics Commission on the use of animals in research of Instituto de Ciências Biomédicas da Universidade de São Paulo, CEUA-ICB USP nº90/2011.

2.2. Parasites and Animals

Two strains of *T. gondii*, RH and ME-49, cryopreserved and maintained by successive passage in mice (Protozoology Lab., Tropical Medicine Institute of São Paulo). RH strain was maintained routinely by intraperitoneal (i.p.) passage in outbreed mice. ME-49 strain was kindly donated by Prof. Dr. Fausto Araújo, UCLA, and was also kept serial passage in C57Bl/6j or Swiss mice-oral gavage. C57Bl/6J and BALB/c mice, were obtained from our colony (Centro de Bioterismo/FMUSP), and maintained in sterilized cages and absorbent media, with commercial food (Nuvital) and water "ad libitum". The management of these animals before or during the experiments was according of "Principles of Laboratory Animal Care" (NIH Publication no 86-23, revised 1996) and the "Principles of Ethics in Animal Experimentation" (COBEA-Colégio Brasileiro de Experimentação Animal).

2.3. Soluble neutral detergent extracts of *T. gondii* tachyzoites (AgTg)

Tachyzoites of the virulent type I RH strain were obtained from the peritoneal exudate of previously infected Swiss mice. The exudate and cell-free tachyzoites were filtered through a polycarbonate filter with a pore size of 3 µM (Millipore®, Billerica, MA, USA) for the removal of inflammatory infiltrate cells, and tachyzoites were recovered from the filtrate by centrifugation at 2000 g for 5 min. The pellet was suspended in hypotonic neutral detergent buffer containing 0.005 M Tris/HCl at pH 7.5, 1 mM EDTA, 1 mM benzamidine, 0.5 mM

PMSF (phenylmethanesulfonyl fluoride, Pierce®, Rockford, IL, USA) and 0.5% DOC (sodium deoxycholate) to lyse the tachyzoites. After vortexing, all intact intracellular organelles and nuclei were removed by centrifugation at 10,000 g for 30 min at 4°C. The cleared supernatant was subjected to size exclusion chromatography using SEPHADEX® G-25 equilibrated with sterile PBS for removal of <5KDa peptides, detergents and other degradation products. Fractions containing large proteins were pooled assayed for protein content by the Bradford Method (18) and adjusted to 200 µg/mL of protein. Aliquots were sterilised with a 0.22 µM filters (Millipore®) and frozen for further processing.

2.4. AgTg irradiation by a homogeneous source of Cobalt-60 (Co-60)

Aliquots of AgTg were subjected to 0.25 kGy, 0.5 kGy, 1.0 kGy, 1.5 kGy, 2.0 kGy and 4.0 kGy by homogeneous exposure to gamma rays of a Cobalt-60 GammaCell source (Gammacel® Atomic Energy of Canada, Pinawa, Canada) in the presence of O_2 and at a dose rate of 1.21 kGy/hour. Control aliquots were transported under the same conditions and kept off camera on ice to mimic the process. At the end of irradiation, each fraction was carefully observed for clouding or precipitate formation.

2.5. SDS PAGE and immunoblotting for the characterization of native and Co-60-irradiated AgTg proteins

Samples of native antigens or irradiated AgTg (20 µg/well) were subjected to SDS-PAGE in 12.5% slabs for the separation of proteins in the extract as described elsewhere (19); the Spectra™ Multicolor Broad Range Protein Ladder (Thermo Scientific®, USA) was used as a molecular standard. Slab gels ere stained by Coomassie Brilliant Blue G250 and scanned to create a graphic jpg file. SDS-PAGE-separated AgTg proteins were transferred to nitrocellulose membranes (Millipore®) using semi-dry transfer. After transfer, membranes were blocked with 5% skim milk (Molico®) and 0.02% Tween 20 PBS (PBSTL) for 1 hour at room temperature with stirring. For antigen, analysis the membranes were reacted with 1/100 dilution of hyperimmune anti *T.gondii* sera from ME-49 strain chronically infected mice.

2.6. Immunization of animals and detection of anti-*T. gondii* IgG production and avidity

Groups of 5 BALB/c mice were immunized biweekly (three doses) subcutaneous in the dorsal region (s.c) with 20 µg (100µL) of protein of native or irradiated AgTg in PBS. Tail blood samples were collected weekly on filter paper, dried and stored at -20 °C. Specific IgG production and avidity was measured by ELISA from the filter papers. Before use, filters were extracted in 24-well plates with 300 µL of buffer containing 10 mM Tris/HCl at pH 7.5, 150 mM NH_4Cl, and 1mM of NaN_3 to lyse erythrocytes and permit conversion to cyanohemoglobin (21), which could be measured at 540 nm in a microplate reader; this allowed volume correction of the extract to a 1:100 serum dilution with PBS.

Antigen specific IgG antibodies were analyzed by enzyme-linked immunosorbent assay (ELISA). ELISA plates were coated overnight at 4 °C with 1 µg protein/mL of *T. gondii* antigen in 0.05 M carbonate buffer, pH 9.0. Plates were washed with PBST (PBS containing 0.05% Tween-20) for 5 min and blocked with 0.3% milk in PBST for 1h at 37 C. After blocking, the samples of sera were added. To test for IgG avidity, an additional step of 15 min incubation with a 6 M urea chaotrope solution was added for the removal of low avidity antibodies. Next, plates were washed and appropriately, diluted anti-mouse IgG peroxidase-conjugated antibodies were added (Sigma®, Sigma-Aldrich Co., St. Louis, MO, USA). After further

washes, bound IgG was revealed with by the *o*-phenylenediamine system for 1h; reactions were stopped by adding 4 M hydrochloric acid (HCl). Absorbance at 492 nm was determined using an automatic microplate reader (Multiskan MS® Labsystems, Vienna, USA). For each serum sample, the assay was done in duplicate. Avidity was expressed as % of resistance of bound IgG (A_{495} from well with urea washing*100/A_{495} from PBSTL well).

2.7. Spleen cell proliferation, phenotype and production of cytokines in response to AgTg in immunized animals.

Spleen cells were obtained from groups of mice, including controls, experimental mice and mice chronically infected with the ME-49 strain of *T. gondii*. Pooled spleens were sterilely dissociated in RPMI 1640 culture medium, and isolated cells were centrifuged at 2800 g for 15 minutes. Erythrocytes were eliminated with RBC lysis solution v/v (0.16 M ammonium chloride and 0.17 M Tris hydroxymethyl aminomethane, pH 7.2). Cells adjusted to $4x10^6$ cells/mL were incubated with 5µM 5-6 carboxyfluorescein diacetate succinimidyl ester (CFSE – BD Biosciences®, East Rutherford, New Jersey) in culture medium at 37 °C for 5 min. Foetal bovine serum (FBS) was added at a concentration of 10%, and CFSE-containing cells were kept on ice for 5 minutes to block CFSE uptake. Cells were recovered by centrifugation (800 g for 8 min) and washed in 10 volumes of RPMI culture medium/10% FBS. For proliferation, cells were adjusted to $1x10^6$ cells/mL in the same media and distributed into wells of 24-well culture plates, adding sterile AgTg at 30 µg/mL or ConA at 10 µg/mL; cells were cultured for 6 days at 5% CO_2 and 37°C. After this, the cells were recovered, washed and labelled with Pacific Blue-conjugated CD3, Horizon V500-conjugated CD4, APC-H7-conjugated CD8 and PE-Cy-7-conjugated CD19 mabs (monoclonal antibody, BD Biosciences®) at adequate dilutions and incubated for 30 minutes at 4 °C in the absence of light. Cells were analysed (10,000 events) on a flow cytometer with adequate filters (LSRFortessa). Seven data parameters were collected with BD FACSDIVA software and analysed with FlowJo X software. A typical analysis of, the results were expressed as a whole or as phenotype-specific proportions of proliferated cells. For measured IFN-γ, supernatant from the cultured cell spleen were collected after 72 h of stimulation with *T. gondii* antigen (10 µg/mL) and assayed for interleukin IFN-γ. IFN- γ concentration were determined using a CBA kit (BD Bioscience®), according to the manufacturer's instructions. Data parameters were obtained with FCAP Array Software (BD Bioscience®).

2.8. Challenge with the cystogenic ME-49 strain (type II) and virulent strain RH (type I)

Groups of 5 immunized or control BALB/c mice at 15 days following the last immunization were challenged i.p. with lethal RH strain tachyzoites or orally with cysts of the non-lethal ME-49 strain. For lethal challenge with the RH strain, groups of 5 mice were challenged i.p. with 10^3 tachyzoites. Acute mortality was recorded daily for 15 days. Cysts of ME-49 *T. gondii* were obtained from brains of chronically infected C57Bl/6J mice. The brains were removed and homogenised in PBS, and 25-µl samples were observed under phase contrast microscopy to count cysts as described elsewhere (8). Groups of mice also were challenged orally with 10 cysts by gavage. After a period of 30 days, brains were recovered, cysts were counted or brain samples were subjected to real-time PCR for *T. gondii* DNA quantification. DNA extraction was performed using a commercial QIAmp ® DNA mini kit (Qiagen®, Germany) according to the manufacturer's instructions. Primers were designed using Primer 3 software after B1 gene sequences were checked in the NCBI Nucleotide Database (B1 sense: 5'GCACCTTTCGGACCTCAACAACCG, anti-sense: 5'TTCTCGCCTCATTTCTGGGT CTAC), and standardised reactions with SYBR Green revealed a 286-bp product. Real-time

PCR assays were performed with the ABI Prism 7500 Sequence Detection System (Applied Biosystems®, USA) using Power SYBR Green PCR Master Mix (Applied Biosystems®) following the manufacturer's protocol. The thermo-cycler was programmed as follows: 95 °C for 10 minutes, 40 repetitions of 95 °C for 15 seconds and 60 °C for one minute, and finally a denaturation step. The results were expressed as Ct values (cycle threshold: number of cycles required to reach the detection threshold). All samples were tested in triplicate.

3. RESULTS

Structural and antigenic effects of gamma radiation on extracts of *T. gondii* tachyzoites (AgTg)

Precipitates or visual aggregates were not observed after irradiation with up to 4.0 kGy. Irradiated samples were subjected to SDS-PAGE, as shown in figure 1, with smaller doses (0.25 kGy (lane 3) and 0.5 kGy (lane 4)) doses maintaining the same pattern of protein bands as the native extract. Higher doses (1.0 kGy at 2.0 kGy) increased the diffuse banding in a dose-dependent manner; this increase was especially pronounced at 4.0 kGy, which resulted in reduced band integrity.

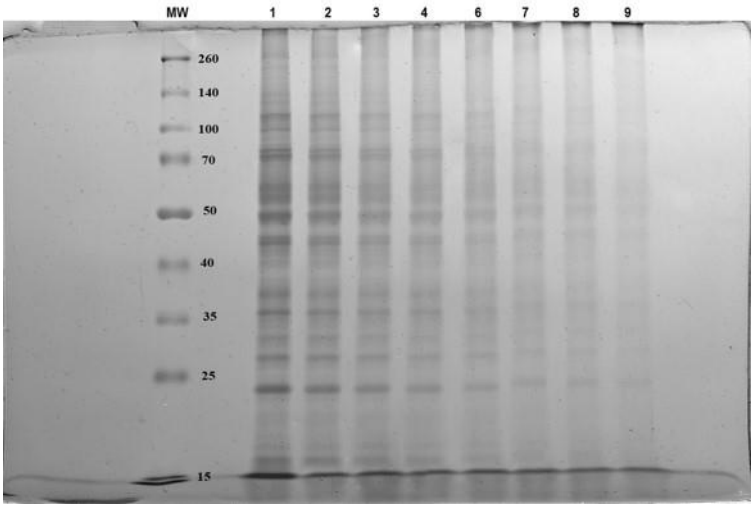

Figure 1. SDS-PAGE of antigen-soluble extracts of tachyzoites of *T. gondii* (AgTg), both native and irradiated at different doses of Co-60. (MW) Molecular Weight; (1) *T. gondii* antigen before purification; (2) Molecular exclusion chromatography native AgTg; (3) 0.25 kGy irradiated AgTg; (4) 0.5 kGy irradiated AgTg; (5) 1.0 kGy irradiated AgTg; (6) 1.5 kGy irradiated AgTg; (7) 2.0 kGy irradiated AgTg; (7) 4.0 kGy irradiated AgTg.

After immunoblotting, Native AgTg and 1.0-2.0 kGy-irradiated AgTg recognized similar antigens, as shown in figure 2, but AgTg irradiated at 4.0 kGy exhibited little reactivity to antibodies, with minor identifiable bands of approximately ~20-25 kDa.

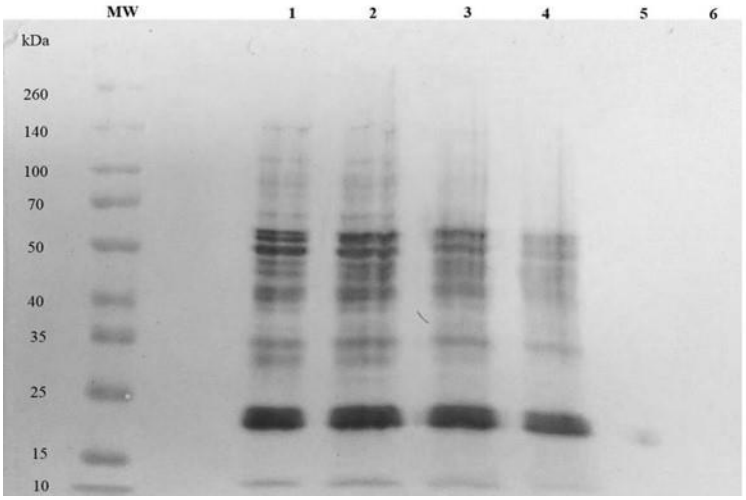

Figure 2. Western blot analysis to assess the immunogenicity of native AgTg and AgTg irradiated at different doses of Co-60, using positive sera from the ME-49 strain of *T. gondii* (dilution 1/100). (MW) Molecular Weight; (1) Native AgTg; (2) 0.5 kGy irradiated AgTg; (3) 1.0 kGy irradiated AgTg, (4) 2.0 kGy irradiated AgTg; (5) 4.0 kGy irradiated AgTg; (6) Negative control (non-immunized).

Antibody production, avidity of IgG, memory cell phenotype and IFN- γ cytokine in immunized mice with irradiated AgTg.

Groups of 5 mice were immunized with three biweekly doses of native or 1.5 kGy-irradiated AgTg, and we obtained sera 15 days after last dose. These sera were assessed with ELISA or reacted with membranes containing AgTg. The serum samples from mice immunized with three doses of 20 µg 1.5 kGy-irradiated AgTg were obtained and assayed with anti-*T. gondii* IgG in AgTg-adsorbed plates. AgTg-specific IgG was present in all vaccinated groups and presented high-avidity IgG antibodies (Figure 3).

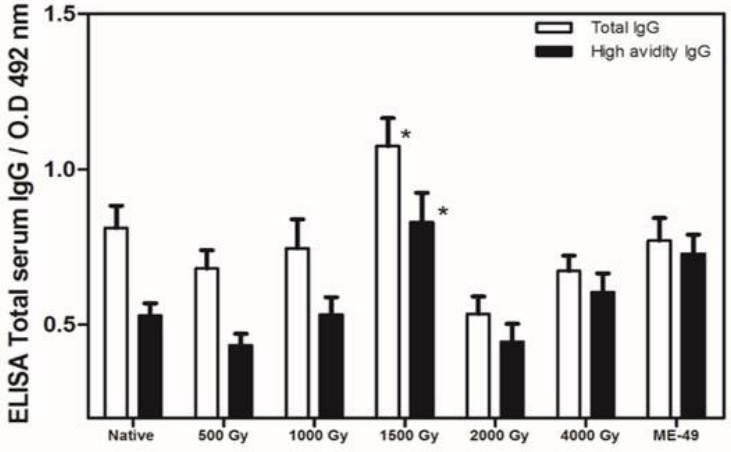

Figure 3. Humoral immune response and hight avidity antibody production in mice immunized with 03 doses (s.c) soluble native *T. gondii* antigen or irradiated to different doses of Co-60, 15 days after the third immunization, demonstrating increased production of anti-*T.gondii* IgG and increased productin of antibodies of hight avidity. Differences between the 1,5kGy irradiated AgTg groups was significant (p<0,05=*)

However, the AgTg 1.5 kGy immunized mice significantly statistically differed ($p<0.05$) in the production of AgTg-specific and high-avidity IgG antibodies compared to the other groups. The antibodies induced by 1.5 kGy-irradiated AgTg could recognize most of the epitopes present in the total extract as well as in hyperimmune sera from ME-49-infected mice. Interestingly, the antibodies induced by native AgTg showed a lower intensity of antigen recognition (figure 4).

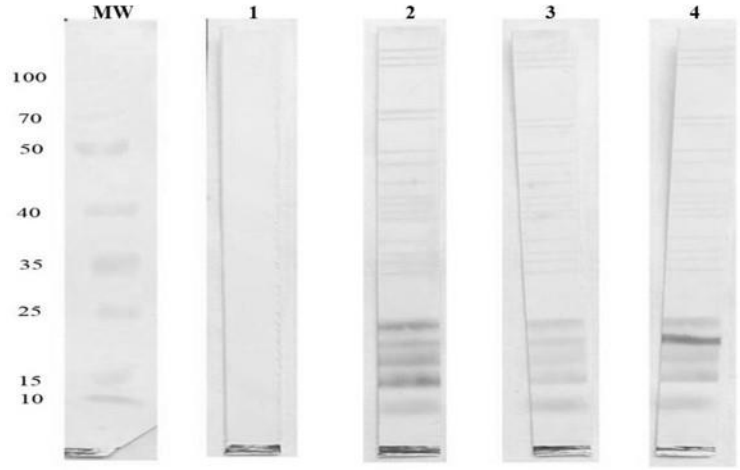

Figure 4. Western blot analysis of recognition of AgTg antigens in the sera of BALB/c mice immunized s.c. with 3 doses of native AgTg or 1.5 kGy-irradiated AgTg). (MW) Molecular Weight; (1) sera from unimmunized BALB/c (negative control); (2) sera from BALB/c infected with the ME-49 strain of *T. gondii* (positive control); (3) sera from BALB/c immunized with native AgTg; (4) sera from BALB/c immunized with AgTg irradiated at 1.5 kGy of Co-60.

The memory cell phenotypes were analyzed in mice immunized with three doses of 1.5 kGy-irradiated AgTg, and these data are shown in table 1. A cell suspensions labeled with CFSE as described in the Methods was maintained in culture in the presence of 10 µg/mL AgTg for 6 days; adequate controls with unspecific mitogens were utilized for both cell viability and responses. Good responses to unspecific ConA mitogens were observed, and the proliferation rates cells from non-immunized BALB/c mice presented were reduced (1%). Infected ME-49 mice responded with approximately one fourth of surviving cells, while native AgTg-immunized mice presented lower than 7,7% surviving cells. Cells from mice immunized with irradiated AgTg exhibited 12.2% splenic cell proliferation, 11.4% $CD19^+$ proliferation, 8.8% $CD3^+CD4^+$ proliferation and 12% $CD3^+CD8^+$ proliferation, reaching proliferation levels that were approximately half of those of infection-induced memory cells.

Table 1 Representation of the proliferation of spleen cells from BALB/c controls, BALB/c mice immunized s.c with native AgTg, BALB/c mice immunized s.c with 1.5 kGy irradiated AgTg and infected ME-49 strain mice. Spleen cells were marked with carboxyfluorescein diacetate succinimidyl ester (CFSE) and stimulated with *T. gondii* protein extract.

	Nonspecific control (ConA)	Unimmunised (Control)	Immunised with native AgTg	Immunised with 1.5 kGy AgTg	Infected with ME-49 strain
Total proliferation	72.5%	1.3%	7.7%	12.2%	26.5%
Total B cell proliferation ($CD19^+$)	60.3%	1.1%	5.4%	11.4%	30.6%
Total helper T lymphocyte proliferation ($CD3^+CD4^+$)	73.5%	1.1%	4.6%	8.8%	27%
Total cytotoxic T lymphocyte proliferation ($CD3^+CD8^+$)	78.5%	0.8%	5.3%	12%	24.3%

The supernatants from cultured spleen cells were used to measure the production of IFN- γ after immunization with 1.5kGy irradiated AgTg and native AgTg, and these levels were compared to the control group. As shown in Figure 5, mice immunized with 1.5 kGy-irradiated AgTg produced significant levels of IFN-γ ($p<0.05$) compared to mice immunized with native AgTg and mice in the control group.

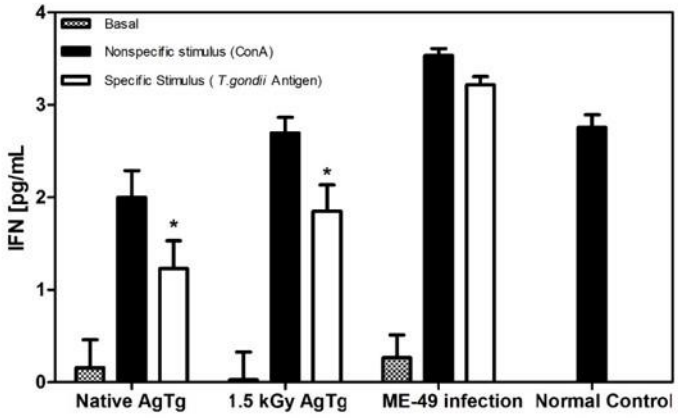

Figure 5. Production of IFN-γ cytokine. Cytokines obtained from the culture supernatant of the spleen cells of BALB/c mice (n=5) immunized with native antigen or irradiated at 1.5 kGy of Co-60, BALB/c mice infected with ME-49 strain and normal mice after 72 hours (n =5). The results are expressed as pg/mL. Asterisks show statistically significant differences between groups ($p<0.05$).

Protection induced in BALB/c mice immunized with 1.5 kGy irradiated AgTg after challenge with different strains of *T. gondii*: ME-49 (type II) and RH (type I)

To assess the protective efficacy of the immunization of BALB/c mice immunized with 1.5 kGy irradiated or native AgTg, we challenged groups of five immunized mice with 10 cysts of the ME-49 strain of *T. gondii* 15 days after the last dose and euthanized the animals 30 days later. In this experiment, protection was defined as the number of cysts present in the brain; the results are depicted in figure 6. The numbers of brain cysts counts and DNA quantification 30 days after challenge or infection are shown in figure 6A and 6B, respectively. BALB/c immunized with 1.5 kGy AgTg exhibited a decrease in brain cyst numbers compared to the Native AgTg-immunized group ($p<0.05$, figure 7A). Similar data were obtained when *T. gondii* DNA was quantified in the brains using real time PCR, as shown in figure 6B ($p<0.05$).

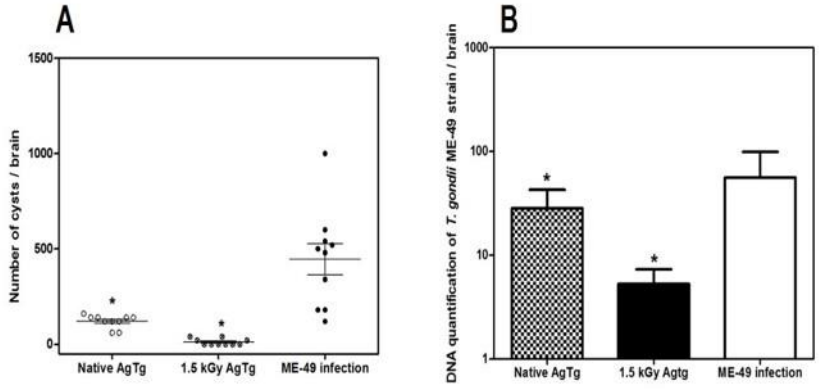

Figure 6. Number of brain cysts of immunized BALB/c mice s.c by native or 1.5 kGy irradiated AgTg (A) and DNA quantification of cysts in brains of immunized BALB/c mice s.c by native or 1.5 kGy irradiated AgTg (B) and challenge with 10 cysts of *T. gondii* ME-49 strain 15 days after immunizations. Data represent the mean of 3 experiments. Bars represent the mean and standard deviation, and the presence of asterisks indicates a statistically significant difference ($p<0.05$) compared to infection.

A lethal challenge with RH tachyzoites was tested by injecting 10^3 tachyzoites i.p. into groups of mice and monitoring the survival daily. The results shown in figure 7 represent the top two groups obtained from three immunized groups. All animals in the control group had died 2, 4 and 6 days after challenge with the lethal RH strain. The animals immunized with native or irradiated 1.5 kGy antigen survived, but the survival rate was higher in mice irradiated 1.5 kGy antigen. In this group, the first deaths occurred 10 days after challenge ($p<0.0001$).

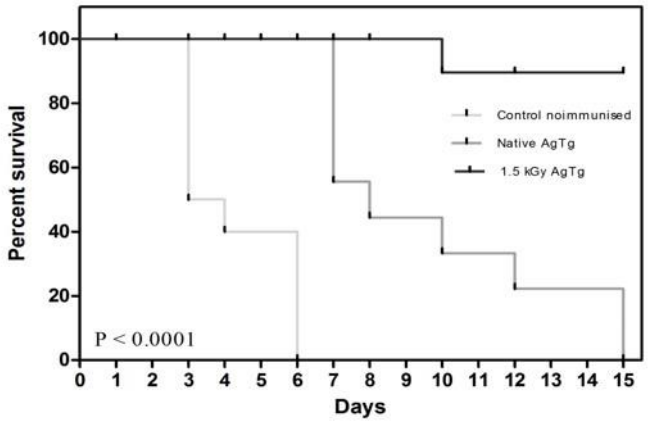

Figure 7. Kaplan-Meir survival analysis of BALB/c mice immunized with 3 doses of native AgTg or 1.5 kGy AgTg irradiated by Co-60 and challenged with 10^3 tachyzoites of *T. gondii* RH strain type I, 15 days after immunizations. Data represent 2 experiments. The comparison of survival curves was significant ($p < 0.0001$).

4. DISCUSSION AND CONCLUSIONS

Our results overall show that gamma irradiation effectively improves the immune response to a soluble extract of *T.gondii*, as evidenced by progressive, dose-dependent changes that affect the molecular weight and protein structure. A loss of antigen immunogenicity and structural properties was observed above 2 kGy, as reported elsewhere by several authors [6,8], including descriptions of the progressive blurring of bands, which indicates breakage and aggregation. Many of the effects of radiation on proteins have been attributed to the products of water radiolysis, which are extremely reactive and capable of interacting with nearby compounds due to extended life [12]. Other authors have shown that water radiolysis products interfere with the active centers in proteins and toxins, making them less toxic and thus more efficient for immunization [13].

We have shown that irradiation at adequate doses exerts few effects on the antigenic properties of AgTg, as determined by antibody recognition. These irradiated proteins were recognized by anti-*T. gondii* antibodies from infected animals due to the recognition of epitopes, which was evident even in degraded proteins [14]. These irradiated antigens could also induce antibodies that recognize intact antigen. The use of ionizing radiation maintained the original antigenic properties but improved the immune response, producing antibodies that were highly responsive and better than native protein, as also reported elsewhere [8]. Our data corroborate that ionizing radiation acts on the protein structure by stimulating the immune system to recognize molecules, which improves immune maturation, as evidenced by the high avidity of antibodies from irradiated AgTg immunized animals compared to animals immunized with native AgTg.

In our study, protein modifications after irradiation increased the numbers of antigen-responsive cells. This increase could be attributed to the enhanced initial binding of antigens to APCs [15]. We demonstrated that both CD8 and B cells proliferated after immunization with irradiated antigens, which suggests that T cells recognized irradiated epitopes. This recognition could be improved to enhance protection; indeed, CD8 cells were also more abundant in response to antigen [16]. In mice, the production of IFN-γ in the presence of TNF-α activates

macrophages to eliminate *T. gondii* [17]. Our results demonstrate a significant increase in the production of IFN- γ, which indicates that immunization with irradiated AgTg to 1.5 kGy Co-60 induces an effective immune response mediated by $CD8^+$ and $CD4^+$ T cells associated with the production of IFN- γ [18]. The proliferative response of antigen-stimulated cells and cytokine production was smaller than that observed in infected animals, but this can be explained by the fact that challenge was performed with 3 pulsed doses of antigen, and this approach usually results in lower immunity than natural infection, which features continuous antigen stimulation.

Our irradiated antigen immunization provided protection. In toxoplasmosis, protection can be measured based on the absence of the eliciting agent to demonstrate the prevention of infection or the presence of fewer agents in the host [8]. When assessing protection from toxoplasmosis, mice immunized subcutaneously with irradiated AgTg and challenged with the ME-49 strain exhibited a smaller number of cysts than mice that were not immunized based on the level of *T. gondii* DNA in the brain. Most authors use challenges with less virulent strains of the parasite that generally produce cysts [16] and avoid stress tests with virulent strains, such as RH, for which only a delay in mortality and no effective protection are observed; this result was also evident in our work.

Diseases caused by Apicomplexa protozoa such as *Toxoplasma* are disseminated and little protection against reinfection but with protection to disease. Since 1967 vaccines with radiated protozoa showed promising results, and the alternatives tested for 50 years had no equivalent success. In toxoplasmosis vaccines with irradiated parasites promote effective immunity to the disease, similar to previous infection. Radiation agent prevents replication, but keep your physiology with intact antigens to respond effectively. Irradiated crude antigens of *Toxoplasma* induced better immunity without adjuvant associated, showing that the radiation acts upon proteins and isolated not only in nucleic acids agent. Using a human-specific vaccine is not always desirable, but targeting the vaccination of the definitive host of *T. gondii*, i.e., the cat, might reduce the oocyst production and thus the contamination of water and vegetables. This reduction would also indirectly decrease the contamination of pastures and ruminants, whose meat is used for human consumption. Vaccine processing with an optimal radiation dose could be a tool to design vaccines without adjuvants and model the use of a toxoplasmosis vaccine for public health.

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