Journal of Dermatological Science 96 (2019) 143–150

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

Original Article

Chronic infection with the protozoan *Toxoplasma gondii* prevents the development of experimental atopic dermatitis in mice★

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ARTICLE INFO

Article history:
Received 14 June 2019
Received in revised form 4 October 2019
Accepted 27 October 2019

Keywords:
Allergy
Atopic dermatitis
Immunomodulation
Toxoplasma gondii

ABSTRACT

Background: Supporting the hypothesis that*T. gondii* infection protects against allergy in humans we previously demonstrated that this infection can modulate not only the susceptibility to develop respiratory allergies in mice but also suppresses allergic responses at systemic level. This latter finding suggests that *T. gondii* infection could prevent the onset of other allergic diseases, such as atopic dermatitis. At present, few studies have investigated the modulation of atopic dermatitis by parasite infections.
Objective: Here, we sought to investigate whether chronic infection with *T. gondii* is capable of modulating the development of atopic dermatitis.
Methods: Chronically infected mice were sensitized by repeated epicutaneous ovalbumin administration. Skin histopathology, humoral response, cytokine production and innate type-II lymphoid cells (ILC2) were assessed.
Results: A marked reduction in epidermal thickness and dermal inflammatory infiltrate along with a reduction in mast cell count was observed in infected mice compared to non-infected mice. These results correlated with a diminished T_H2 and T_H1 allergen specific response. Reduced type-II IL-4 and IL-5 cytokines were already detected during the first 24 h of allergen sensitization in splenocytes and draining lymph nodes from infected mice. Moreover, this reduced type-II profile in chronically infected animals correlated with diminished ILC2 number in draining lymph nodes.
Conclusion: Chronic infection with*T. gondii* prevents the development of atopic dermatitis. The diminished susceptibility seems to result from changes in type-II innate immune response that may lead to the induction of a deficient T_H2 response and consequently to a lower susceptibility to develop atopic dermatitis.

1. Introduction

Atopic disorders are allergic conditions mediated by allergen-specific IgE. These diseases include asthma, rhinoconjunctivitis and atopic dermatitis (AD) among others. Susceptibility to their development depends on the interaction between genetic and environmental factors [1]. The rapid increase in the prevalence of these disorders during the last 50 years points to environmental changes as the main responsible factors for the observed increment [2]. Epidemiological data allowed to establish a negative correlation between the increase in allergies and the prevalence of certain infections, leading to the elaboration of the hygiene hypothesis which postulates that exposure to certain micro-organisms or their components is necessary for the development of non-allergic phenotypes [3]. A decrease in such exposure would lead to changes in T_H1, T_H2 and Treg balance triggered by an absent or altered activation of the innate immune system, predisposing it to the development of allergic disorders [4,5].

An inverse correlation between atopic diseases and *Toxoplasma gondii* infection has been reported [6–8]. This globally distributed intracellular protozoan establishes an asymptomatic chronic

★ This study was supported by the Agencia Nacional de Promoción Científica y Tecnológica (PICT2014-1377, PICT2015-0592, PICT201-0582, ANPCyT, Argentina), (http://www.agencia.mincyt.gob.ar/).
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https://doi.org/10.1016/j.jdermsci.2019.10.007
0923-1811/

infection in immunocompetent individuals. The first week after infection tachyzoites define the acute phase. Bradyzoites, found inside tissue cysts mainly in brain, appear around the third week and mark the beginning of the chronic phase [9]. Felines serve as definitive hosts and dissemination of the highly infectious oocysts into the environment provides a major route of transmission through food and water [10,46]. One third of the world's population is seropositive to Toxoplasma. Prevalence varies by geographical region from < 10% to > 90% [10–12]. Still, a decrease has been observed during the last decades [12–16]. *T. gondii* has unique features to be considered as an allergy-modulating pathogen: (i) it establishes a chronic infection; (ii) infection leads to the induction of a highly polarized T_H1 response which is, though lower, maintained during chronic infection; (iii) counter-regulatory mechanisms are simultaneously induced by the parasite [17]; and (iv) the elicited immune response prevails, indeed, experimental models have shown *T. gondii* ability to modulate the immune response of co-administered proteins or co-infected parasites [18–20].

The vast majority of the experimental studies that allowed to support the hygiene hypothesis and to study the mechanisms involved were based on respiratory allergies [21–24]. In this regard, we have demonstrated that both acute and chronic *T. gondii* infection diminishes the susceptibility to develop allergic lung inflammation [24]. The immunological mechanisms involve a deviation towards a T_H1 profile [25] and the induction of cells with suppressive activity [25,26], depending on whether the sensitization is carried out in the acute or chronic stage of the infection. The immunomodulatory effects are not confined to the lung alone but also encompass systemic responses [27]. This would suggest that infection with this protozoan could also prevent the onset of other allergic disorders, such as AD.

AD is one of the most common cutaneous inflammatory disorders, affecting 10–20% of children and 1–3% of adults worldwide [1]. It's a chronic relapsing disease characterized by skin hyperreactivity, rash, thickened and parched epidermis, and intense itching [28]. AD is orchestrated by a strong type-II response, characterized by high levels of IL-4, IL-5, IL-9 and IL-13, cytokines associated with both adaptive immune response of $CD4^+$ T_H2 cells, as with type-II innate lymphoid cells (ILC2) [29]. The chronic phase of AD is characterized by a mixed T_H1 and T_H2 local response and tissue remodeling that produces dermal thickening and increased collagen deposition.

At present, few studies investigate regulation by micro-organisms in AD models. Regarding *T. gondii* infection, Jeong et al. reported the ability of acute infection to prevent the development of AD [30]. However, since the acute stage lasts only 10–14 days, it's very likely that the epidemiological studies are based on people sensitized during the chronic phase of infection. Thus, the aim of the present work was to evaluate whether chronic infection with *T. gondii* is capable of modulating the development of this disorder.

2. Materials and methods

2.1. Animals

BALB/c mice (6–8 weeks-old) were obtained from the animal facilities of the School of Veterinary Sciences, UBA, Argentina, and maintained in our animal facilities for use throughout the experiments. All procedures were approved by the Independent Ethics Committee for the Care and Use of Experimental Animals of National University of San Martin (CICUAE protocol ID # 005/16).

2.2. Experimental protocol and AD model

Mice were orally infected with 25 cysts of the low virulence *T. gondii* ME49 strain obtained from brains of infected C57BL/6 mice. Infection was confirmed by the presence of parasite-specific IgG antibodies. Thirty days later mice were sensitized by epicutaneous allergen administration. Mice's back hair was shaved with an electric clipper and completely removed with depilatory cream. 150 μg of ovalbumin (OVA) (grade V; Sigma-Aldrich) in 100 μl of PBS was placed on a gauze and secured to the skin with a hydrocolloid patch (Comfeel® Plus, Coloplast, BsAs, Argentina). Each mouse had a total of three 1-week exposures to OVA patch separated from each other by 2-week intervals [31] (Fig. 1). Mice were analyzed the last day of the third week of sensitization. Negative controls include *T. gondii* infected and non-infected mice both treated with PBS.

2.3. Assay of serum immunoglobulin

ELISA plates (Nunc Maxisorp, Boston, MA, USA) were coated with OVA (10 μg/ml). Mouse sera were diluted 1:10 (IgE), 1:10000 (IgG1) and 1:1000 (IgG2a). Biotinylated anti-IgE mouse antibody (BD, Biosciences) or HRP-conjugated goat anti-mouse IgG1 or IgG2a (BD, Biosciences) were used as secondary antibodies. For IgE determination, streptavidin coupled to peroxidase enzyme (Zymed, 1/4000) was added [32]. The presence of *Toxoplasma gondii*-specific antibodies was analyzed by direct ELISA assays.

2.4. Histopathology

Skin samples were fixed in 10% buffered formalin, embedded into paraffin, sectioned, stained with H&E, and examined by light microscopy. Changes in the degree of inflammatory infiltration in the dermis, hyperkeratosis, hyperplasia and epidermal thickening were analyzed. Epidermal hyperplasia was evaluated by measuring the height of epidermis at 400X magnification in at least ten fields per mouse of each experimental group. The analysis was performed using microscope software platform Leica Application Suite (LAS4). Giemsa-stained sections were used for the quantification of mast cells (MC); 10 fields at a magnification of 400X were counted at random. MC morphology appearance was divided into

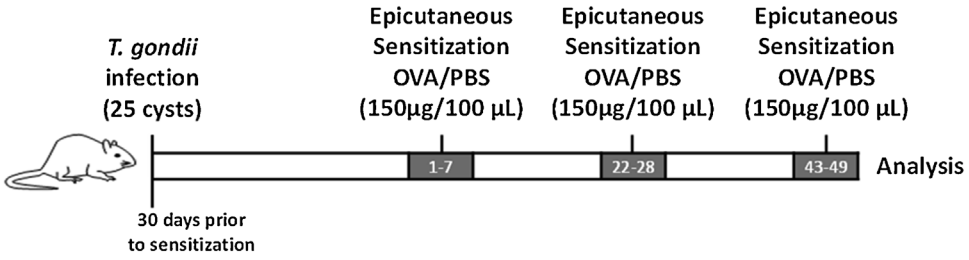

Fig. 1. Schematic of infection and sensitization protocol. BALB/c mice were orally infected with T. gondii cysts. Thirty days later mice were sensitized by epicutaneous OVA administration (TDA). Controls include uninfected and OVA-sensitized mice (DA), T. gondii infected (T) and non-infected (N) mice both treated with PBS.

three categories namely intact, partiality and extensively degranulated as previously reported [33]. Briefly, intact MC had a round to oval shape, had a clearly defined cell membrane, and were associated with none or fewer than 10 granules in the immediate extracellular space. Partially degranulated MC had an oval or irregular shape and their cell membrane was obscured by numerous granules both in the cytoplasm and in the extracellular space. Extensively degranulated MC had a thin rim of granules around the cell membrane, but no granules in the remaining more central cell cytoplasm.

2.5. Ex vivo *cytokine production*

5×10^5 splenocytes were cultured in 200 μl of medium RPMI-1640 (Thermo Fisher Scientific) supplemented with 20% FBS (NATOCOR, Argentina), 1% antibiotic-antimycotic (Thermo Fisher Scientific) and 5×10^{-5} M 2-mercaptoethanol in the presence of OVA (200 μg/ml) for 72 hs.

Explants obtained from skin were incubated for 30 min at 37 °C in 96 well flat-bottomed culture plates allowing the tissue to get attached to the well. Afterwards, explants were cultured in 200 μl of medium RPMI-1640 supplemented with 20% FBS, 5×10^{-5} M 2-mercaptoethanol and Primocin (100 μg/ml) (InvivoGen) in the presence of OVA (200 μg/ml) (grade V; Sigma-Aldrich) for 72 hs.

To assess cytokine production during first stages of allergen sensitization $2{,}5 \times 10^5$ splenocytes or draining brachial lymph node (DLN) cells were *ex vivo* stimulated with PMA (30 ng/ml) (Sigma-Aldrich) plus ionomycin (500 ng/ml) (Sigma-Aldrich) for 72 hs.

Cytokine production was measured in supernatants by capture ELISA commercial kits (BD Biosciences, OptEIA™ kit).

2.6. Flow cytometry analysis

For ILC2 determination, single-cell suspensions were obtained from brachial DLN. Cells were stained with fluorescently labeled antibodies to CD45 (Clone 30-F11), CD3 (Clone 17A2), B220 (Clone RA3-6B2), CD11c (Clone N418), ICOS (Clone C398.4A) and ST2 (Clone DIH4) (all BioLegend) and acquired on a FACScan cytometer (Becton Dickinson, Mountain View, CA). Data were analyzed with FlowJo software (Tree Star Inc.). ILC2s were characterized as $CD45^+$, Lin^- ($CD3^-$, $CD11c^-$, $B220^-$) and $ST2^+$ $ICOS^+$.

2.7. Statistical analysis

Each experimental group had at least four mice and each experiment was repeated at least 4 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 the extent of degranulation was analyzed by non-parametric Kruskal Wallis'test with Dunn's test *a posteriori*. Kinetic studies were analyzed by a two-way ANOVA with Sidak's test *a posteriori*. Statistical significance was accepted when $p < 0.05$.

3. Results

3.1. Chronic infection blocks the development of atopic dermatitis

To evaluate the ability of chronic *T. gondii* infection to modulate AD, mice were experimentally infected by ingestion of *T. gondii* cysts. Thirty-days later, mice were sensitized by epicutaneous allergen administration. Each mouse had a total of three 1-week exposures to OVA patches separated by a 2-week interval (Fig. 1).

Changes in skin structure are hallmarks of AD. Histological sections of the treated skin were analyzed. Mice treated with PBS (N) presented the histopathology of a healthy skin: orthokeratosis, non-thickened epidermis and dermis, and little dermal lymphocytic infiltrate. Uninfected and sensitized mice (AD) exhibited all signs of a dermal inflammatory disorder: hyperkeratosis, parakeratosis, thickening of the epidermis and an increase of the dermal leucocyte infiltrate composed by numerous eosinophils, scanty neutrophils, and few lymphocytes. Chronically infected mice treated with PBS (T) presented a histopathology similar to the N group. Mice infected with *T. gondii* before allergic sensitization (TAD) showed a similar appearance to negative controls N and T; the corneal layer exhibited a normal thickness with a slight detachment of corneal lamellae, non-thickened dermis and epidermis, and a slight inflammatory infiltrate of lymphocytes (Fig. 2).

Another key parameter in AD is the increase of mast cell (MC) infiltrate in dermis during the inflammatory process. MC evaluation was performed in Giemsa-stained skin sections (Fig. 3A). AD mice presented a higher number of MC than non-sensitized animals (N). *T. gondii* chronic infection previous to sensitization (TAD) resulted in a decrease in this cell population compared to the AD group. These observations were supported by a quantitative scoring (Fig. 3B). Moreover, we analyzed MC morphological appearance as an indicator of the activation status. When MC were classified as resting, partially degranulated and extensively degranulated, a significant decrease in extensively degranulated MC percentage was observed in TAD mice compared to the allergic group (AD), suggesting that chronic infection results in decreased MC activation (Fig. 3C).

3.2. Circulating allergen-specific antibodies

High levels of IgE is an AD hallmark, particularly specific for dietary and aeroallergens [34]. To study whether the infection is able to alter the humoral response characteristic of AD, we analyzed serum allergen-specific IgE and IgG subtypes (Fig. 4). Although there were no significant differences in OVA-specific IgE levels between the TAD and AD group, a significant decrease in IgG1 levels was observed in the TAD group compared to the AD group. On the other hand, there were no significant differences in T_H1 IgG2a antibody levels between the TAD and the AD group, indicating that there is no T_H1-deviation of the allergen-specific humoral response.

3.3. Chronic infection inhibits T_H2 and T_H1 allergen specific cytokines

To evaluate whether chronic infection modulates T_H1/T_H2 balance, IL-4, IL-5 and IFN-γ cytokine levels were analyzed in supernatants from OVA-stimulated splenocyte cultures. Epicutaneous sensitization resulted in increased levels of IL-4, IL-5 and IFN-γ compared to non-sensitized (N) and infected (T) controls. Mice sensitized during chronic infection showed significant decreases in both T_H2 and T_H1 cytokines compared to the allergic group (Fig. 5A). The same effect was observed when cytokines were locally analyzed. Skin explants from allergic mice *ex vivo* stimulated with OVA showed high levels of IL-4, IL-5 and IFN-γ. This response was reversed when the animals were previously infected with *T. gondii* (Fig. 5B).

No increases were detected in IL-10 and TGF-β regulatory cytokines or in $CD4^+FoxP3^+$Tregs in spleens from chronically infected mice (Supplementary Fig. A.1). Moreover, as for T_H2 and T_H1 cytokines, a decrease in OVA-specific IL-10 levels was observed in the TAD group. These data suggest that allergy inhibition was not achieved by a T_H1 immune deviation but rather that chronic

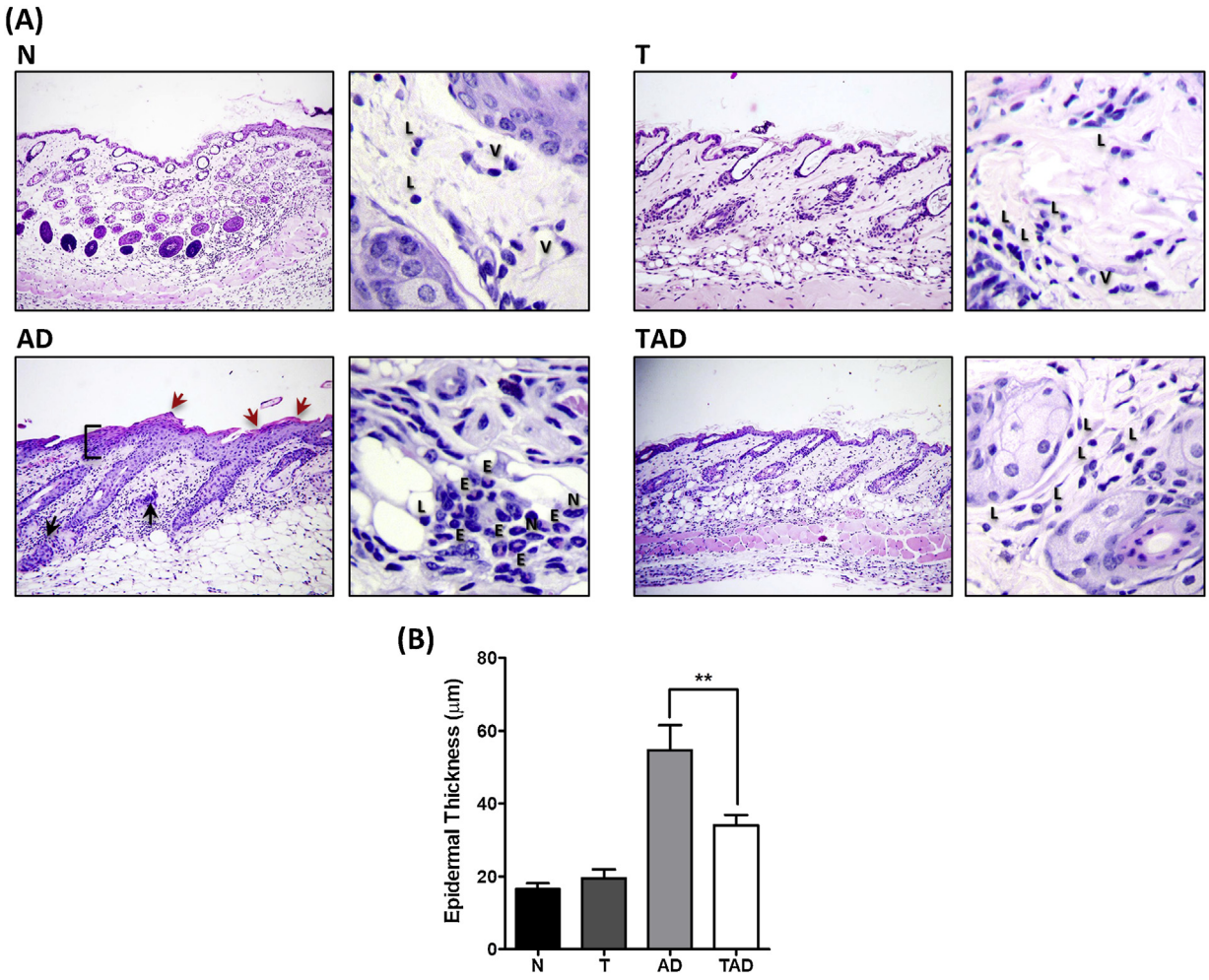

Fig. 2. Histopathological analysis. Following paraffin embedding, skin sections for microscopy were stained with H&E. (A) Representative photographs of each group are shown at an original magnification of 100 ×. N: Orthokeratosis, healthy epidermis and dermis with little inflammatory infiltrate. T: Orthokeratosis, epidermis and dermis have the expected thickness for healthy skin, with little cellular infiltrate. DA: Hyperkeratosis and parakeratosis (red arrows), thickened epidermis (black brackets) and dense inflammatory infiltrate in dermis (black arrows). TDA: As for N and T, a normal disposition of keratin, healthy epidermis and dermis, and little inflammatory infiltrate is observed. Representative photographs with an original magnification of 600X showing the cell infiltrate in each experimental group. N: few lymphocytes; T: few lymphocytes and isolated neutrophils; AD: numerous eosinophils, neutrophils, and few lymphocytes; TAD: slight lymphocytic infiltrate. L: lymphocytes, N: neutrophils, E: eosinophils, V: Vessels. (B) Epidermal hyperplasia was quantified by measuring the height of epidermis at 400X magnification in at least 10 randomly selected fields on sections stained with H&E per mouse of each experimental group. Results for each group are expressed as the mean ± SEM.**$p < 0.01$; ANOVA with Bonferroni's test a posteriori.

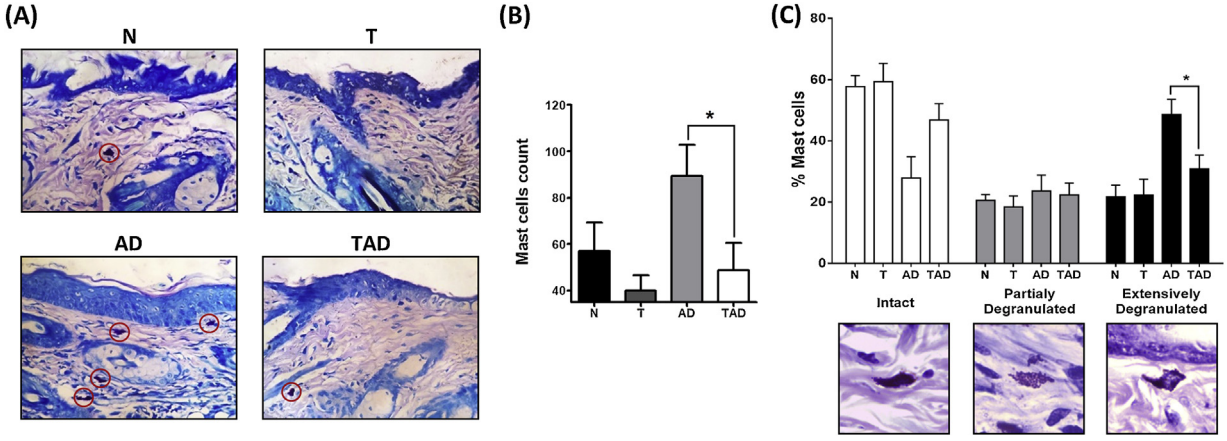

Fig. 3. Mast cell count. Skins sections were stained with Giemsa. MC were identified by their deep purple staining. (A) Images of a representative sample of each experimental group where red circles indicate MC presence. Original magnification 400 ×. (B) MC count was assessed by quantification of 10 fields. (C) MC were analyzed by morphologic appearance based on the extent of their degranulation dividing them into three categories: i) intact (white bars), ii) partiatly degranulated (grey bars) and iii) extensively degranulated (black bars). Representative pictures of the morphological appearance are shown at an original magnification of 1000 ×. Results for each group are expressed as the mean ± SEM.*$p < 0.05$; Kruskal-Wallis with Dunn's test a posteriori.

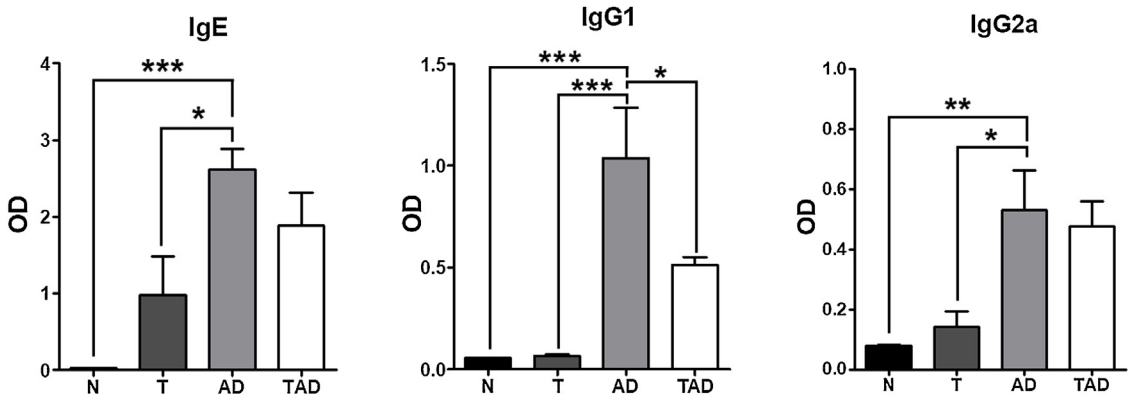

Fig. 4. Allergen-specific humoral response. Serum levels of OVA-specific IgE (dilution, 1:10), IgG1 (dilution, 1:10000) and IgG2a (dilution, 1:1000) antibodies were quantified in all experimental groups.
Results are expressed as the mean OD ± SEM. ***$p < 0.001$; **$p < 0.01$ and *$p < 0.05$; ANOVA with Bonferroni's test a posteriori.

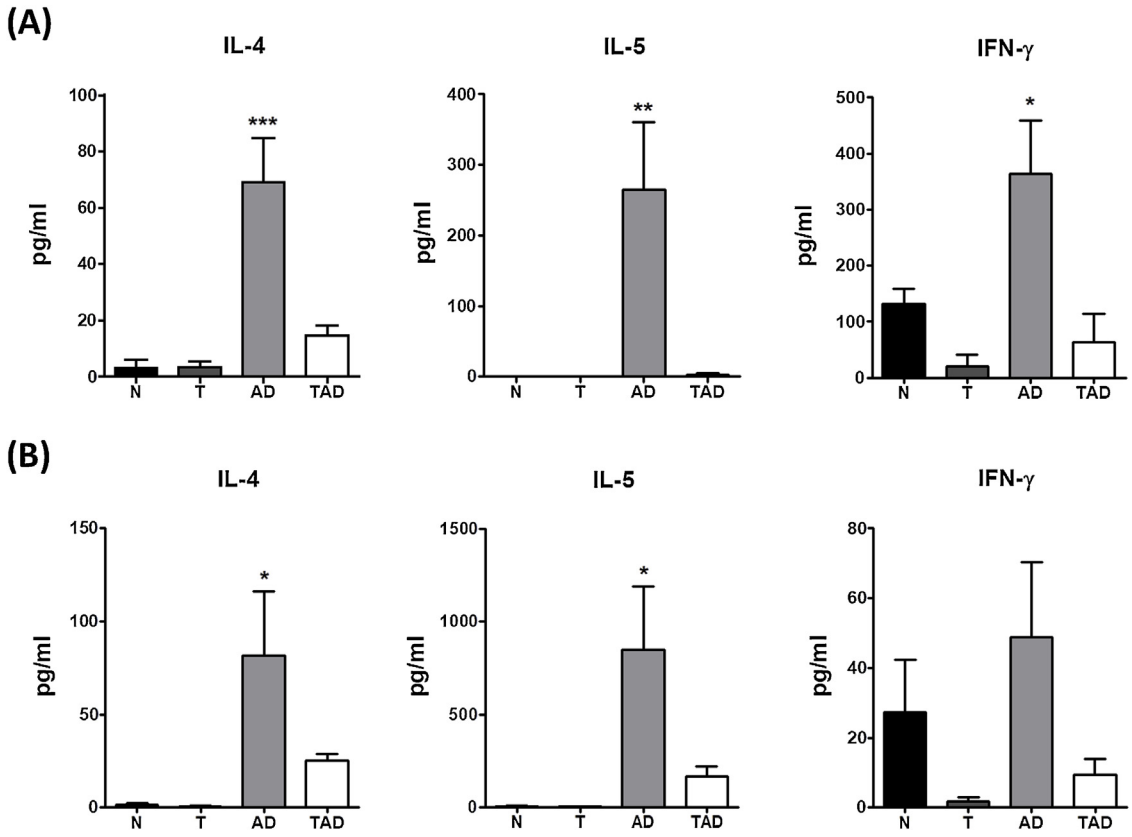

Fig. 5. TH1/TH2 cytokine profile. Cytokine production by spleen cells (A) and skin explants (B) ex vivo stimulated with OVA. ANOVA test with Bonferroni's a posteriori; ***$p < 0.001$, **$p < 0.01$ and *$p < 0.05$ DA vs all other groups.

infection might inhibit the establishment of an allergen specific T cell response.

3.4. *Chronic* T. gondii *infection suppresses innate type-II immune responses during allergen sensitization*

We hypothesized that infection could modulate allergic-sensitization by regulating early innate immune responses, leading to the induction of a deficient T_H2 response and consequently to a lower susceptibility to develop AD. To test this, we moved to the first hours of OVA-sensitization and assessed the responses at 0, 2 and 24 h after the first encounter with the allergen. Splenocytes and DLN cells from naive and *T. gondii* infected mice sensitized with OVA were stimulated with PMA/ionomycin. No increase in type-II cytokines was detected in spleen and DLN from TAD mice (Fig. 6A and B). Conversely, higher IFN-γ levels were observed (Fig. 6A and B). Although IL-10 was decreased in splenocytes (Fig. 6A), no differences were registered in DLN (Fig. 6B). Regarding type-III response, we found differences in IL-17 only in DLN and at 2 h after sensitization.

Since OVA-specific T_H2 cells are not yet differentiated, it's unlikely to attribute type-II cytokine production to this population, suggesting that the source of secreted cytokines in the AD group would mainly come from cells of innate immunity. Therefore, we assessed ILC2 numbers in DLN (Fig. 6C). In agreement with the results obtained for DLN type-II cytokines, ILC2 peaked at 24 h post

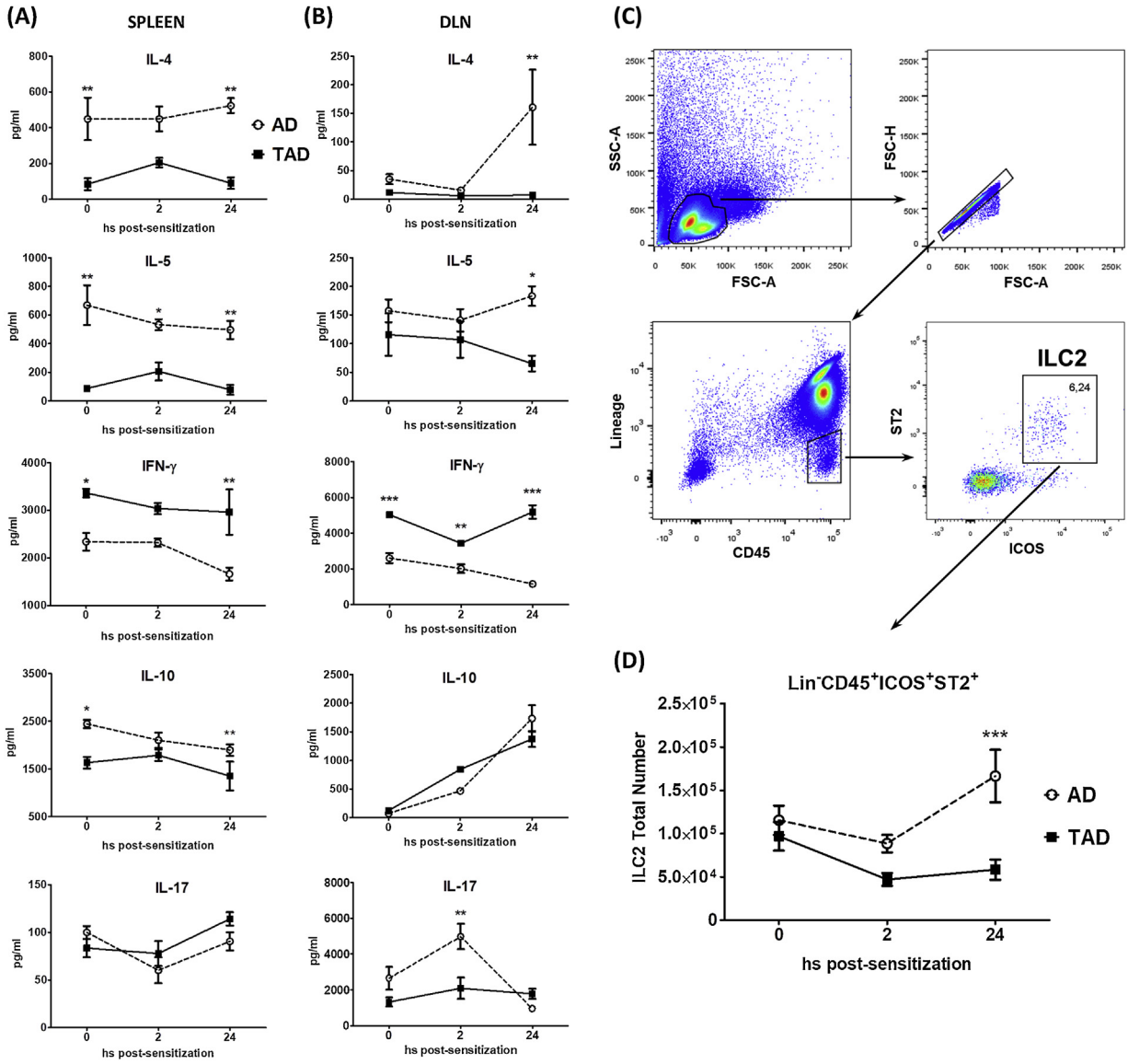

Fig. 6. Immune response during the first stage of allergen sensitization. Cytokine production by splenocytes (A) and DLN cells (B) ex vivo stimulated with PMA/Ionomycin. Flow cytometry gating strategy for group 2 innate lymphoid cells (ILC2) selection (C). Total ILC2 in DLN identified as CD45+Lin-ICOS + ST2+(D). Two-way ANOVA with Sidak's test a posteriori; ***$p < 0.001$; **$p < 0.01$ and *$p < 0.05$.

sensitization in AD mice. Conversely, no increase in this subset could be detected in TAD mice (Fig. 6D). These results strongly suggest that chronic infection would result in a modulation of this innate cell population.

4. Discussion

The hygiene hypothesis postulates that exposure to certain microorganisms, or their products, is necessary for developing non-allergic phenotypes, and that a decrease in such exposure would lead to an imbalance of the immune system predisposing it for the development of allergic disorders [35]. In this context, our previous finding showing that *T. gondii* infection can modulate the susceptibility to develop respiratory allergies and also allergic responses at systemic level [24,27] opened the possibility that this protozoan could also modulate other allergies such as AD. This study aimed to investigate this hypothesis by employing an AD model induced by epicutaneous sensitization with the allergen [36].

In line with our hypothesis, Jeong et al. showed that sensitization with *Dermatophagoides farinae* of mice that are in the acute stage of *T. gondii* infection, resulted in the improvement of skin lesions [30]. Since acute stage of infection lasts only between 10–14 days, epidemiological studies are likely to be based on people sensitized during the chronic phase. Therefore, we focused this study on this stage of infection.

We herein demonstrate that chronic infection decreases all histopathological signs of dermatitis. Moreover, infected mice that were subsequently allergen-sensitized (TAD) presented a histopathology similar to untreated controls (N). A marked reduction in both epidermal thickness and dermis inflammatory infiltrate was observed, compared to the sensitized but uninfected mice (AD). In addition, the TAD group showed a significant reduction of MC cell count, a population that is critically involved in the pathogenesis of AD. Through TNF-α, IL-4 and IL-13, MC induce the expression of cell adhesion molecules in the endothelium which may participate in leukocyte recruitment. MC can also contribute to T cell differentiation and migration either directly through chemoattractants or indirectly by inducing endothelial cell's adhesion molecules expression [34]. Thus, MC decrease in infected mice could explain the improvement in skin histopathology.

When MC were discriminated by their activated status, by identification of resting or degranulated cells, the decrease was particularly detected in the extensively degranulated population. Accordingly, we previously showed in an asthma model, that sensitization during acute and chronic infection resulted in a lower anaphylaxis reaction [27], indicating that infected animals show lower vasodilatation and vascular permeability as a consequence of less degranulation of MC. All these results may suggest that infection with *T. gondii* could regulate MC degranulation. Moreover, *T. gondii* has been shown to inhibit antigen-stimulated degranulation in infected MC [37].

T_H2 cells play a central role in the onset and perpetuation of the inflammatory response by secreting cytokines that activate MC and eosinophils. Besides, IL-4 is essential for B cell IgM-to-IgE class switch. However, in contrast to atopic asthma, while in the acute phase of AD there is a predominant T_H2 response, the chronic phase is characterized by a mixed T_H1 and T_H2 response [38]. Our dermatitis model showed a mixed profile since significant increases in IL-4, IL-5 and IFN-γ secretion in skin explants and splenocytes stimulated with OVA were detected. These results are consistent with the marked inflammation of the dermal and epidermal layers observed in the histopathological analysis. Both T_H2 and T_H1 cytokines were diminished when epicutaneous sensitized mice were previously infected with the parasite. Also, OVA-specific humoral response showed a significant decrease in IgG1 with no increases in IgG2a. These results would discard a deviation towards an allergen-specific T_H1 profile as observed by Jeong et al when sensitizing during acute *T. gondii* stage [30]. The decrease in T_H1 and T_H2 cytokines let us to hypothesize that lower allergic sensitization and/or regulatory cell/cytokine induction may account for the decreased susceptibility to dermatitis of chronically infected mice. It was reported that Tregs have an important role in controlling AD-like inflammation [39]. However, and contrary to Wagner et al. who detected a significant increase in $CD4^+FoxP3^+$ cells in the spleen of mice chronically infected with *T. gondii* [40] we didn't find changes in Tregs. Moreover, in our dermatitis model, mice sensitized during the chronic stage showed a decrease in OVA-specific IL-10 compared to the allergic group. Likewise, no differences were observed in allergen-specific TGF-β. These results agree with the murine model of atopic asthma, where the chronically infected and allergen-sensitized group presented systemic OVA-specific TGF-β levels similar to the asthmatic group [27].

Although other regulatory cells could account for the observed suppression, these last results prompted us to evaluate our second hypothesis. As mentioned above, *T. gondii* infection might modulate allergic sensitization by regulating early innate immune response, leading to the induction of a poor T_H2-type response and consequently to a lower susceptibility to develop AD. Even though several inflammatory mediators influence the replication of *T. gondii*, IFN-γ has the most prominent function in the elimination of the parasites. Between all the proinflammatory cytokines, IFN-γ is mostly expressed by T_H1 cells, ILC1 and NK cells [41]. Recently, Park et al. found an increase in ILC1-like cell number in splenocytes from chronically infected BALB/c mice. Moreover, *T. gondii* infection induced expansion of ILC1-like cells, which persisted independent of ongoing parasite replication, suggesting a long-lasting change [42]. On the other hand, it has been observed that cytokines associated with type-I immunity, such as IFN-γ, inhibit the activation of ILC2, involved in the onset of allergic sensitization [29]. Accordingly, it's been shown that NK cells down-regulate ILC2 population during early stages of pulmonary inflammation [43]. Hence, we assessed cytokine responses in the first few hours of epicutaneous OVA-sensitization. Interestingly, we found in infected mice, a potent suppression of IL-4 and IL-5 release in splenocytes and DLN cells stimulated with PMA/ionomycin. In spleen, this difference was detected even before applying OVA. Conversely increased IFN-γ secretion was observed in *T. gondii* infected mice. Remarkably, the decrease in type-II cytokines correlated with reduced ILC2 in DLN. To our knowledge, this is the first work that demonstrates that chronic *T. gondii* infection is able to modulate this cell population. These results suggest that significant changes in type-II innate immune response appear to be manifested in chronically *T. gondii* infected mice and might account for the decreased susceptibility to AD development. Accordingly, McSorley et al. showed that the reduced airway allergic inflammation mediated by the secreted products of *H. polygyrus* was associated with a diminished innate type-II cytokines production and ILC2 population [44].

Allergic march states that allergy frequently starts during childhood with AD that evolve, later in life, into respiratory allergies such as rhinitis and allergic asthma [45], suggesting that the first allergen sensitization would be by epicutaneous route. Hence, our results showing that *T. gondii* is capable to block the development of AD could also explain, in part, the epidemiological data showing that individuals infected with this parasite have less incidence of rhinitis and asthma [6–8].

In conclusion, our current and previous data show that chronic infection with the protozoan *T. gondii* leads not only to amelioration of allergic airway inflammation but also to AD. This outcome is associated with a diminished ILC2 in skin draining lymph nodes from chronically infected mice during early sensitization. Alterations in type-II innate responses during sensitization leading to fewer allergic sensitization might arise as a new explanation of the hygiene theory. Knowledge of the mechanisms underlying this phenomenon will contribute valuable data to the ongoing evaluation of the 'hygiene hypothesis' and its potential application to human health.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

Acknowledgements

We especially thank Dr. Mariana Fragner and Mrs. Irina García for their excellent technical assistance. We acknowledge the funding from Agencia Nacional de Promoción Científica y Tecnológica (PICT2014-1377, PICT2015-0592, PICT201-0582, ANPCyT, Argentina), (http://www.agencia.mincyt.gob.ar/).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jdermsci.2019.10.007.

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