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SHORT COMMUNICATION

IgG from Non-atopic Individuals Induces In Vitro IFN-γ and IL-10 Production by Human Intra-thymic γδT Cells: A Comparison with Atopic IgG and IVIg

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Abstract

Matured in the thymus, γδT cells can modulate the development of allergy in humans. The main γδT cell subsets have been described as interleukin (IL)-17A or interferon (IFN)-γ producers, but these cells can also produce other modulatory cytokines, such as IL-4 and IL-10. Here, we aimed to evaluate whether IgG can modulate the profile of cytokine production by γδT cells during their maturation in the thymus and after its migration to peripheral tissues. Thymic tissues were obtained from 12 infants, and peripheral blood mononuclear cells (PBMCs) were obtained from adults (both groups without an atopic background). IgG was purified from atopic and non-atopic volunteers. Thymocytes and PBMCs were cultured with purified atopic or non-atopic IgG, and intracellular cytokine production and phenotype were assessed. Mock and IVIg conditions were used as controls. IgG from non-atopic individuals induced IFN-γ and IL-10 production by thymic γδT cells, and no effect was observed on peripheral γδT cells. IL-17 production was inhibited by non-atopic IgG on thymic γδT cells and augmented by atopic IgG on peripheral γδT cells. Modulated thymic γδT cells did not produce IFN-γ and IL-10 simultaneously. We additionally evaluated the phenotype of intrathymic γδT cells and observed that IgG from all groups could induce CD25 expression and could not influence the CD28 expression of these cells. This report describes evidence revealing that IgG may influence the production of IFN-γ and IL-10 by intrathymic γδT cells depending on the donor atopic state. This observation is unprecedented and needs to be considered in further studies in the IgG immunotherapy field.

Keywords Allergy · IgG · IFN-γ · IL-10 · γδT cells · Thymus

Introduction

γδT cells represent a minority of mature T cells present in secondary lymphoid organs and peripheral blood (Ferreira 2013). Unlike most mature T cells in the thymus, γδT cells express T cells receptors (TCRs) composed of γ and δ chains, which do not have well-defined ligands (Brenner et al. 1986; Lanier et al. 1987; Satyanarayana et al. 1988)

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and are not subject to the same selection processes during intrathymic maturation as other T cells, thus, representing a link between innate and adaptive immune responses (Holtmeier and Kabelitz 2005).

Although the ligands of these TCRs have not been well described, γδTCR signalling seems to be important in determining the functional activity of γδT cells (Mahtani-Patching et al. 2011; Turchinovich and Pennington 2011), and although two major γδT cell subsets have been described based on their capacity to produce interleukin (IL)-17A (hereafter referred to as IL-17) or interferon (IFN)-γ (Ribot et al. 2009), these cells can produce several cytokines with modulatory potential in allergy development, including IL-4 (Krug et al. 2001) and IL-10 (Exley and Boyson 2011).

Additionally, γδT cells can exert some functions mediating human and murine allergy development, including eosinophilic infiltration during allergic inflammation (Pang et al. 2012), the inhibition of allergen-specific IgE production (de Oliveira Henriques and Penido 2012; Huang et al. 2013), the induction of murine dermatitis (Gray et al. 2013) and the enhancement of allergic inflammation in a murine model (Ullah et al. 2015). More recently, it was also demonstrated that γδT is implicated in the amplification of the murine and human allergic response to dust mites (Belkadi et al. 2019).

As evidenced in 2003, the preconceptional immunization of mice with allergens can inhibit offspring allergy development, and this effect was proportional to the levels of allergen-specific IgG detected in offspring sera (Victor et al. 2003). Later, it was observed in a similar murine model that the passive transference of IgG purified from allergen-immunized females to non-immunized pregnant females could mediate the inhibitory effect observed in offspring derived from immunized females (Victor et al. 2010). This work also demonstrated that the passive transference of purified IgG could modulate offspring T cell cytokine production.

Based on this evidence, it was hypothetically proposed in 2014 that the mechanism by which maternal allergen immunization can mediate offspring allergy inhibition possibly involves the passive transference of maternal soluble factors that can mediate interactions within offspring primary organs (Victor 2014). This hypothesis was improved in 2017, when it was suggested that the main soluble factor responsible for mediating offspring allergy inhibition is IgG and that this interaction occurs mainly in the thymus of offspring and involves several lymphocyte populations with regulatory and/or modulatory functions, including B and T cells (Victor 2017). This hypothesis was called the MatIgG primary modulation theory.

More recently, this hypothesis was experimentally verified with the demonstration that murine allergen-specific IgG can induce regulatory B10 cells in offspring, and a similar profile was observed in human peripheral B cells (de Oliveira et al. 2017; Lira et al. 2018). It was also demonstrated that IgG from atopic individuals can inhibit IFN-γ production by human thymic TCD4 and TCD8 cells (Sgnotto et al. 2017) and that IgG from atopic dermatitis patients can induce IL-17 and IL-10 production by thymic TCD4 cells (Sgnotto et al. 2018). Together, these results reinforce the MatIgG primary modulation theory. However, only B cells and αβTCR-expressing T cells were evaluated; thus, no results for γδTCR-expressing T cells have been obtained.

Since γδT cells are also subjected to IgG interactions in the thymus and the acquisition of their functional properties has not been fully elucidated, we aimed to evaluate whether IgG molecules purified from non-atopic and atopic individuals can differentially modulate the functional properties of γδT cells and analyse their cytokine production profile.

Materials and Methods

Patient Samples

Thymic tissues were obtained from 14 patients who underwent corrective cardiac surgery at the Hospital do Coração (HCor), São Paulo, Brazil. The evaluated patients did not exhibit signs of immunodeficiency, genetic syndromes or allergic reactions, and patient age of fewer than 7 days was used as an inclusion criterion [patient age, mean ± standard error (SE): 3.2 ± 1.52 days]. The parental allergic background was evaluated, and only children from non-atopic mothers were included in this study.

Additionally, blood samples were collected from subjects who were previously clinically classified as non-atopic individuals (without any clinical allergy symptoms and not reactive to any tested allergen on the skin prick test (SPT), $n = 14$, patient age, mean ± SE: 28.2 ± 3.11).

Each sample of thymic tissue or peripheral blood mononuclear cells (PBMCs) was provided from a different donor and was analysed in three independent experiments. The ethics committees at the HCor and the School of Medicine at the University of São Paulo approved this study.

SPT and Blood Sample Collection

The SPTs were performed in accordance with European standards (Heinzerling et al. 2013) with an adapted panel of allergens that included the profile of Brazilian allergens (i.e., *Blomia tropicalis, Canis familiaris, Periplaneta americana, Aspergillus fumigatus, Penicillium notatum, Alternaria alternata, Cladosporium herbarum, Dermatophagoides pteronyssinus, Dermatophagoides farinae,* and *Felis domesticus*) as described previously (Sgnotto et al. 2017).

Thereafter, two blood samples were obtained from each non-atopic individual via venipuncture and placed in tubes

without anticoagulants. After the blood samples were centrifuged at 940×*g* for 10 min, the serum was fractionated, pooled and stored at − 80 °C.

Separation of Thymocytes and PBMCs

Thymocytes were released from the tissue samples using enzymatic dissociation, and PBMCs were obtained from non-atopic individuals via centrifugation in a density gradient as described previously (Bento-de-Souza et al. 2016; Sgnotto et al. 2018).

IgG Purification

IgG was purified from pooled serum according to the specifications of the Melon Gel IgG Spin Purification Kit (Thermo, USA). Purified IgG was collected, sterilized and stored at − 80 °C for subsequent use in cell culture experiments. The IgG concentration was determined using Coomassie Protein Assay Reagent (Pierce, USA) according to the manufacturer's instructions. The purity of IgG was above 90%; thus, this method is more effective than using protein A, but we cannot exclude the possibility that other antibody isotypes were potentially present as contaminants at low or undetectable concentrations.

Cell Culture and Flow Cytometry

A total of 1×10^6 viable thymocytes or PBMCs were placed in each well of a 96-well culture plate (CoStar, USA) and cultured in the absence of IgG (Mock condition) or with 100 µg/mL of IgG purified from pooled serum samples from atopic or non-atopic individuals or IVIg (Endobulin, Baxter, AT). The culture plate was incubated at 37 °C in 5% CO_2 for 2 or 6 days, as required for the kinetic assessment. Subsequently, 1 µg/mL Brefeldin A (Sigma, Israel) was added to each well of the culture plate, and after 12 h, cell staining was performed to evaluate cell labelling via flow cytometry.

To evaluate cell viability and the kinetic rate of the investigated populations, thymocytes or PBMCs were cultured in RPMI 1640 culture medium containing 10% fetal bovine serum (FBS), and the cell viability of each examined cell population was evaluated via flow cytometry at time zero (after thawing) and after 3 or 7 days of culture.

For the cell viability analysis, extracellular staining was performed as described above, and the cells were incubated with Live/Dead (PE-Texas red) fluorescent reagent (ThermoFisher, USA). All analyses were performed using viable cells.

To perform extracellular staining, thymocytes or PBMCs at a concentration of 0.5×10^6 cells/mL were transferred to test tubes, and 1 µg of each antibody was added to the cells (except to the unlabelled tubes). Then, the samples were incubated for 30 min at 4 °C while protected from light. Thereafter, 500 µL of 1 × phosphate buffered saline (PBS) solution was added, and the tubes were centrifuged at 400×*g* for 5 min. The supernatant was discarded by inverting each tube. Then, PBS was added, followed by fixation in 200 µL of 1% formaldehyde for at least 10 min. Thymocytes or PBMCs were stained with mouse anti-human CD3, TCRγδ or isotype control antibodies (BD Pharmingen, NJ, USA) to identify populations of γδT cells ($CD3^+TCR\gamma\delta^+$).

To perform intracellular labelling, tubes were centrifuged at 400×*g* for 5 min. The supernatant was discarded, and 1 µg of each antibody was added to the cells (except to the unlabelled tubes). Then, 100 µL of 1 × PBS containing 0.05% saponin was added, and the tubes were stored at 4 °C for 30 min while protected from light. After centrifugation at 400×*g* for 5 min, the supernatant was discarded by inverting each tube, and the cells were resuspended in 300 µL of 1 × PBS solution. Thymocytes and PBMCs were stained with mouse anti-human IFN-γ, IL-17A, IL-4, IL-10 or isotype control conjugated with the corresponding fluorochromes (BD Pharmingen, NJ, USA).

Using an LSRII Fortessa flow cytometer (BD Biosciences, USA), 500,000 events per sample were acquired in the quadrant of lymphocytes (as determined by their relative size/granularity). Compensation was performed using adsorbed microspheres (CompBeads, BD Biosciences, USA) treated with the same antibodies used for extra- and intracellular staining. Cell gating was based on the specific isotype control values as well as the fluorochrome minus one setting when needed. Data analysis was performed using FlowJo software (Tree Star, Ashland, OR, USA), and only the extra- and intracellular staining of viable cells was analysed.

Statistical Analysis

Differences were considered significant at $P \leq 0.05$, as assessed by one-way ANOVA (Kruskal–Wallis test, comparisons among three or more groups).

Results

Purified Non-atopic IgG Can Induce IFN-γ and IL-10 Production by Human Intrathymic and Not Peripheral γδT Cells

To evaluate the in vitro effect of atopic and non-atopic IgG, infant thymocytes were evaluated after culture in the mock condition or in the presence of IgG purified from atopic and non-atopic individuals for 3 or 7 days. As an additional control, we used IVIg (intravenous immunoglobulin—IgG purified from thousands of donors used as an in vivo human therapy). We found that γδT cells represented nearly 4% of

all thymocytes, and a similar percentage was observed after 3 and 7 days in culture (Fig. 1a, c); however, the viability of these cells decreased from approximately 50–20% at 7 days of culture. No differences were observed between the analysed conditions.

Next, we evaluated the intracellular production of IFN-γ, IL-17, IL-4 and IL-10 by γδT cells at both time points (gating strategy can be found in Fig. S1 and S2) and found that treatment with IgG from non-atopic individuals induced elevated production of IFN-γ and IL-10 at both time points compared with the mock condition or IVIg and atopic-IgG treatment (Fig. 1b, d). No influence of IgG was observed on IL-4 production.

After 3 days in culture, we also observed that the treatment with IgG from non-atopic individuals reduced the production of IL-17 compared with that resulting from all other conditions, but this effect was not observed after 7 days (Fig. 1b–d).

We also evaluated whether peripheral γδT cells show a similar profile of cytokine responses to that observed in intrathymic γδT cells. For this purpose, we cultured PBMCs from healthy non-atopic individuals under the same conditions adopted for infant thymocytes and evaluated the production of the same cytokines after 3 days of culture, corresponding to the time point when the effects were most pronounced (gating strategy can be found in Fig. S3). None of the evaluated conditions influenced the frequency and viability of these cells (Fig. 2a), and no effect of non-atopic IgG on the production of IFN-γ, IL-17, IL-4 and IL-10 was observed (Fig. 2b). However, IgG from atopic individuals increased IL-17 levels in peripheral γδT cells (Fig. 2b).

As the modulatory effect of non-atopic IgG could exert a similar effect on thymic IFN-γ- and IL-10-producing γδT cells, we additionally evaluated whether this may be due to simultaneous production of these cytokines (gating strategy can be found in Fig. S4). This evaluation revealed that the frequency of IFN-γ and IL-10 co-production by thymic γδT cells was low to undetectable at both evaluated time points (Fig. 3a). Finally, to better understand the effect of IgG on γδT cells, we also evaluated the expression of

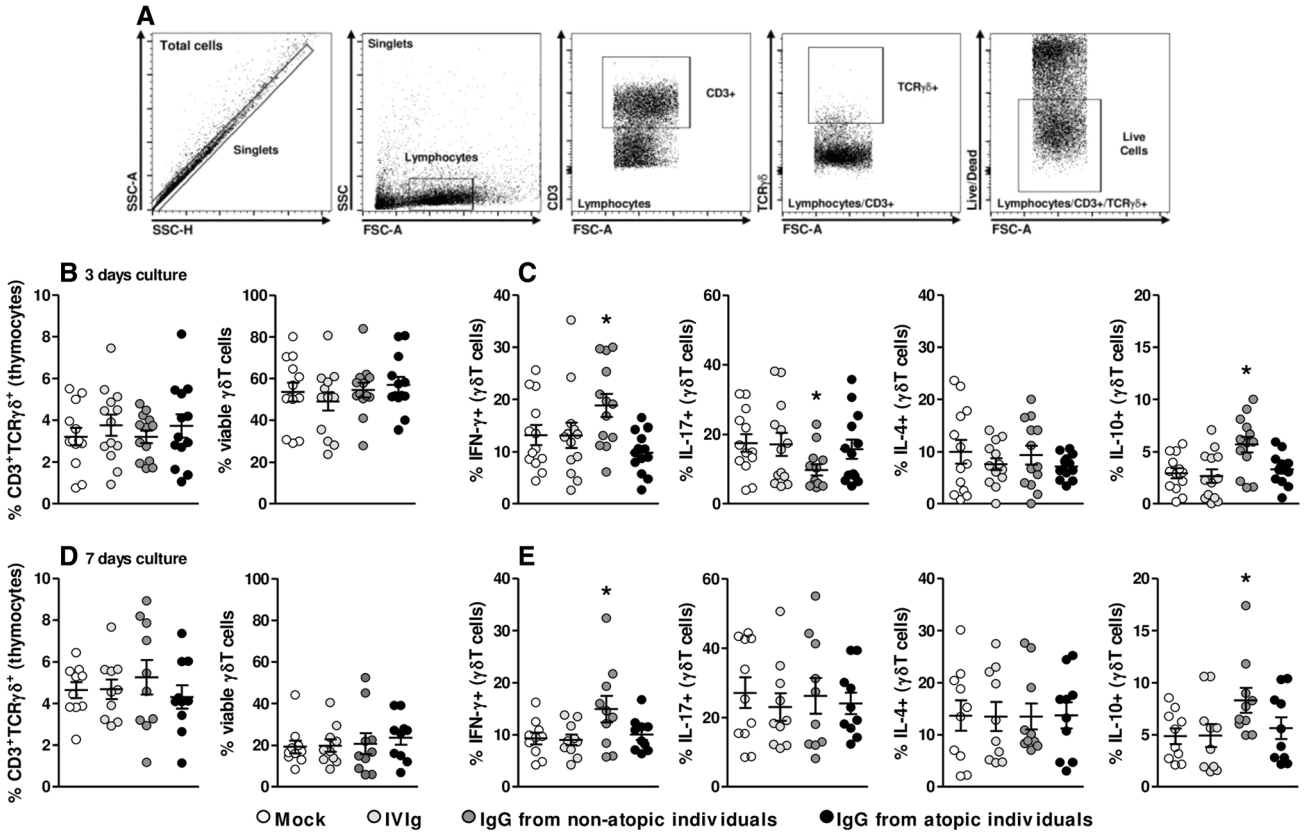

Fig. 1 Effect of purified IgG on cytokine production by intrathymic γδT cells. Illustrative dot plots of the gating strategy used to identify thymic γδT cells and to evaluate viability are generically demonstrated in the upper panels (**a**). Thymocytes from children less than 7 days old ($n = 12$) were evaluated after three (**a**, **b**) or seven (**c**, **d**) days in culture in RPMI medium supplemented with FBS in the absence (mock) or presence of 100 µg/mL IgG from IVIg, from atopic or non-atopic individuals. At each time point, γδT cells were evaluated to assess the frequency, viability and intracellular IFN-γ, IL-17, IL-4 and IL-10 production by flow cytometry. Data are presented as individual values, means, and SEMs. $^*P \leq 0.05$, compared with mock, IVIg and IgG from atopic individual conditions

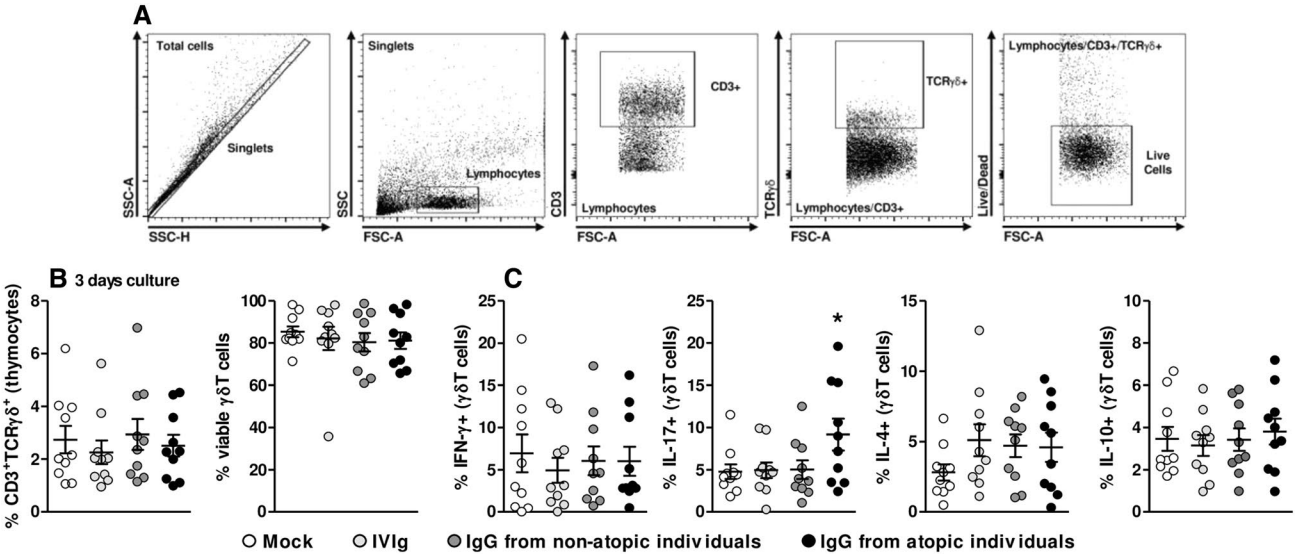

Fig. 2 Effect of purified IgG on cytokine production by peripheral γδT cells. Illustrative dot plots of the gating strategy used to identify peripheral γδT cells and to evaluate viability are generically demonstrated in the upper panels (**a**). γδT cells from non-atopic adults were evaluated after 3 days in culture in RPMI medium supplemented with FBS in the absence (mock) or presence of 100 µg/mL IgG from IVIg, from atopic or non-atopic individuals. γδT cells were evaluated to assess the frequency, viability (**a**) and intracellular IFN-γ, IL-17, IL-4 and IL-10 production (**b**) by flow cytometry. Data are presented as individual values, means, and SEMs. $*P \leq 0.05$, compared with mock, IVIg and IgG from atopic individual conditions

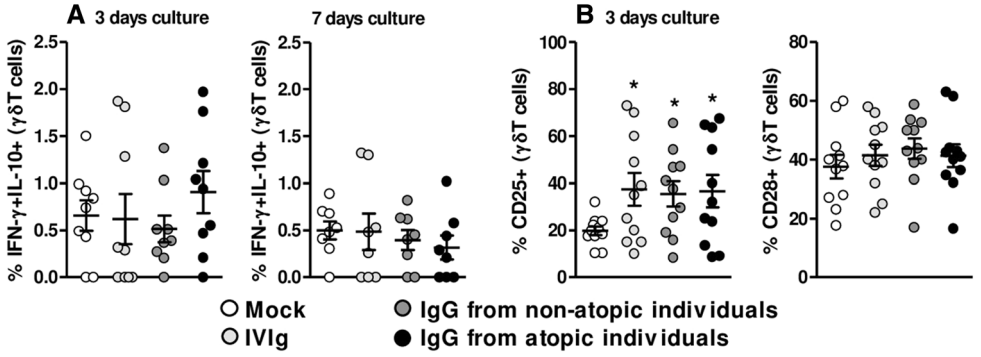

Fig. 3 Co-production of IFN-γ and IL-10 and phenotypic analyses of intrathymic γδT cells. Thymocytes from children less than 7 days old ($n = 12$) were evaluated after three or 7 days in culture in RPMI medium supplemented with FBS in the absence (mock) or presence of 100 µg/mL IgG from IVIg, from atopic or non-atopic individuals. At each time point, γδT cells were evaluated to assess the frequency of intracellular IFN-γ and IL-10 co-production (**a**) or were evaluated after 3 days in culture to assess the frequency of CD25 and CD28 expression (**b**) by flow cytometry. Data are presented as individual values, means, and SEMs. $*P \leq 0.05$, compared with the mock condition

CD25 and CD28 by thymic γδT cells after a shorter period of culture and observed that all IgG conditions (IVIg, non-atopic IgG and atopic-IgG) could induce an increase in CD25 expression compared with that resulting from the mock condition, and no difference was observed in CD28 expression between the culture conditions.

Discussion

To evaluate the effect of IgG antibodies on cytokine production by γδT cells in the human thymus, we collected human thymi from infants born to mothers without an allergic background and purified IgG using a method

involving the removal of non-relevant proteins present in human sera without selective IgG binding to avoid interfering with its affinity, which is broadly used in the literature (Ankeny et al. 2009; Dugast et al. 2014; Mahan et al. 2015; Mara-Koosham et al. 2011; Rigato et al. 2012; Victor et al. 2010).

This culture protocol is described in the literature as an in vitro model to elucidate the direct effect of interactions between soluble IgG and cell populations in the human thymus and PBMCs (Sgnotto et al. 2017, 2018).

As discussed in the previously cited studies, in the adopted culture system, the main interactions that can occur between γδT cells and IgG are mediated by idiotypic interactions and membrane clonal receptors (including γδTCR) expressed by immature lymphocytes (Bogen et al. 1993; Borghesi and Nicoletti 1996; Ghosh and Chakrabarti 1993; Vakil et al. 1986) since γδT cells do not express FcγRs (IgG receptors). In the present study, we observed that IgG from non-atopic individuals could modulate cytokine production in human intrathymic γδT. These observations are unprecedented in the literature and reveal a difference between the effects of IgG on atopic and non-atopic individuals.

In 2010, most γδTCRs were still considered orphans (Kreslavsky and von Boehmer 2010), but it was described that γδTCR signalling is important during maturation promoting the specific homing properties of γδT cells (Jin et al. 2010). More recently, it was demonstrated that γδTCR signalling participates in the regulation of γδT cell production of the two main cytokines produced by this cell, namely, IL-17 and IFN-γ (Muñoz-Ruiz et al. 2016).

Here, using the absence of IgG or purified human IgG antibodies [IVIg—purified human IgG used in the treatment of patients with primary immunodeficiencies (Lemieux et al. 2005)] as controls, we observed the induction of IFN-γ in thymic γδT cells mediated by non-atopic IgG. This evidence corroborates the hypothesis that the IgG effect can be mediated by γδTCR signalling.

Furthermore, after a short period of culture, we observed the opposite effect of non-atopic IgG on IL-17 production of γδT cells. These results reinforced the involvement of γδTCR inducing a dichotomy between IL-17 and IFN-γ, but this effect was not observed in the later period of culture.

The evaluation of peripheral γδT cells revealed an unprecedented and pronounced augmentation of IL-17 production in response to IgG from atopic individuals. The role of IL-17-producing γδT cells at allergic inflammation sites was described in a murine model (Ullah et al. 2015), but its role in human allergy induction remains unclear. Here, our results suggest that IL-17-producing peripheral γδT cells can be induced by human atopic IgG, but the role of these cells in allergy development still needs to be elucidated.

Some related data can be found in a similar cell culture model where the production of IL-17 in response to IgG was evaluated in αβT cells. In this study, IgG from atopic and non-atopic individuals could induce the production of IL-17 by thymic TCD4 and TCD8 cells at similar levels, but this study did not evaluate peripheral and/or γδT cells (Sgnotto et al. 2017).

Data on the effect of IgG production on IL-17 production by γδT cells were obtained by assessing the relationship between the production of *Helicobacter pylori* urease-specific IgG and an increased γδT cell count and IL-17 production in patients with gastritis (Futagami et al. 2006), an observation also found in the present study. However, possible mechanisms that could directly relate IgG to γδT cell regulation were lacking.

Similar to the effect on IFN-γ production, we observed the induction of IL-10 production in γδT cells after culture with non-atopic IgG. γδT cells are capable of producing IL-10, and it was demonstrated in a murine model of tumour lesion that infiltrating γδT cells can produce IL-10 and suppress the proliferation of cytotoxic T lymphocytes (Seo et al. 1999). In humans, high amounts of IL-10 can be detected in γδ T cells from early pregnancy deciduas, suggesting that γδT cells can promote immune tolerance during pregnancy (Fan et al. 2011; Nagaeva et al. 2002).

In some recent and similar studies, in which intracellular cytokine production was observed after the culture of thymocytes with purified IgG, IL-10 production was observed by TCD4 and TCD8 cells (αβT cells) after culture with purified IgG from both atopic and non-atopic individuals (Sgnotto et al. 2017) or from atopic dermatitis patients (Sgnotto et al. 2018). In accordance with our results, this evidence suggests an unprecedented mechanism for T cell regulatory property induction that can be mediated by IgG, can affect αβ and γδ T cells, and is dependent on the donor's atopic state. To date, there is no in vivo evidence of this mechanism; however, we believe that, due to its importance, this will be investigated in the near future.

Currently, there is no evidence in the literature that correlates IL-10-producing γδT cells with allergy development; thus, our work may represent the first evidence to suggest this relationship.

An interesting observation was generated with the evaluation of PBMCs under the same culture conditions. It was observed that the pronounced modulatory effect of non-atopic IgG on IFN-γ and IL-10 production by γδT cells cannot be induced in peripheral mature cells, suggesting that the mechanism mediated by IgG occurs only in the initial stages of γδT cell ontogeny.

In the present work, we did not perform experiments that can precisely elucidate the mechanism by which IgG can interact with immature thymic γδT cells modulating its functional properties; however, our observations regarding the expression of CD25 and CD28 lead us to suggest some possibilities. The overexpression of CD25 in response to

all IgG culture conditions suggests that the mechanism can indeed, as suggested above, involve the activation of γδT cells via TCR. This is because the expression of CD25 is induced mainly as a response to the engagement of this receptor (Dopfer et al. 2014; Juraske et al. 2018). In this case, the diverse functional activities observed in response to IgG from each group of donors possibly occur due to additional factors related to IgG repertoires, including specificity and affinity. Finally, the absence of an effect on CD28 expression by γδT cells suggests that the survival and proliferation of these cells were not influenced by the presence of IgG, as this molecule can control both properties in murine and human γδT cells (Ribot et al. 2012).

Taken together, our results indicated that IgG from individuals with divergent atopic states can modulate γδT cell functions during thymic maturation. Our observations cannot fully elucidate this immunoregulatory mechanism; however, it is possible that individuals predisposed to becoming non-atopic can develop an IgG repertoire that favours the acquisition of a γδT cell cytokine profile capable of collaborating with the control or inhibition of an allergic response.

We can also translate our findings to human IVIg immunotherapy, which suggests that the atopic state of donors can represent a factor to be considered to elucidate the immunomodulatory effects in response to treatment. Finally, it is important to consider a possible effect of IVIg treatment on thymic cell maturation, especially in the context of neonates.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no conflicts of interest.

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Title: *IgG from non-atopic individuals induces in vitro IFN-γ and IL-10 production by human intra-thymic ¥¢T cells: a comparison with atopic IgG and IVIg*

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SUPLEMENTAL FIGURES S1 TO S4

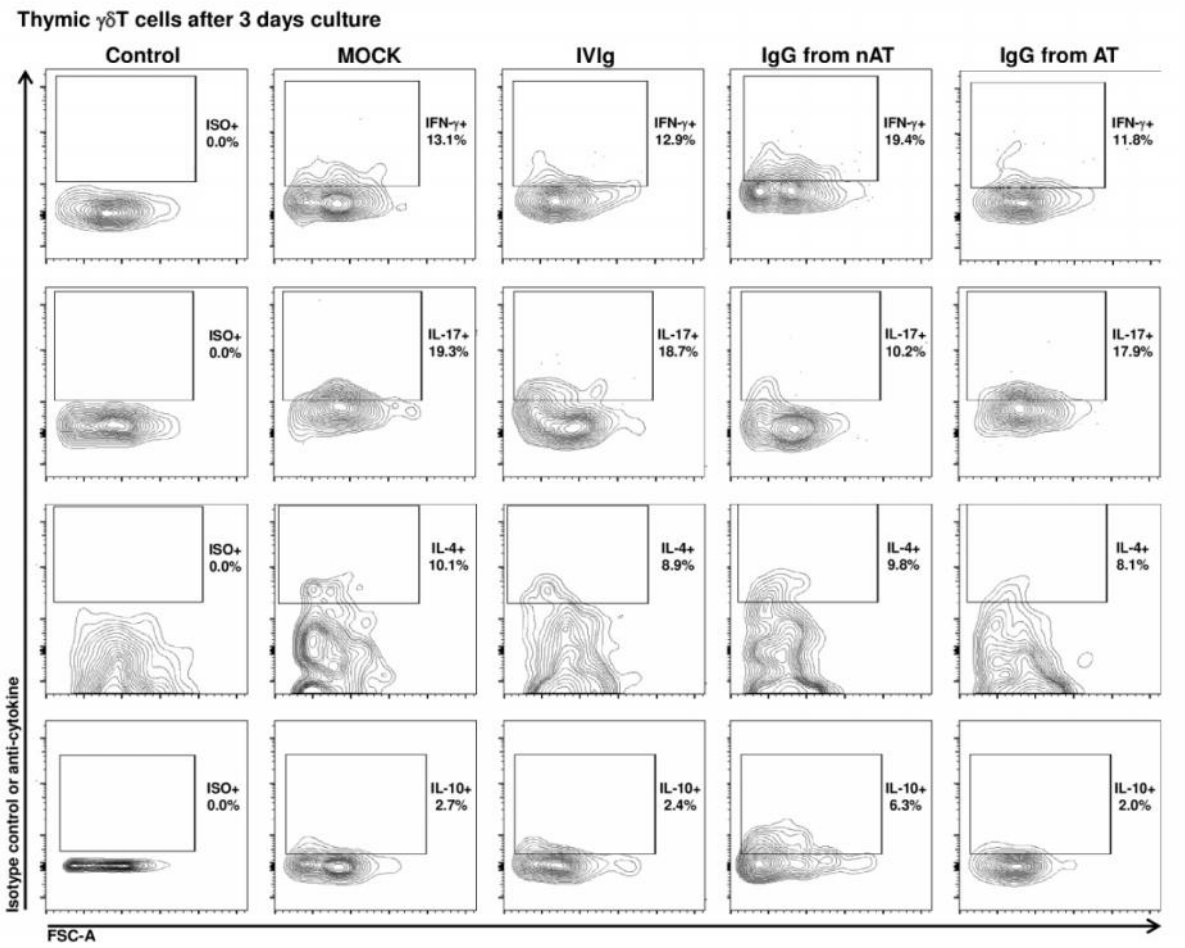

Fig. S1 Illustrative contour plots of the effect of purified IgG on cytokine production by intra-thymic ¥¢T cells after 3 days of culture. Each sample was acquired using the singlet cell gate (determined by SSC-A/SSC-H parameters), then the lymphocyte gate (determined by their relative size/granularity) and then the viable cell gate; samples were then acquired gated as CD3+TCRγδ+ (γδT cells). Cytokine gating was determined using isotype controls on cells from mixed culture conditions (Left panels), and cytokines of cells from each culture condition: mock, IVIg, non-atopic IgG (nAT) and atopic IgG (AT) cultures were measured accordingly, as indicated on the right panels.

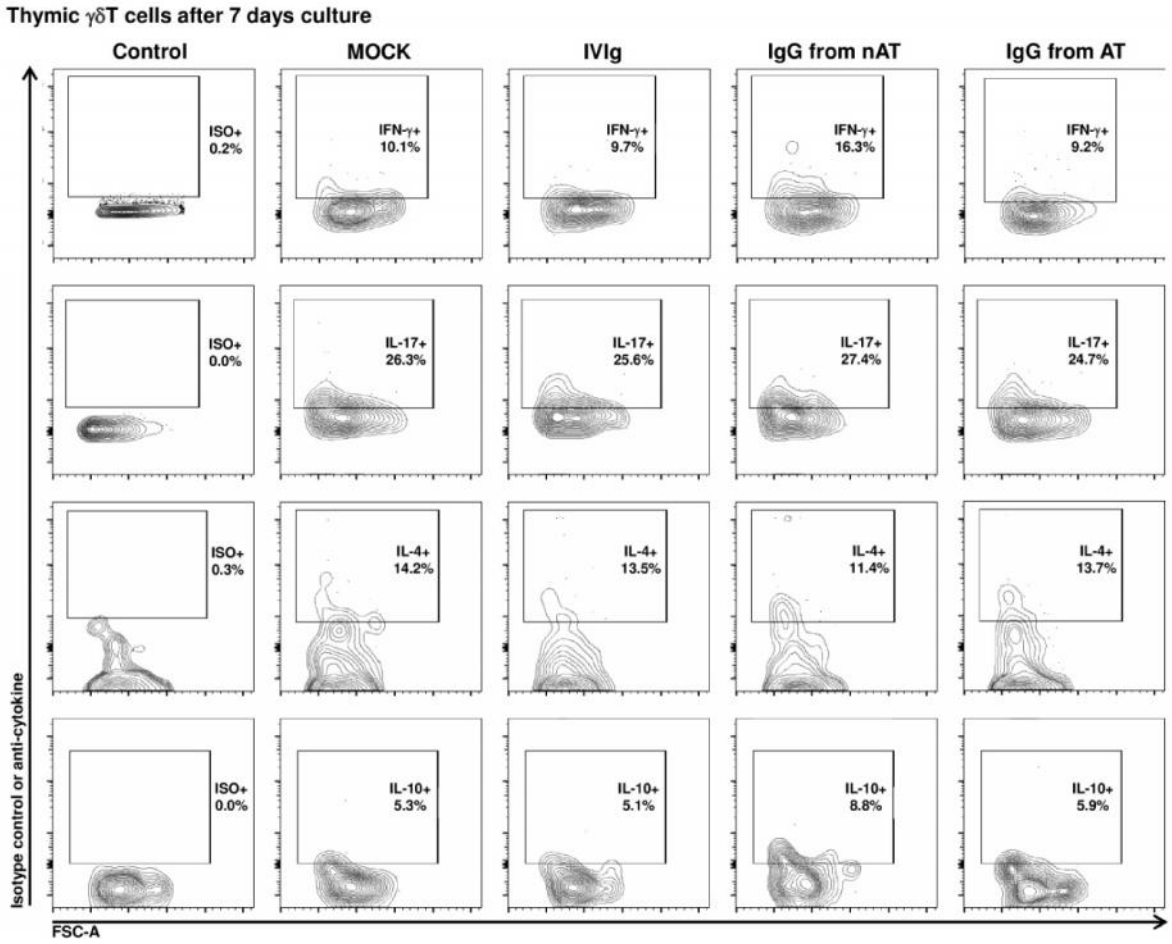

Fig. S2 Illustrative contour plots of the effect of purified IgG on cytokine production by intra-thymic ¥¢T cells after 7 days of culture. Each sample was acquired using the singlet cell gate (determined by SSC-A/SSC-H parameters), then the lymphocyte gate (determined by their relative size/granularity) and then the viable cell gate; samples were then acquired gated as CD3+TCRγδ+ (γδT cells). Cytokine gating was determined using isotype controls on cells from mixed culture conditions (Left panels), and cytokines of cells from each culture condition: mock, IVIg, non-atopic IgG (nAT) and atopic IgG (AT) cultures were measured accordingly, as indicated on the right panels.

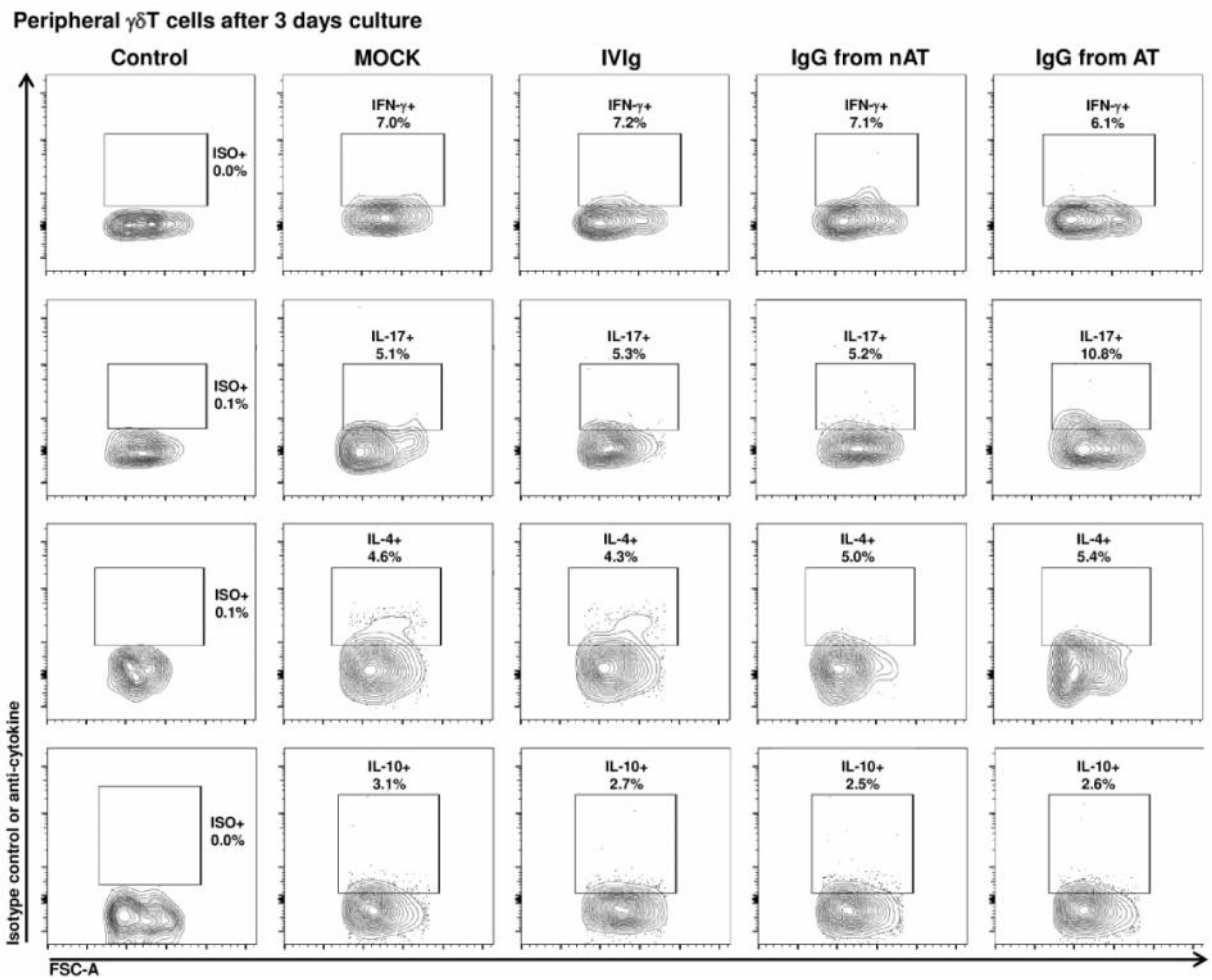

Fig. S3 Illustrative contour plots of the effect of purified IgG on cytokine production by peripheral ¥¢T cells after 3 days of culture. Each sample was acquired using the singlet cell gate (determined by SSC-A/SSC-H parameters), then the lymphocyte gate (determined by their relative size/granularity) and then the viable cell gate; samples were then acquired gated as CD3+TCRγδ+ (γδT cells). Cytokine gating was determined using isotype controls on cells from mixed culture conditions (Left panels), and cytokines of cells from each culture condition: mock, IVIg, non-atopic IgG (nAT) and atopic IgG (AT) cultures were measured accordingly, as indicated on the right panels.

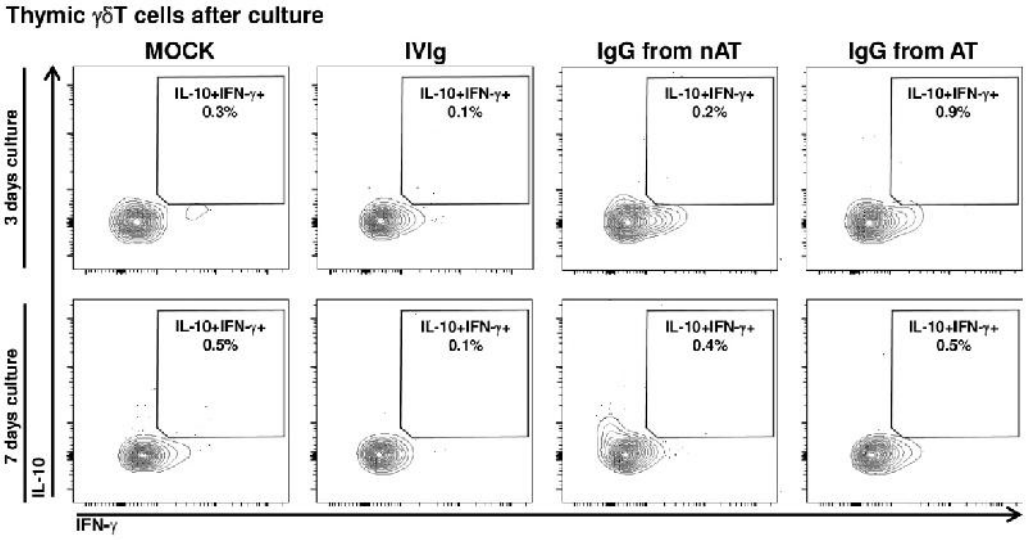

Fig. S4 Illustrative contour plots of the effect of purified IgG on IL-10 and IFN-¥ co-production by intra-thymic ¥¢T cells after 3 and 7 days of culture. Each sample was acquired using the singlet cell gate (determined by SSC-A/SSC-H parameters), then the lymphocyte gate (determined by their relative size/granularity) and then the viable cell gate; samples were then acquired gated as CD3+TCRγδ+ (γδT cells). The frequency of double positive IL-10+IFN-γ+ γδT cells were evaluated in each culture condition; mock, IVIg, non-atopic IgG (nAT) and atopic IgG (AT) cultures were measured accordingly, as indicated on the panels.