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Research Brief

Toxoplasma gondii infection modulate systemic allergic immune response in BALB/c mice

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HIGHLIGHTS

- *T. gondii* immune-modulation at systemic level in an asthma mouse model.
- Infection before allergic sensitization results in a lower Th2 cytokine response.
- Sensitization during acute infection results in increased IFN-γ and TGF-β levels.
- Infection results in a decreased T cell proliferation and anaphylaxis reaction.
- *T. gondii* infection prevents an allergic reaction beyond the lung.

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ABSTRACT

The increased prevalence of allergies in developed countries has been attributed to a reduced exposure to some microbes. In agreement with epidemiological studies, we previously showed that *Toxoplasma gondii* infection prevents allergic airway inflammation. The mechanisms would be related to the strong Th1 response induced by the parasite and to regulatory cell induction. Herein we further characterized whether *T. gondii* allergy modulation extents to a systemic level or if it is limited to the lung. Parasite infection before allergic sensitization resulted in a diminished Th2 cytokine response and, when sensitized during acute infection, an increased in TGF-β production was detected. Allergen specific T cell proliferation was also reduced. Sensitization during both acute and chronic phases of infection resulted in a decreased anaphylaxis reaction. Our results extend earlier work and show that, in addition to lung airway inflammation, *T. gondii* infection can suppress allergic responses at systemic level. These results open the possibility that this protozoan infection could modulate other allergic disorders such as atopic dermatitis or oral allergies. Understanding the mechanisms by which different microorganisms regulate inflammation may potentially lead to the development of strategies aimed to control atopic diseases.

1. Introduction

The original version of the hygiene hypothesis postulated a link between decreased childhood infections and increased allergy in Western societies (Strachan, 1989) and was supported by epidemiological data such as the inverse relationship of BCG infection to atopy (Shirakawa et al., 1997). Among epidemiological studies supporting this hypothesis many showed that respiratory allergy is less frequent in people exposed to orofecal and foodborne microbes such as *T. gondii* and hepatitis A virus, but not to viruses transmitted through other routes (Ellertsen et al., 2008; Fernandes et al., 2010; Matricardi et al., 2000). We and others (Fenoy et al.,

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2008; Wagner et al., 2009) provided experimental support for these epidemiological data by showing that *T. gondii* infection can block the development of allergic airway inflammation in adult BALB/c mice. This effect operates during both acute and chronic phases of infection. Parasite infection before allergic sensitization also significantly decreased the synthesis of Th2-directed IgE and IgG1 antibodies when compared with allergic mice. In parallel, an enhancement of OVA-specific IgG2a, an IgG isotype driven by Th1 lymphocytes, was detected (Fenoy et al., 2008). The mechanisms involved in allergy protection by *T. gondii* infection include both immune deviation (Fenoy et al., 2008) and regulatory cells induction in thoracic lymph nodes (Fenoy et al., 2012).

Exposure to microorganisms or their products diminish the susceptibility to develop different atopic disorders, including not only respiratory allergies but also atopic dermatitis (Flohr and Yeo, 2011; Ryozawa et al., 2007). Diverse experimental models of systemic allergy, food allergy and atopic dermatitis support those epidemiological data and demonstrate that different infectious or commensal microorganisms could suppress the development of these disorders (Castro et al., 2012; Kim et al., 2012; Schiavi et al., 2011). With this in mind, and considering the observed allergen specific humoral immune deviation induced by *T. gondii* infection, we decided to analyze whether this protozoa also modulates allergy at systemic level or if it is only confined to the lung.

2. Materials and methods

2.1. Animals

BALB/c (H-2^d) mice were obtained from the animal facilities of the Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (UBA), Argentina and maintained in our animal facilities for use throughout these experiments. Mice were used at the age of 6–8 weeks. All procedures requiring animals were in accordance with the guidelines established by the National University of General San Martin and the National Research Agency (PICT 562).

2.2. Infection, sensitization and exposure

The Beverley strain of *T. gondii* was used in this study. For infection, BALB/c mice were orally infected with 25 cysts. One week (acute phase) or one month later (chronic phase), sensitization was achieved by two i.p. injections of 0.2 ml PBS containing 20 mg of chicken egg white albumin (OVA) (grade V, Sigma-Aldrich) and 2 mg of alum hydroxide (Sigma-Aldrich) one week apart. One week later, mice were exposed to aerosols of allergen (3% (w/v)) OVA in PBS for 20 min on 3 consecutive days (TO group). Aerosol exposure was performed within individual compartments of a mouse pie chamber using a nebulizer (SAN-UP, Argentina, OVA solution flux 0.33 ml/min in air flux of 6–8 l/min). Mice were analyzed 48 h after the last exposure. Negative controls include *T. gondii* infected (T) and non-infected (naive).

2.3. Proliferation assays and cytokine production

Spleen and lung were removed, single cell suspensions were made using a cell strainer and 3×10^5 cells were cultured in 200 ml of medium RPMI 1640 supplemented with 20% FBS (GIBCO), 1% antibiotics (GIBCO) and 5×10^{-5} M 2-mercaptoethanol alone or in the presence of OVA (200 mg/ml) (grade V, Sigma-Aldrich) (Sigma-Aldrich). Cytokine production was measured in supernatants at 72 h by capture ELISA commercial kits (IL-4, IL-5, IFN-γ TGF-β: Pharmingen, BD Bioscience OptEIA™ kit, IL-10: BioLegend ELISA MAXTM kit). Proliferative responses of splenocytes cultured in 96 wells round bottom plates with medium or OVA (100 μg/well) were determined after addition of methyl-^{3}H thymidine (1 μCi/well, PerkinElmer, Argentina) for the last 18 h of a 5 day culture period. Proliferation is shown as the difference in incorporation of [^{3}H]thymidine between stimulated and non-stimulated cells (Δ cpm).

2.4. Active cutaneous anaphylaxis (ACA)

ACA was induced in the six experimental groups by intradermic challenge with the allergen. Briefly, an intravenous injection of 1% w/v Evans Blue (50 μl) was administered by the tail vein, and ACA was then elicited in the right ear by intradermic inoculation of 50 μl OVA (1 mg/ml). PBS was inoculated in the left ear of each animal as a control reaction. Animals were observed after 30 min. To confirm the qualitative changes, a semi-quantitative scoring was developed by ranking the reaction observed on each ear from 1 to 100 considering the color intensity and the extent of the blue area. The index was calculated by dividing the score assigned to the ear inoculated with the allergen by the one inoculated with PBS. The scoring was generated without knowing the experimental group each mice belonged to.

2.5. Statistical analysis

Each experimental group had at least four mice and each experiment was repeated at least 3 times. Data are presented as mean ± SEM. Statistical analysis was performed using ANOVA analysis of differences among groups with Bonferroni test *a posteriori* as indicated in the figure legends. Statistical analysis for semi-quantitative scoring was done using Kruskal–Wallis with Dunn's test a posteriori. Statistical significance was accepted when $p < 0.05$.

3. Results and discussion

3.1. Local and systemic cytokine profile

We first analyzed lung and systemic cytokine profiles. The reduced allergic lung inflammation induced by *T. gondii* infection that we have previously reported (Fenoy et al., 2008), correlates with a diminished Th2 cytokine production in lung cell cultures upon *in vitro* allergen stimulation (Fig. 1A). Splenocytes from the different experimental groups were *ex-vivo* stimulated with OVA in order to evaluate *T. gondii* modulation at systemic level. As expected, allergic mice showed higher production of both IL-4 and IL-5 compared with normal animals (Fig. 1B). Similar to the data previously obtained with thoracic lymph node cells (Fenoy et al., 2008) and herein with lung, this predominant Th2 immune response was reversed when mice were previously acutely or chronically infected with the parasite (Fig. 1A and B). An increase in IFN-ɣ production was detected only when mice were sensitized during acute infection (Fig. 1A and B). These results agree with those obtained by Wagner and colleagues (Wagner et al., 2009).

3.2. Splenocyte proliferation and regulatory cytokines

Sensitization during both acute and chronic *T. gondii* infection (TO groups) resulted in a dramatic decrease in allergen-specific T cell proliferation compared with allergic mice (O), as previously observed for thoracic lymph node cells (Fenoy et al., 2012). Moreover, this decrease was so remarkable that no significant differences were observed in splenocyte proliferation levels between the TO group and the naive (N) and non-sensitized *T. gondii* infected animals (T group) (Fig. 2A). The decrease in the proliferative response could be explained by a less allergic sensitization, consequence of the high IFN-γ levels characteristic of the *T. gondii* infection or by the presence of regulatory cytokines elicited to control the strong immune response mounted against the parasite. One mediator responsible for the down-regulation of allergies might be the anti-inflammatory

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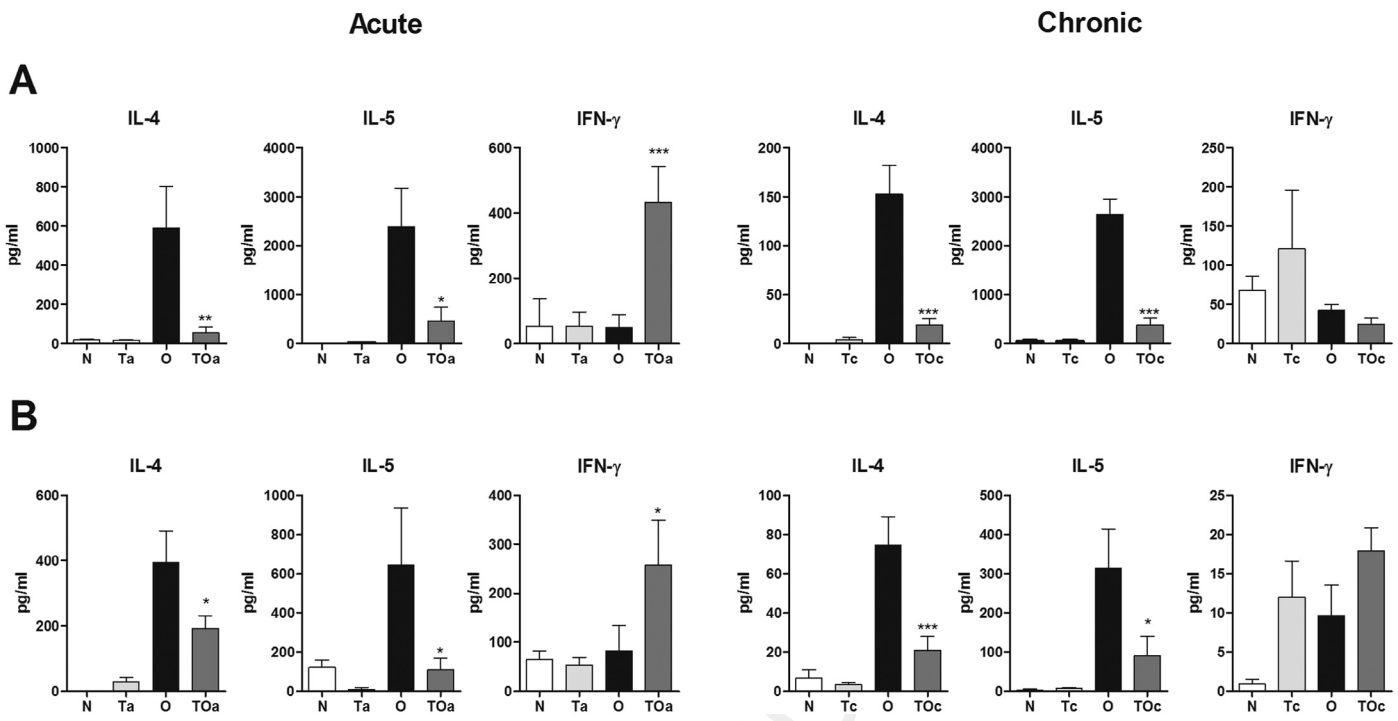

Fig. 1. Th1/Th2 cytokine profile. Cytokine production by lung (A) and spleen (B) cells *ex vivo* stimulated with OVA was measured in mice OVA-sensitized (O), *T. gondii* infected and OVA sensitized during acute (TOa) or chronic (TOc) infection (both groups aerosolized with OVA), *T. gondii* acute (Ta) and chronic (Tc) or naive mice (N) (both negative groups aerosolized with PBS). *$p \leq 0.05$, **$p < 0.01$, ***$p < 0.001$ vs O group ANOVA with Bonferroni *a posteriori*.

cytokine IL-10 (Urry et al., 2006). Although an apparently reduced allergen specific IL-10 production in previously infected mice, the differences were not statistically significant (Fig. 2B and C). Hence, similarly to what we have previously observed in lung (Fenoy et al., 2012), IL-10 would not be involved in the *T. gondii* systemic immunomodulation. However, increased levels of TGF-β were detected in splenocytes from mice sensitized during acute infection (Fig. 2B) suggesting that in addition to a deviation toward a Th1 response, this cytokine could be involved in *T. gondii* allergic modulation during this phase of infection.

3.3. *Active cutaneous anaphylaxis*

Mast cell degranulation induced by the allergen crosslinking of cell surface FcεRI is critical to the development of allergic diseases (Galli, 1997). Active and passive cutaneous anaphylaxis are commonly used in animal models for demonstrating *in vivo* IgE-mediated mast cell degranulation and also to evaluate potential anti-allergic compounds (Finkelman, 2007; Inagaki and Nagai, 2009). The active cutaneous anaphylaxis is a localized cutaneous allergic response resulting from increased vascular permeability induced by allergens and plasma extravasation, and represents the clinical features of urticarial (Inagaki and Nagai, 2009). Sensitization during both acute and chronic infection resulted in a lower anaphylaxis reaction compared with allergic mice. As shown in Fig. 3A, the vascular permeability specifically induced by OVA injection was lower in both acute and chronic *T. gondii* infected and sensitized mice compared with allergic mice as observed by the smaller colored area and lower intensity of blue on the ear. The results of semiquantitative scoring support these qualitative changes (Fig. 3B). These results are consistent with the diminished levels of serum IgE previously reported (Fenoy et al., 2008). However, the decrease in anaphylaxis is much more pronounced than the decrease in allergen specific IgE (Fenoy et al., 2008). In this context, it has been reported that in addition to IgE and antigen other molecules such as TSLP can trigger mast cell secretion (Theoharides et al., 2012). Also, it has been shown that

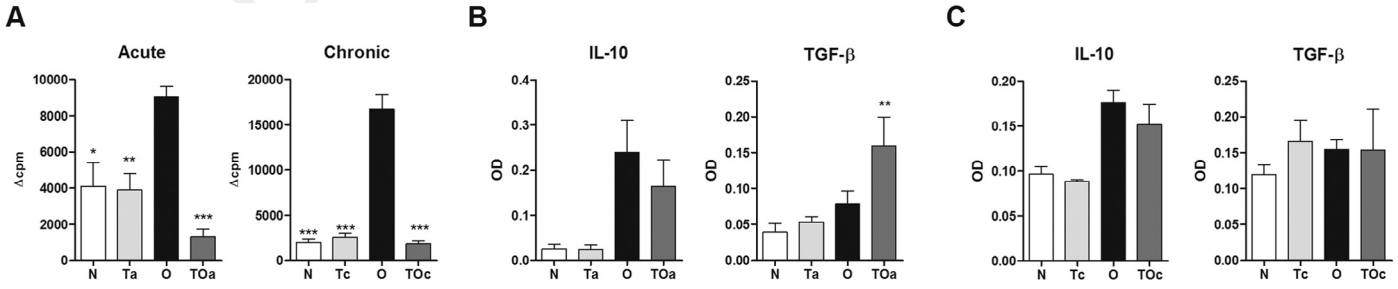

Fig. 2. Reduced allergen specific T cell proliferation and production of systemic regulatory cytokines. (A) Proliferative responses of splenocytes (3×10^5) were determined by [^{3}H]thymidine incorporation after a 5-day culture period upon stimulation with OVA. Results are expressed as Δcpm. (B and C) Cytokine production by splenocytes cultured with OVA was measured in all groups. OVA-sensitized (O), *T. gondii* infected and OVA sensitized during acute or chronic phase (TOa and TOc), acute or chronic *T. gondii* infected (Ta and Tc) or naive (N) mice. *$p \leq 0.05$, **$p < 0.01$, ***$p < 0.001$ vs O group ANOVA with Bonferroni *a posteriori*.

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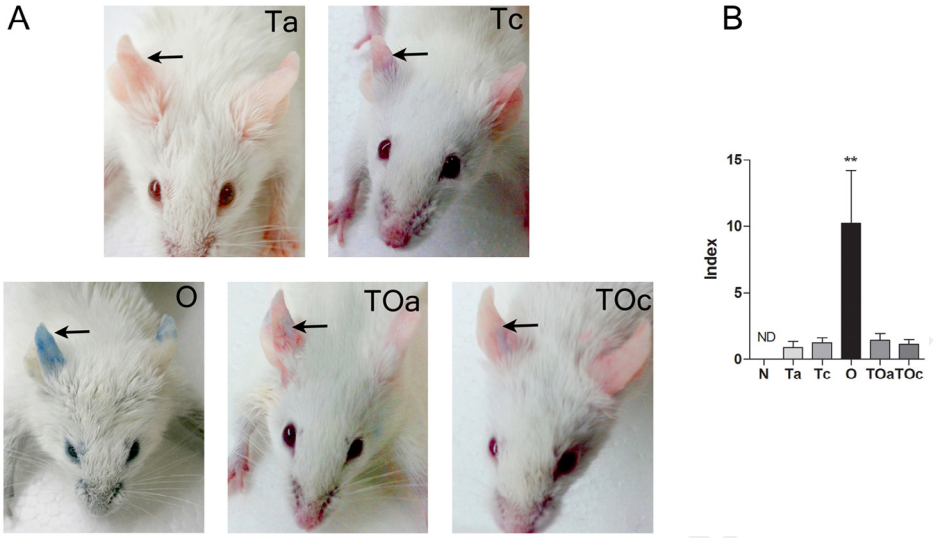

Fig. 3. Diminished active cutaneous anaphylaxis. ACA was induced in naive (N), non-sensitized *T. gondii* infected (Ta and Tc), allergic (O) and infected and acute or chronic sensitized (TOa and TOc) mice by intradermic challenge with the allergen (OVA) in the right ear. PBS was inoculated in the left ear of each animal as a control reaction. (A) Representative pictures from the different experimental groups sacrificed 30 min post inoculation. (B) A semi-quantitative scoring was developed by ranking the reaction observed on each ear from 1 to 100 by considering the color intensity and the extent of the blue area. ND: not done. $^{**}p < 0.01$ vs all other experimental groups, Kruskal–Wallis with Dunn's test *a posteriori*.

T. gondii inhibits antigen-stimulated degranulation in mast-infected cells. This effect correlates with reduced cytoplasmic Ca^{2+} mobilization, particularly antigen-mediated Ca^{2+} release from intracellular stores (Smith et al., 2013).

Our work allows concluding that *T. gondii* infection can modulate not only the susceptibility to developing respiratory allergies but also systemic IgE-dependent allergic diseases. These results open the possibility that this protozoan infection could modulate other allergic disorders such as atopic dermatitis or oral allergies.

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