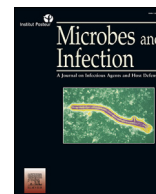




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Original article

Helicobacter pylori pediatric infection changes FcεRI expression in dendritic cells and Treg profile *in vivo* and *in vitro*

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ABSTRACT

H. pylori infection shows an inverse relationship with allergies. Dendritic cells regulate mucosal immune responses including the induction of T regulatory cells which are fundamental in *Helicobacter pylori*-induced dampening of allergies. In this respect expression of high-affinity IgE receptor (FcεRI) has been associated with a regulatory dendritic cell profile. Therefore we aimed to evaluate possible mechanisms by which *H. pylori* infection might modify atopy in pediatric patients. Here we show that *H. pylori*-infected children exhibited both increased expression of FcεRI on peripheral myeloid and plasmacytoid dendritic cells and higher levels of Foxp3 and Latency Associated Peptide on T regulatory cells. Moreover, exposure to *H. pylori* drove increased FcεRI expression and IL-10 secretion by both pediatric *H. pylori*-exposed monocyte derived dendritic cells and T cells. Finally, we show a positive correlation between expression of FcεRI in circulating myeloid DCs and total Treg cells, suggesting that in children, *H. pylori* infection may have a modulating role in atopy, mediated by both altered surface expression of FcεRI on children's DC and an increased T regulatory cell profile.

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While the burgeoning allergy epidemic continues to gain momentum [1], proponents of the hygiene hypothesis have attempted to explain this phenomenon as a consequence of decreased microbial or environmental exposures and/or a changing host microbiota, both leading to the development of an immature immune system in atopic individuals [2,3].

An inverse relationship has been described between *Helicobacter pylori* infection and atopy. During infection, *H. pylori* colonizes the stomach and is the predominant cause of chronic gastritis, gastroduodenal ulceration, and gastric malignancy [4]. The infection is acquired in childhood and can last within its host for decades [5]. Epidemiological data has shown that *H. pylori* seropositivity was inversely associated with asthma [6,7]. Our group has reported that *H. pylori* infection was associated with lower levels of allergy markers using a combined score that

includes skin test and total seric IgE. This inverse relationship was more pronounced on pediatric than adult patients [8]. Moreover, in the mice model, therapeutic administration of an *H. pylori* extract prior to allergen challenge in C57BL/6 mice significantly reduced airway inflammation [9].

Dendritic cells (DCs) are the main orchestrators of immune responses and constitutive expression of the high affinity IgE receptor (FcεRI) in human DCs has been described [10]. Traditionally the interaction between FcεRI and IgE in DCs has been considered a pro-inflammatory event, mainly based on the role their interaction has upon mast cells [11] and on the observation that patients with atopic diseases show increased levels of the receptor on the surface of circulating DCs [12–14]. In opposition, Platzer et al. reported that in a humanized mouse model of DCs expressing FcεRI, IgE/FcεRI interaction fails to induce DC maturation, as determined by CD80 and CD83 DC surface expression and production of pro-inflammatory mediators, such as TNF-α and IL-6. Transgenic mice with FcεRI⁺ DCs showed decreased severity in experimental models of food allergy and asthma, suggesting a regulatory effect of FcεRI on human DCs [15].

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Data from murine models suggest a key role for T regulatory (Treg) cells as one of the mechanisms associated with the dampening of allergic diseases in response to *H. pylori* infection [16,17]. *H. pylori*-infected children show increased numbers of Treg cells suggesting a modulating role of these cells in the reduced Th1 and Th17-mediated gastritis and ulceration in children. The same Treg cells or associated cytokines such as TGF- β and IL-10 might affect phenotypic expression of allergies [18,19]. In addition, human IL-10 secreting T cells have been associated with *H. pylori*-mediated protection from allergy in an adult cohort of patients [20]. Significantly lower levels of blood circulating IgE in serum were also described in infected patients from the same cohort [20] suggesting a role of this bacterium in IgE mediated responses.

To date, no data is available that relates Fc ϵ RI expression with *H. pylori* infection in pediatric population, and little is known regarding circulating Treg cells. Therefore, our aim was to evaluate potential mechanisms by which *H. pylori* infection might modify atopy in pediatric patients.

1. Patients and methods

1.1. Patient recruitment

Peripheral blood samples were obtained from children less than 16 years old undergoing upper gastrointestinal endoscopy. This study was performed in accordance with the Declaration of Helsinki and the recommendations of local IRB CEC-MED UC (Project numbers #PUC 12–204 and 14–065). Written informed consent was obtained from all parents/guardians and children over 8 years old gave assent to participate in this study.

1.2. *H. pylori* status determination

H. pylori infection status was determined in gastric biopsies by Rapid Urease Test (RUT) (Hepytect[®], BiosChile, Chile) or by serum levels of anti-*H. pylori* IgG antibodies using the *H. pylori* IgG Antibody ELISA Test Kit (Demeditec Diagnostics, Germany). *H. pylori* infection was defined as either a positive RUT or the presence of IgG antibodies over the cut-off suggested by the kit manufacturer.

1.3. Serum IgE determination

Total IgE concentration in serum samples was measured using the total IgE ELISA according to the manufacturer instructions (Ebiosciences, San Diego, CA). OD values were determined on an ASYS Hitech Expert 96 microplate reader (Biochrom, UK) at 450 nm. Patients were classified according to their level of serum IgE, used as a surrogate for atopy at a cut-off above 100 UI/ml in serum IgE titers.

1.4. Bacterial strains and culture conditions

H. pylori ATCC26695 strain originally obtained from American Type Culture Collection (ATCC) was kindly provided by Dr. Alejandro Venegas (Universidad Diego Portales, Santiago, Chile). *H. pylori* were grown on Brucella Agar plates supplemented with 7% v/v inactivated horse serum and 1% v/v DENT (Oxoid, Basingstoke, UK) at 37 °C under microaerophilic conditions, using the CampyGen[™] system (Oxoid, Basingstoke, UK). Bacteria colonies were collected in Brucella broth and OD600 nm values were determined. To calculate infection doses we used the LIVE/DEAD[®] BacLight[™] Bacterial Viability Kit (ThermoFisher, Carlsbad, CA) that correlates bacterial cell numbers with OD at 600 nm.

1.5. Immune cell isolation

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation. For circulating DC analysis PBMCs were stored at –80 °C until use. For monocyte-derived dendritic cells (MoDCs) generation and DC:T cell co-culture fresh cells were used. CD14⁺ monocytes were isolated using MACS anti-CD14-MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer instructions. Naïve CD4⁺ T cell isolation was performed using the Naïve CD4⁺ T cell isolation kit II (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer instructions. Naïve T cells were stored in liquid nitrogen until DC:T co-culture.

1.6. Dendritic cell generation, *H. pylori* exposure and DC:T co-culture

Freshly isolated CD14⁺ Monocytes were cultured in complete media (RPMI 1640 medium supplemented with 10% human AB serum (Cellgro, Manassas, VA), 2 mM glutamine, 1 mM pyruvate, 1 mM HEPES, 10 mg/ml Penicillin, 100 mg/ml streptomycin, 50 mg/ml gentamycin), in presence of recombinant human GM-CSF (25 ng/ml) and rhIL-4 (7 ng/ml). Non-adherent cells were harvested as MoDCs by vigorous pipetting after 5 days of culture. For MoDC response, on day 5 of culture, MoDCs were exposed to *H. pylori* for 48 h at an MOI of 10 PFU per cell in complete media without cytokines. As controls, DCs were left untreated. Forty-eight hours post-infection, surface marker expression levels were analyzed by FACS and IL-10 release was analyzed by ELISA.

For DC:T co-culture, MoDCs were prepared as described above. On day 5 of culture they were exposed to *H. pylori* for 2 h at an MOI of 10 PFU per cell in complete media without cytokines. MoDCs were washed twice with DPBS to remove *H. pylori*. MoDCs and syngeneic T cells were plated at 1:10 cell ratio in complete media for 3 days. For controls no *H. pylori*-exposed MoDC and T cells activated with activation/expansion kit (Miltenyi Biotec, Bergisch Gladbach, Germany) were used. Supernatants were collected for ELISA assays and cells for FACS analysis.

1.7. Flow cytometry

To identify and characterize circulating DC analyses in human PBMC populations, cells were stained with anti-Human Lineage Cocktail 1 (Lin 1)-FITC (BD Fastimmune), anti-HLA-DR-PerCP (clone L243; BD Biosciences), anti-CD11c-APC/Cy7 (clone Bu15; Biolegend), anti-CD123-APC (clone 7G3; BD Pharmingen), and anti-Fc ϵ RI α -PE (clone 773704; R&D Systems). In vitro MoDCs were stained with anti-CD11c-APC/Cy7, anti-HLA-DR-PerCP, anti-Fc ϵ RI α -PE, plus Anti-CD83-PE (clone HB15e; BD Pharmingen), Anti-CD86-PE (clone IT2.2; BD Pharmingen), Anti-CD40-FITC (clone 5C3; BD Pharmingen) and Anti-PD-L1-FITC (clone MIH1; BD Pharmingen). Treg cells were stained with anti-Human CD4-FITC (clone RPA T4; BD Pharmingen), anti-CD25-PE (clone M-Ac51; BD Pharmingen), anti Foxp3-APC (clone PCH101; Ebiosciences) and anti-LAP-PE-Cy7 (clone FNLAP; Ebiosciences). Appropriate isotype controls were used in this analysis.

All samples were stained in 1X PBS containing 1% FBS as a blocking solution and fixed using BD Cytofix (BD Biosciences), according to the manufacturer instructions. For Foxp3 staining, cells were fixed/permeabilized, and stained using the Foxp3 staining set (Ebiosciences, San Diego, CA). All samples were acquired on a FACS Canto II flow cytometer (BD Biosciences, San Jose, CA) and analyzed using FlowJo 7.6 software.

1.8. Statistical analysis

Comparisons between *H. pylori* infected or non-infected groups were performed using the Student's t test for continuous parametric data and the Mann–Whitney test for continuous non-parametric data. For the in vitro assays, paired analysis using parametric tests with or without Welch correction for differences in variances or non-parametric tests were used depending on the data. Spearman non parametric correlation was used for correlation analysis. GraphPad Prism v5 was used for all the analyses. A p value < 0.05 was considered statistically significant.

2. Results

2.1. Clinical characteristic of pediatric blood donors

Twenty-seven pediatric donors with a mean age of 8 ± 5 years old were included in the circulating DC analysis. Forty-one percent of the analyzed patients were male. *H. pylori* infection was present in 19% of the analyzed patients. Although not significant, total serum IgE levels, as well as the percentage of patients with IgE >100 UI/ml levels, were higher in non-infected donors in comparison to *H. pylori* infected donors (Table 1).

2.2. Circulating pediatric dendritic cells from *H. pylori* infected patients show increased levels of FcεRIα

Since increased FcεRIα surface expression on circulating myeloid (Lin[−]HLA-DR⁺CD11c⁺ cells) and plasmacytoid (Lin[−]HLA-DR⁺CD123⁺) DCs has been related with atopic disease [12], and concomitant *H. pylori* infection in pediatric patients might help reduce the burden of allergic disease, we analyzed the expression of FcεRIα receptor on the surface of circulating myeloid and plasmacytoid DCs obtained from PBMCs of pediatric donors. A significantly increased percentage of cells expressing FcεRIα was detected in *H. pylori*-infected pediatric patients compared to non-infected patients for both myeloid (Fig. 1A and B) ($p = 0.02$) and plasmacytoid DC subsets (Fig. 2A and B) ($p = 0.03$). In addition, median fluorescence intensity (MFI) was significantly increased in *H. pylori*-infected pediatric donors, compared to non-infected donors (Fig. 1C,D; Fig. 2C and D) ($p = 0.03$).

2.3. *H. pylori* exposure increases FcεRIα expression and imprints a tolerogenic profile in pediatric in vitro-generated MoDC

We first determined whether the increased expression of FcεRIα on circulating DCs of *H. pylori*-infected pediatric patients was recapitulated by an in vitro *H. pylori* exposure of MoDCs generated from *H. pylori* non-infected pediatric donors. A significant increase in the numbers of CD11c⁺HLA-DR⁺ MoDC expressing FcεRIα was found in *H. pylori*-treated, compared to non-treated MoDCs ($p = 0.03$) (Fig. 3A). Next, we determined the expression of surface maturation/activation markers. *H. pylori*-exposed pediatric DCs exhibited a significant increase in the maturation marker CD83 (Fig. 3B), as previously described in presence of this bacterium [21] ($p = 0.04$). In contrast, the activation markers CD86 and CD40 were unchanged between the treatments (Fig. 3C and D). Interestingly,

the tolerogenic marker PD-L1 was also significantly increased in *H. pylori* treated cells (Fig. 3E; $p = 0.003$). A protective role in the development of allergies for the T helper cytokine IL-10 has been described in models of atopy [20]. Consistent with these reports, significantly higher levels of IL-10 were detected in the culture supernatants of MoDCs after *H. pylori* exposure, compared to non-treated MoDCs ($p = 0.003$) (Fig. 3F).

2.4. Circulating levels of Foxp3⁺Treg cells are increased in *H. pylori* infected pediatric patients

One of the hallmarks of pediatric *H. pylori* infection is a pronounced infiltration of the gastric mucosa with Foxp3⁺ Treg [18], although scarce data is available regarding circulating Foxp3Tregs.

Circulating levels of Treg cells were higher in *H. pylori* infected patients (Fig. 4A). Moreover, increased MFI of Foxp3 transcription factor as well molecules associated with suppressor capability, such as Latency-Associated Peptide (LAP), were higher in circulating Treg cells of infected patients, compared to non-infected patients ($p = 0.04$; 0.03) (Fig. 4B and C).

2.5. In vitro generated MoDC exposed to *H. pylori* directed pediatric naïve CD4⁺T cells to secrete IL-10

To determine if pediatric *H. pylori*-exposed MoDCs directed naïve CD4⁺ T cells to a regulatory phenotype, we determined the percentage of CD4⁺CD25⁺FOPX3⁺ Treg cells after a 3 days co-culture of *H. pylori*-exposed MoDCs with purified naïve CD4⁺T cells from the same donor. *H. pylori*-exposed MoDCs were not able to drive an increase in the percentage of CD4⁺CD25⁺Foxp3⁺ Treg cells in comparison to the activation control (Fig. 5A). However, *H. pylori*-exposed MoDCs were able to induce a potent regulatory cytokine response in the T cells characterized by significantly higher levels of IL-10 than non-activated and antiCD3/CD28-activated T lymphocytes ($p < 0.05$) (Fig. 5B). Moreover, the same lymphocytes showed a reduced secretion of IFN-γ, a Th1 cytokine and IL-13, a Th2 cytokine in response to *H. pylori*-exposed MoDCs, in comparison to antiCD3/CD28 activated T lymphocytes (Fig. 5C and D).

2.6. FcεRIα expression on circulating DCs correlates with Treg cell numbers in peripheral blood of pediatric patients

To evaluate whether FcεRIα surface expression on circulating myeloid (Lin-HLA-DR + CD11c + cells) peripheral blood DCs correlated with Treg cells numbers, we analyzed the MFI intensity of FcεRIα as a surrogate of expression of this receptor, and correlated with the numbers of CD4⁺CD25⁺Foxp3⁺ Treg cells. Indeed, FcεRIα expression in myeloid DCs correlated strongly with the numbers of circulating Tregs in our patients (Spearman $r = 0.7$; $p = 0.0005$) (see Fig. 6).

3. Discussion

The inverse relationship between *H. pylori* and allergies has been well documented. This association tends to be more pronounced in children and on neonate mice, compared to adults

Table 1
Clinical characteristic of pediatric blood donors.

	Non-infected subjects (N = 22)	<i>H. pylori</i> -infected subjects (N = 5)	p value
Age, X ± SD	8 ± 5	12 ± 3	0.08
Sex, N(%) of males	10 (45)	1 (20)	0.3
Total serum IgE Titers UA/ml, X ± SD	88.3 ± 123	31.7 ± 28	0.1
Patients with serum IgE > 100 IU/ml, N (%)	5 (23)	0 (0)	0.3

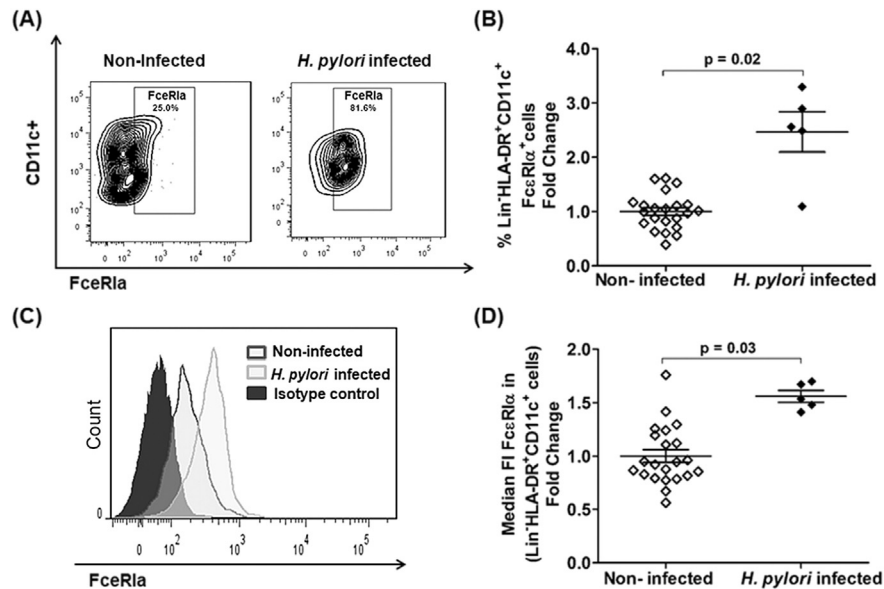


Fig. 1. FcεRIα expression on circulating myeloid dendritic cells from pediatric donors with and without *H. pylori* infection. A) Representative contour plots showing % of Lin⁺HLA-DR⁺CD11c⁺ cells that express FcεRIα from *H. pylori*-infected and non-infected donors. B) Percentage of Lin⁺HLA-DR⁺CD11c⁺ cells that express FcεRIα. Data are presented as fold change from non-infected patients with media plus SEM. $p < 0.05$ unpaired t test. C) Representative histograms of Median Fluorescence Intensity (MFI) of FcεRIα on Lin⁺HLA-DR⁺CD11c⁺ cells from *H. pylori*-infected and non-infected donors MFI of FcεRIα on Lin⁺HLA-DR⁺CD11c⁺ cells. D) MFI of FcεRIα on Lin⁺HLA-DR⁺CD11c⁺ cells. Data are presented as fold change from non-infected patients with media plus SEM. $p < 0.05$ unpaired t test.

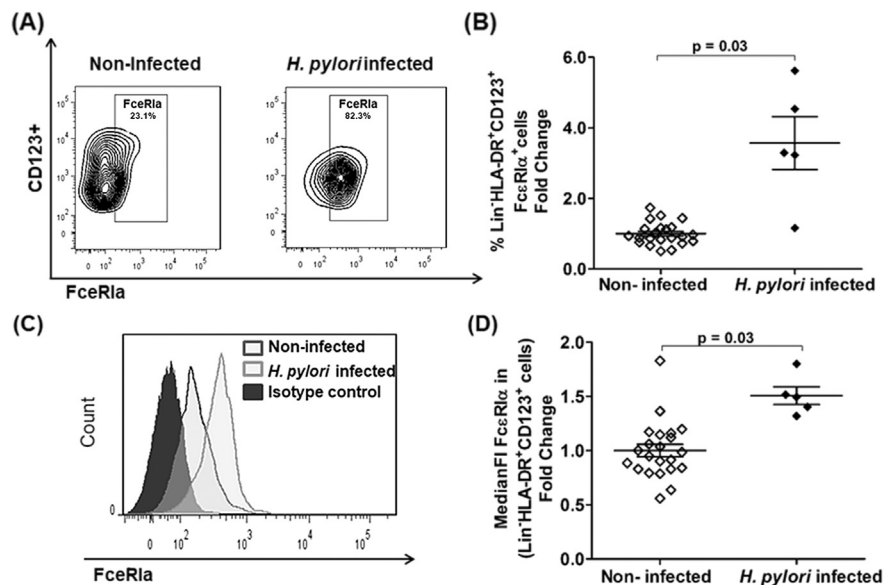


Fig. 2. FcεRIα expression on circulating plasmacytoid dendritic cells from pediatric donors with and without *H. pylori* infection. A) Representative contour plots showing % of Lin⁺HLA-DR⁺CD123⁺ cells that express FcεRIα from *H. pylori*-infected and non-infected donors. B) Percentage of Lin⁺HLA-DR⁺CD123⁺ cells that express FcεRIα. Data are presented as fold change from non-infected patients with media plus SEM. $p < 0.05$ unpaired t test. C) Representative histograms of Median Fluorescence Intensity (MFI) of FcεRIα on Lin⁺HLA-DR⁺CD123⁺ cells from *H. pylori*-infected and non-infected donors MFI of FcεRIα on Lin⁺HLA-DR⁺CD123⁺ cells. D) MFI of FcεRIα on Lin⁺HLA-DR⁺CD123⁺ cells. Data are presented as fold change from non-infected patients with media plus SEM. $p < 0.05$ unpaired t test.

[6,16]. In this study, we evaluated in peripheral blood obtained from children, both the innate immune response compartment represented by circulating myeloid and plasmacytoid DCs, as well as the adaptive immune compartment, with emphasis on circulating Treg cells in *H. pylori*-infected and non-infected pediatric donors. Furthermore, we determined the effect of *H. pylori* exposure on pediatric MoDCs and T cells *in vitro*.

Since IgE and its cell surface receptors are key components in the phenotypic expression of allergic reactions, we determined the

surface expression of the IgE high affinity receptor on peripheral blood circulating DCs and correlated it with the presence of *H. pylori* infection. FcεRI on monocytes and DCs is expressed in a trimeric isoform which contains one FcεRIα subunit and two FcεRIγ subunits, with the former α subunit being indispensable for successful IgE binding [10]. Expression of this receptor on pediatric DCs has been described by Dehlink et al. [14,22].

We describe an increased percentage of cells that express FcεRIα as well as increased MFI of the receptor in both myeloid and

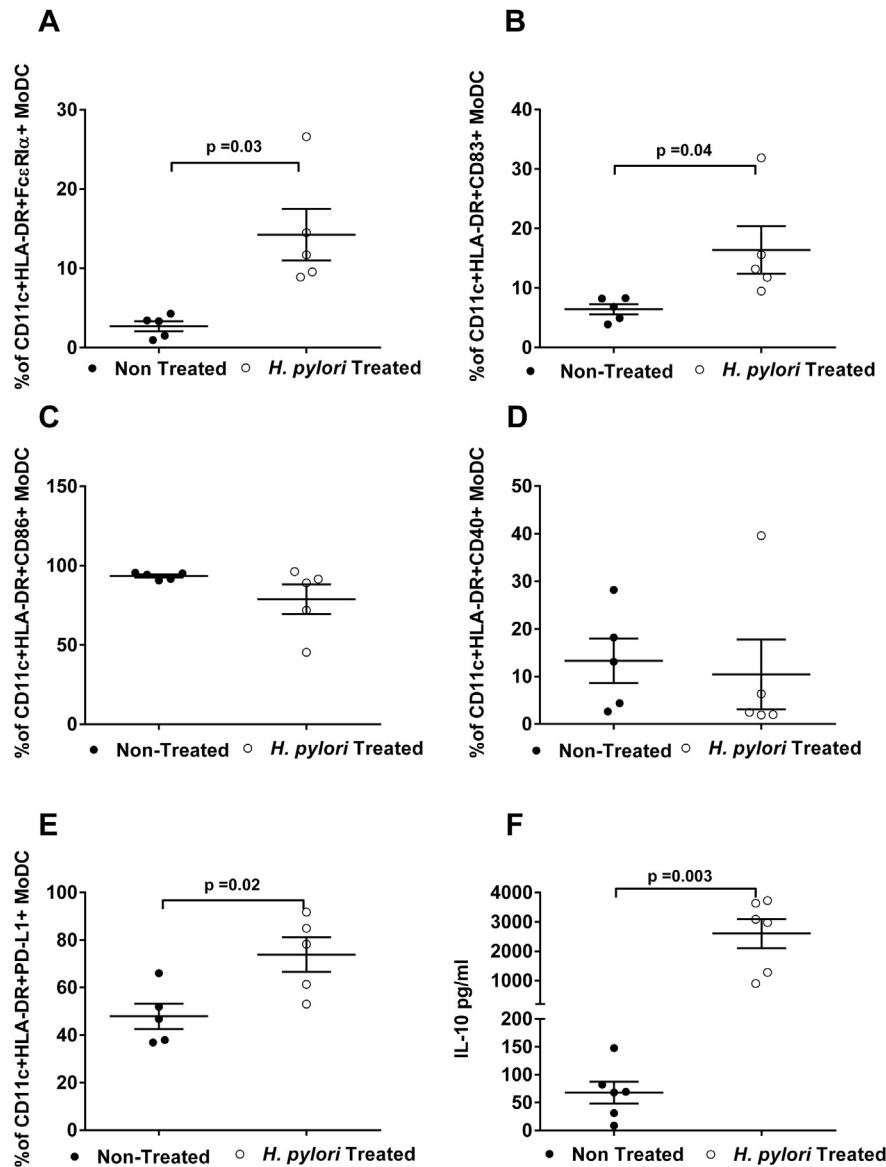


Fig. 3. FcεRIα expression on pediatric Monocyte derived DC from *H. pylori* non-infected donors upon in vitro *H. pylori* exposure. A) Representative contour plots showing % of CD11c⁺HLA-DR⁺ MoDCs that express FcεRIα with and without *H. pylori* exposure in vitro. B) Percentage of CD11c⁺HLA-DR⁺FcεRIα⁺ MoDC. Data from individual patients with media plus SEM are shown. $p < 0.05$ paired t test. C) Representative dot and contour plots showing % of CD11c⁺HLA-DR⁺ MoDCs that express activation marker CD83 with and without *H. pylori* exposure in vitro. D) Percentage of CD11c⁺HLA-DR⁺ CD83⁺ MoDC. Data from individual patients with media plus SEM are shown. $p < 0.05$ paired t test. E) IL-10 secretion levels determined in control and *H. pylori* exposed MoDC supernatant. Data from individual patients with media plus SEM are shown. $p < 0.05$ Paired t test.

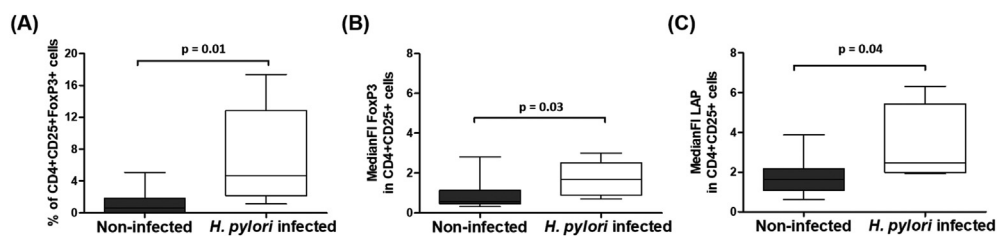


Fig. 4. Circulating levels of Treg cells in peripheral blood from pediatric donors with and without *H. pylori* infection. A) Percentage of CD4⁺CD25⁺Foxp3⁺ Treg cells from *H. pylori*-infected and non-infected donors. Box and whiskers graph showing max and min values. Mann–Whitney test. B) Median fluorescence intensity (MFI) for Foxp3 transcription factor in CD4⁺CD25⁺ Treg cells from *H. pylori*-infected and non-infected donors. Box and whiskers graph showing max and min values. $p < 0.05$ Mann–Whitney test. C) MFI for latency associated peptide (LAP) in CD4⁺CD25⁺ Treg cells from *H. pylori*-infected and non-infected donors. Box and whiskers graph showing max and min values. $p < 0.05$ Mann–Whitney test.

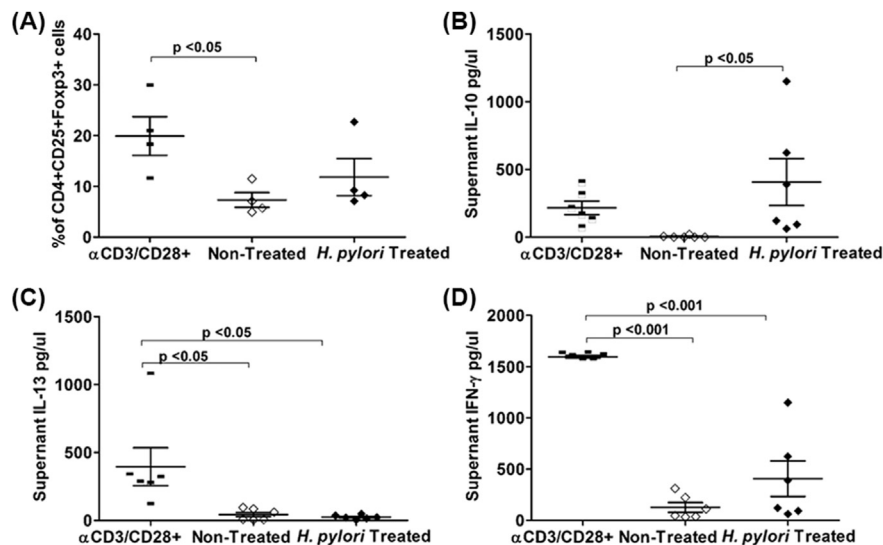


Fig. 5. Treg cells induction and cytokine secretion from naïve T cell co-cultured with pediatric Monocyte derived DC exposed to *H. pylori*. A) Percentage of CD4⁺CD25⁺Foxp3⁺ Treg cells after 3 day co-culture with *H. pylori* exposed MoDC from the same donors. Anti-CD3/CD28 stimulation was used as positive control. Naïve CD4⁺ T cells were used as negative controls. Data from 4 different *H. pylori* non-infected donors are shown plus media and SEM. Data were analyzed by Repeated measures Anova and Bonferroni multiple comparison post-test. B, C, D) IL-10, IL-13 and IFN- γ secretion into MoDC: T cell co-culture supernatant. Anti-CD3/CD28 stimulation was used as positive control. Naïve CD4⁺ T cells were used as negative controls. Data from 5 different *H. pylori* non-infected donors are shown plus media and SEM. Data were analyzed by Repeated measures Anova and Bonferroni multiple comparison post-test.

plasmacytoid DCs of *H. pylori*-infected pediatric patients. Moreover, we showed that *H. pylori* exposure of monocyte derived DC in culture generated from *H. pylori* naïve non-infected pediatric donors also results in Fc ϵ R1 α up-regulation, suggesting that *H. pylori* infection might alter Fc ϵ R1 α expression patterns on circulating DCs. Berings et al. [23] showed that specific allergen signaling induces a temporary increase in Fc ϵ R1 expression on DCs, suggesting that the expression of this receptor might be involved in immunomodulatory properties of circulating DCs in the context of allergic sensitization.

Platzer et al. described a regulatory effect on allergies related to Fc ϵ R1 expression on DC characterized by low levels of DC activation markers and increased levels of IL-10 secretion [15]. Consistent with these findings, we report that following *H. pylori* stimulation, pediatric in vitro-generated MoDCs expressed increased Fc ϵ R1 α levels. Moreover, while maturation marker CD83 was increased as

previously described for adult MoDCs [21], activation markers CD86 and CD40 were not stimulated by *H. pylori* exposure. Interestingly, tolerogenic marker PD-L1 was increased in the *H. pylori* exposed DCs. Further, secretion of IL-10 is significantly increased upon *H. pylori* exposure. Dendritic cell impaired maturation in *H. pylori* infection and the predominant role of IL-10 has been established in DCs [24,25], although most of the experimental data comes from murine models and adult DCs. The effect of this bacterium on pediatric DCs has remained elusive. This study provides evidence that the previously described effects also apply when DCs are obtained from pediatric patients.

Animal models as well as human cell based studies have suggested a role for Treg cells and IL-10 [16,20,26,27] as a possible mechanism underlying the inverse association between allergic disease and *H. pylori*. Consistently, our study in pediatric primary cells detected increased numbers of circulating CD4⁺CD25⁺Foxp3⁺ Treg cells in the peripheral blood of infected children but also increased MFI of Foxp3 transcription factor as well as functionality molecule LAP in *H. pylori*-infected children. Taken together, these results suggest that the numbers and functionality of these cells might be altered in *H. pylori*-infected children. The number and functionality of Treg cells in target tissues such as respiratory mucosa is not addressed in this paper and remains to be determined in the context of pediatric *H. pylori* infection. In the pediatric co-culture system, *H. pylori* was not able to induce a significant number of CD4⁺CD25⁺Foxp3⁺ Treg cells in comparison to the activation control, although a regulatory cytokine secretion profile was observed. *H. pylori*-treated MoDC drive T cells to secrete low levels of IFN- γ and IL-13 and high levels of IL-10 asserting the fundamental role of IL-10 regardless if it is produced by DCs or T cells in the *H. pylori* induced immune response.

A direct link between Fc ϵ R1 α expression on DCs, Treg induction and IL-10 secretion has not been demonstrated in this study; further functional experiments are required to assert a causal relationship between them. However, our findings demonstrate a strong correlation between Fc ϵ R1 α expression and circulating Treg cell numbers as well as higher expression of this receptor during the in vitro *H. pylori* exposure assay and are highly suggestive of a

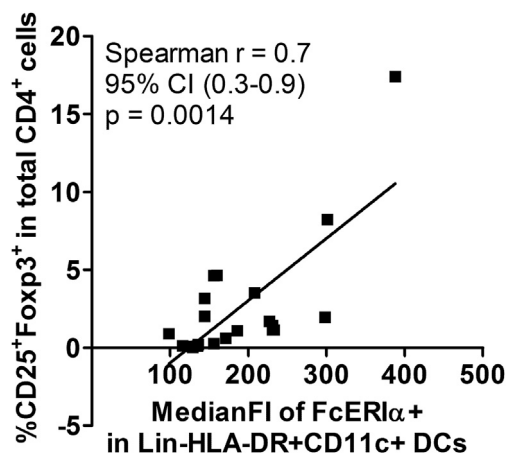


Fig. 6. Fc ϵ R1 α expression on circulating DCs correlation with Treg cell numbers in peripheral blood of pediatric patients. Correlation between MFI of Fc ϵ R1 α determined in myeloid Lin-HLA-DR⁺CD11c⁺ DCs and Treg cell number determined as CD4⁺CD25⁺Foxp3⁺.

tolerogenic profile imprinted by *H. pylori* on both DCs and T cells. Our findings in primary pediatric cells contribute to elucidate the mechanisms by which *H. pylori* contributes to the regulation of the phenotypic expression of allergies supporting the already well-established clinical association between this infection and phenotypic expression of allergies in children.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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