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Children's Adenoids Show A Connection Between Foxp3+ Regulatory T cells and Th17 cell Expression And Pneumococcal Carriage.

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Abstract

Pneumococcal illness requires pneumococcal carriage in the nasopharynx, which is the main route of transmission. Our goal was to determine the relationship between the expression of Foxp3+ regulatory T (Treg) cells and Th17 cells and pneumococcal carriage in the adolescent populations of both pneumococcal-positive and -negative children. From kids having an adenoidectomy, we took samples of their adenoidal tissue and nasopharyngeal swabs. A D39 bacterial strain's culture concentrated supernatant (CCS) was used to isolate, cultivate, and activate adenoidal mononuclear cells. Adenoidal mononuclear cells isolated from the pneumococcus-positive group showed populations of Foxp3+ Treg cells elevated and Th17 cells downregulated. Following CCS stimulation, the pneumococcus-positive group had a large increase in Foxp3+ Treg cells compared to the pneumococcus-negative group, whereas the increase in Th17 cells was significantly lower. These findings were in line with changes in adenoidal mononuclear cell levels of Foxp3 mRNA and retinoic acid receptor-related orphan receptor-t mRNA.The pneumococcus-negative group had higher levels of IL-17A and IL-6 in the adenoid tissue than the pneumococcus-positive group did, while the pneumococcus negative group had lower levels of TGF- than the pneumococcus-positive group did. Foxp3+ Treg and Th17 cell expression in the adenoid was closely correlated with pneumococcal carriage in children. Increased Th17 cell production in the adenoid may be suppressed by Foxp3+ Treg cell upregulation, resulting in decreased scavenging of Streptococcus pneumoniae and persistent pneumococcal carriage.

Keywords: Foxp3+ Regulatory T cells, Adenoids, Streptococcus pneumoniae

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INTRODUCTION

The most frequent cause of community-acquired pneumonia, which is the primary infectious cause of morbidity, mortality, and severe pneumonia in children younger than 5 years old in poor and middle income countries, is streptococcus pneumoniae (pneumococcus) [1]. According to estimates, there were 120 million bouts of pneumonia in children under the age of five in 2010 (14 million of which proceeded to severe episodes), and 1.3 million of these instances resulted in fatalities in 2011 [2]. The nasopharynx is frequently colonised by pneumococcus, which can then travel through the airway to the lower respiratory tract, where it can cause pneumonia, or to the sinuses or middle ear, where it can induce a variety of morbidities [1]. The principal site of pneumococcus colonisation in the nasopharynx, the adenoid, is a mass of nasal lymphoid tissue. Invasive pneumococcal illness is transmitted mostly through pneumococcal carriage, and among young children, a high pneumococcus carriage rate is associated with the development of pneumococcal disease [3, 4]. Therefore, encouraging the elimination and reducing the duration of carriage are effective preventative interventions for invasive pneumococcal illness [5].

Th1, Th2, and Th17 subsets of effector CD4+ T cells, which provide defence against various pathogen types, can develop from CD4+ T cells. These cells may function as immune effectors that destroy infected cells by inducing the production of several sets of cytokines and other soluble and cell-bound products [6]. Th17 cells and IL-17 have lately been demonstrated to work during infections to get rid of pathogens including Helicobacter pylori, Mycobacterium tuberculosis, and Toxoplasma, among others [7, 8]. The quantity and activation of CD4+ T cells, particularly Th17 cells, may have a negative effect on the cytokine network, the prognosis of patients with pneumococcal pneumonia, and their capacity to eradicate streptococci [9, 10].

In the past 10 years, it has been clear that regulatory T (Treg) cells, which lower inflammation and play a crucial regulatory role during infections and are crucial for the upkeep of immunological homeostasis, are immune and inflammatory suppressors. However, an infection can become persistent if there are too many Foxp3+ Treg cells [6]. Another investigation revealed that Foxp3+ Treg cells can control inflammation caused by Th17 cells [11]. In some disorders, an imbalance between Foxp3+ Treg cells and Th17 cells may be crucial. A host organism may be more susceptible to bacterial and fungal infections when there are problems with the Th17 cell differentiation axis.Furthermore, TGF- plays a crucial role in both Treg and Th17 cell development mechanisms, which are closely connected [12].

Adenoid tissue's efficient immune response promotes pneumococcus clearance and can shield against related disorders. According to a recent study, the quantity and functionality of Foxp3+ Treg cells in the adenoid may be related to persistent pneumococcal carriage in the nasopharynx [13]. However, there is weak support for a link between the proportion of Foxp3+ Treg and Th17 cells and pneumococcal carriage, and the precise mechanism is still unknown. We postulated that increasing the number of Foxp3+ Treg cells could reduce the number of Th17 cells produced in the adenoid. This could prevent Th17 cells from scavenging, which would promote pneumococcal carriage.In the current study, the mechanism of pneumococcal carriage in the nasopharynx was explored as well as the link between Foxp3+ Treg and Th17 cells in the adenoid tissues of children positive and negative for pneumococci.

MATERIALS AND METHODS

Specimen Collection

On the day of their adenoidectomy at the Children's Hospital of Chongqing Medical University, 47 children (aged 3 to 8 years) provided nasopharyngeal swabs and samples of adenoid tissue. Patients who underwent surgery within three weeks after receiving antibiotics or systemic steroids, or who had a known immune deficiency, chronic illness, or upper respiratory infection at the time of or two weeks prior to hospital admission, were excluded from the study. No kids in our research had ever received a pneumococcus vaccination. The Children's Hospital at Chongqing Medical University's Ethics and Human Research committees gave their approval to the study protocol. Each patient's legal guardian had to sign an informed consent form before they could participate in the study.

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Identification of Streptococcus pneumoniae

Nasopharyngeal swabs were put onto Columbia blood agar-containing culture plates and grown overnight at 37°C in a 5% CO2 environment. A second plate of Columbia blood agar with an optochin test disc in its centre was then used to inoculate a grey, wet colony surrounded by a grass green hemolysis ring. The size of the hemolysis ring was measured after this plate had been incubated for the same amount of time at 37°C with 5% CO2. Pneumococcus positivity was indicated by a hemolysis ring larger than 14 mm in diameter, while pneumococcus negativity was indicated by a ring smaller than 14 mm.

Isolation and Culture of Adenoidal Mononuclear Cells

Adenoid tissue samples were brought to our lab in minimal essential medium supplemented with glutamine, penicillin (100 U/ml), and streptomycin (100 g/ml), and processed within an hour of the procedure. The tissue samples from the adenoid were divided into pieces, put through a 300mesh strainer, and then given phosphate-buffered saline washings. Adenoidal mononuclear cells were isolated by gradient centrifugation through a lymphocyte separation medium at 2,000 rpm for 20 minutes at 20°C (TBD, Tianjing, Chi- na). Then, four layers of the cell suspension were created.The second layer's adenoidal mononuclear cells were rinsed twice with phosphatebuffered saline before being cultured in RPMI 1640 medium, which contains penicillin, streptomycin, glutamine, and 10% foetal bovine serum (Gibco, Grand Island, N.Y., USA). Using trypan blue staining, adenoidal mononuclear cells' vitality was assessed. A final adjustment brought the cell concentration to 4 106 cells/ml.

Stimulation of Adenoidal Mononuclear Cells

The typical encapsulated type 2 pneumococcal strains were those employed in this study (D39, NCTC7466). Adenoidal mononuclear cells were activated using concentrated culture supernatant (CCS) fraction from cultured D39 bacteria. Briefly stated, strain D39 bacteria were injected into a trypticase-soy broth culture medium and grown to logarithmic phase (108 cfu/ml) at 5% CO2 at 37 °C. After that, the inoculum was collected and centrifuged for 30 minutes at 4°C at 3,000 g/ min. A 0.2-m sterile filter was used to separate the culture supernatant, and a Vivaspin concentrator was used to concentrate it (Millipore, Boston, Mass., USA).Following the manufacturer's instructions, the Bio-Rad protein assay was used to measure the CCS's protein concentration, which was then corrected to 1 g/l. After that, the adenoidal mononuclear cells received aliquots of CCS. In 96-well plates with antibiotics and RPMI medium supplemented with 10% foetal bovine serum, some adenoidal mononuclear cells were cultivated for 6 hours and others for 24 hours in the presence or absence of CCS (1 g protein/ml).

Flow Cytometry

By using flow cytometry, the expressions of Foxp3 and IL-17A in the adenoidal mononuclear cells of each group were examined. Ad-enoidal mononuclear cells were cocultured for 6 hours with phorbol myristate acetate + ionomycin, CCS, or media, with the addition of golgi blockers (BD Bioscience, San Jose, Calif., USA) for the final 4 hours. After that, the cells were stained with fluorescence-labeled mouse anti-human CD4 and CD25 antibodies (CD4-FITC and CD25-PE; 4A Biotech, Beijing, China), and flow cytometry was used to examine the intracellular expression of IL-17A (IL-17A-PE; BD Bioscience). After then, the IL-17A-expressing cells were fixed and made permeable (BD Bio- science).Foxp3 was stained intracellularly with the Foxp3-PEcy5 antibody from eBioscience in San Diego, California, and monoclonal antibody nonspecific binding was avoided by using fixation/permeabilization solutions and 10 permeabilization solution from the same company. Preincubation with purified rat IgG was performed on all samples. The resulting data were examined using FlowJo software on a FACSCalibur flow cytometer from BD Bioscience.

RNA Analysis

Real-time quantitative PCR analysis was used to identify the expression of Foxp3 mRNA and retinoic acid receptor-related orphan receptor-t (ROR-t) mRNA in adenoidal mononuclear cells in each group. According to the manufacturer's recommendations, total RNA was isolated from various samples using Trizol reagent (Invitrogen; Carlsbad, Calif., USA). The RNA was reverse transcribed using a PrimeScriptRT reagent kit following measurement (TaKaRa, Shiga, Japan). In order to determine the relative expression levels of Foxp3 and ROR-t, real-time quantitative PCR was used.The particular primer

sequences for Foxp3 were 5′-ATTCCCAGAGTTCCTC-CAA-3′ for the forward direction and 5′-ATTGAGTGTCCGCTGCTTCT-3′ for the reverse direction. Forward: 5′-AGTGGTGCTGGTTAG- GATGTG-3′; reverse: 5′-AGGGAGTGGGAGAAGTCAAAG-3′ were the sequences for ROR-t. The housekeeping gene glyceraldehyde 3-phosphate dehydrogenase had the sequences 5′-AAGAAGGTGGT- GAAGCAGGC-3′ and 5′-TCCACCACCCTGTTGCTGTA-3′ in both the forward and reverse directions.

Enzyme-Linked Immunosorbent Assay Analysis

Two groups of adenoidal mononuclear cells—the control group and the CCS group—were created. After the mononuclear cells were cultivated and stimulated for 24 hours, the levels of IL-17A, IL-6, and TGF- in the cultured cells' supernatants were determined using ELISA.

eBioscience provided the ELISA kits used to quantify IL-17A, whereas Neobioscience provided the ELISA kits used to detect IL-6 and TGF- (Beijing, China).

Statistical Analysis

The mean value and standard deviation are used to display data. The Student's t test and the 2 test were used to examine statistical differences. p values 0.05 were regarded as statistically significant for all analyses, which were carried out using PASW Statistics for Windows, version 18.0, SPSS Inc., Chicago, Illinois, USA.

RESULTS

Nasopharyngeal Pneumococcal Carriage Rate

47 nasopharyngeal swab samples were collected from kids between the ages of 3 and 8; the overall pneumococcus carriage rate was 32%. Male participants (30.8 percent of the subjects) and female subjects' carriage rates did not differ significantly from one another (33.3 percent of subjects). Children aged 3-5 years had a carriage rate of 35.5%, while those aged 5-8 years had a carriage rate of 25% (p = 0.465).

Foxp3+ Treg and Th17 Cell Expression in Adenoid Tissues from Positive and Negative Pneumococcus Groups when compared to the pneumococcus-negative group, adenoidal mono-nuclear cells from the pneumococcus-positive group contained significantly more Foxp3+ Treg cells (6.70 0.53 vs. 4.49 0.24 percent, p 0.001). When compared to the pneumococcus-negative group, the adenoidal mononuclear cells from the pneumococcus-positive group included significantly less Th17 cells (1.70 0.14 vs. 3.84 0.51 percent, p 0.01). These findings showed a correlation between nasopharyngeal carriage of S. pneumoniae and higher numbers of Foxp3+ Treg cells and lower numbers of Th17 cells (fig. 1, 2).

Expressions of Foxp3 and ROR-t mRNA in Pneumococcus-Positive and Negative Group Adenoid Tissue Foxp3 mRNA expression was substantially higher in adenoidal mononuclear cells from the pneumococcus-positive group than from the pneumococcus-negative group (2.15 0.35 vs. 1.01 0.23, p 0.05). Although much lower than in mononuclear cells from the pneumococcus-negative group (0.19 0.03 vs. 0.52 0.09, p 0.01), ROR-t mRNA expression was nevertheless present in adenoidal mononuclear cells from the pneumococcus-positive group. These Foxp3 mRNA and ROR-t mRNA expressions correlated with the numbers of Foxp3+ Treg and Th17 cells, demonstrating a relationship between rising Foxp3 mRNA and falling ROR-t mRNA levels and nasopharyngeal carriage of S. pneumoniae (fig. 3).

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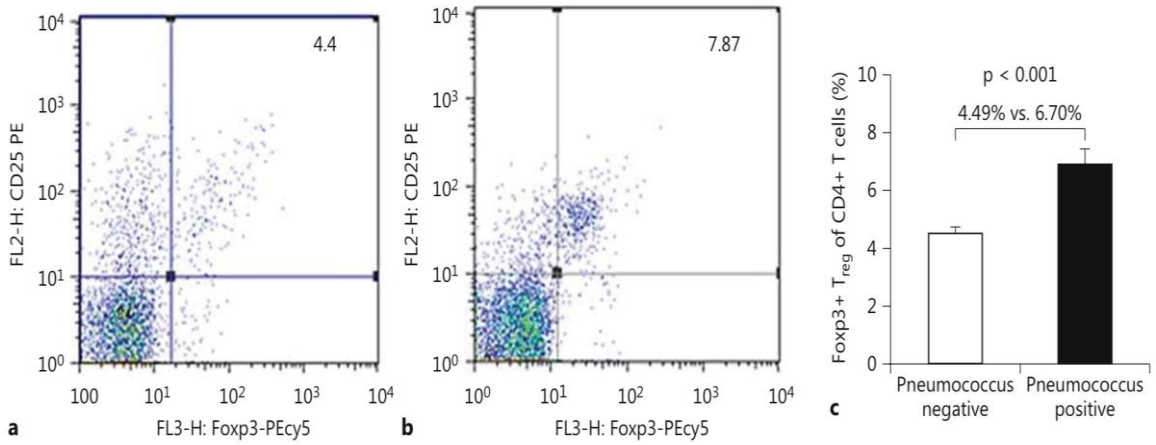
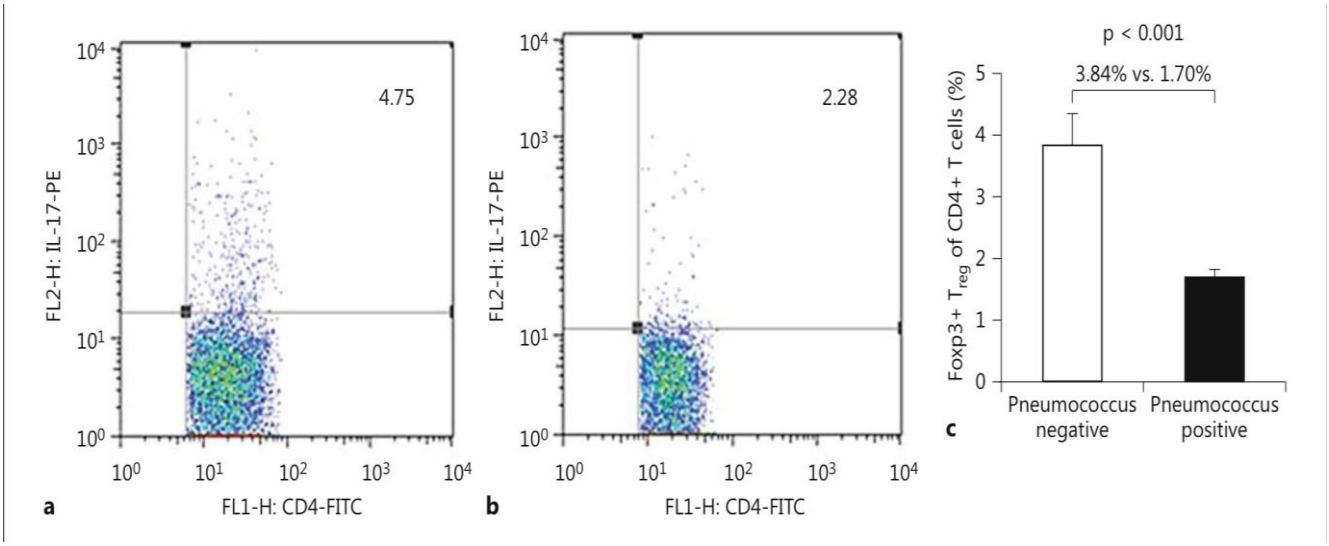


Figure 1- among CD4+ adenoidal mononuclear cells, the proportion of Foxp3+ Treg cells. Pneumococcus-negative sample (a) and pneumococcus-positive sample (b) findings from flow cytometry are displayed, respectively. c Populations of CD4+ adenoidal mononuclear cells from the pneumococcus-negative and pneumococcus-positive groups were compared for the proportion of Foxp3+ Treg cells (pneumococcus-negative group: n = 10; pneumococcuspositive group: n = 10).

Figure 2- The percentage of CD4+ adenoidal mononuclear cells that are Th17 cells. Pneumococcus-negative sample (a) and pneumococcus-positive sample (b) findings from flow cytometry are displayed, respectively. c Comparison of the frequencies of Th17 cells among CD4+ adenoidal mononuclear cells from the pneumococcus-positive and pneumococcus-negative groups (n = 10 for each group).

Greater Foxp3+ Treg Cell and Foxp3 mRNA Production Was Induced by CCS Stimulation in the Pneumococcus-Positive Group.



Pneumococcus-positive and -negative groups' CD4+ adenoidal mononuclear cells were stimulated by CCS, and the productions of Foxp3+ Treg cells and Foxp3 mRNA were subsequently examined. The increments in Foxp3+ Treg cells and Foxp3 mRNA in the pneumococcus-positive group after CCS stimulation were significantly higher than those in the pneumococcus-negative group, according to our findings (3.37 0.31 vs. 1.54 0.50 percent, p 0.01, and 0.97 0.26 vs. 0.14 0.21 percent, p 0.05, respectively; see fig. 4 According to these findings, the pneumococcus-positive group produced more Foxp3+ Treg cells and Foxp3 mRNA in response to CCS stimulation than the pneumococcus-negative group did.

Figure 3- Expressions of Foxp3 and ROR-t mRNA in pneumococcus-positive and pneumococcus-negative adenoidal mononuclear cells. a Adenoidal mononuclear cells from pneumococcus-negative and

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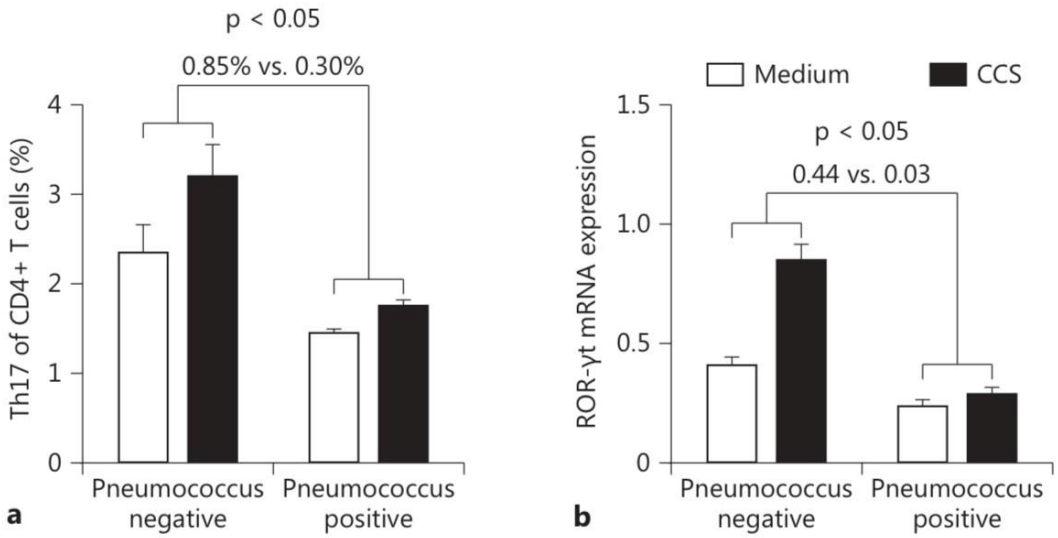
pneumococcus-positive groups cells. b Foxp3 mRNA levels rose in both the pneumococcus-positive (n = 8) and pneumococcus-negative (n = 8) groups. Less Th17 cells and ROR-t mRNA were produced by the Pneumococcuspositive group after CCS stimulation. After CCS stimulation, we examined the Th17 cell and ROR-t mRNA production in CD4+ adenoidal mononuclear cells taken from the pneumococcus-positive and -negative groups. The increases in Th17 cells and ROR-t mRNA in the pneumococcus-positive group after CCS stimulation were significantly smaller than those in the pneumococcus-negative group, according to our findings (0.3 0.07 vs. 0.85 0.14 percent and 0.03 0.02 vs. 0.44 0.16 percent, respectively; see fig. 5). These findings showed that, in comparison to the pneumococcus-negative group, the pneumococcus-positive group did not produce as many Th17 cells or ROR-t mRNA in response to CCS stimulation.



Before and after CCS Stimulation, the IL-17A, IL-6, and TGF-β Levels in Adenoids from the Pneumococcus-Negative and -Positive Groups.

The cytokines in each group's supernatant fraction of adenoidal mononuclear cells were then looked at. Prior to CCS stimulation, we discovered that IL-17A and IL-6 levels were significantly higher in the pneumococcus-negative group than in the pneumococcus-positive group (81.85 17.20 vs. 28.72 5.61 pg/ml and 149.67 19.12 vs. 33.85 6.11 pg/ml, respectively, and 741.02 48.53 vs. 9 However, following CCS stimulation, both the pneumococcus-positive and -negative groups displayed similar alterations in the levels of IL-17A, IL-6, and TGF-β (fig. 6).

Fig 4- The pneumococcus-positive group produced more Foxp3+ Treg cells and Foxp3 mRNA in response to CCS stimulation. a In both the pneumococcus-positive group (n = 10) and the pneumococcus-negative group (n= 10), there was an increase in Foxp3+Treg cells. b Foxp3 mRNA levels rose in both the pneumococcus-positive (n = 8) and pneumococcus- negative (n = 8) groups. Less Th17 cells and ROR-t mRNA were produced by the Pneumococcuspositive group after CCS stimulation. After CCS stimulation, we examined the Th17 cell and ROR-t mRNA production in CD4+ adenoidal mononuclear cells taken from the pneumococcus-positive and -negative groups. The increases in Th17 cells and ROR-t mRNA in the pneumococcus-positive group after CCS stimulation were significantly smaller than those in the pneumococcus-negative group, according to our findings (0.3 0.07 vs. 0.85 0.14 percent and 0.03 0.02 vs. 0.44 0.16 percent, respectively; see fig. 5). These findings showed that, in comparison to the pneumococcus-negative group, the pneumococcus-positive group did not produce as many Th17 cells or ROR-t mRNA in response to CCS stimulation.

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Before and after CCS Stimulation, the IL-17A, IL-6, and TGF-β Levels in Adenoids from the Pneumococcus-Negative and -Positive Groups.

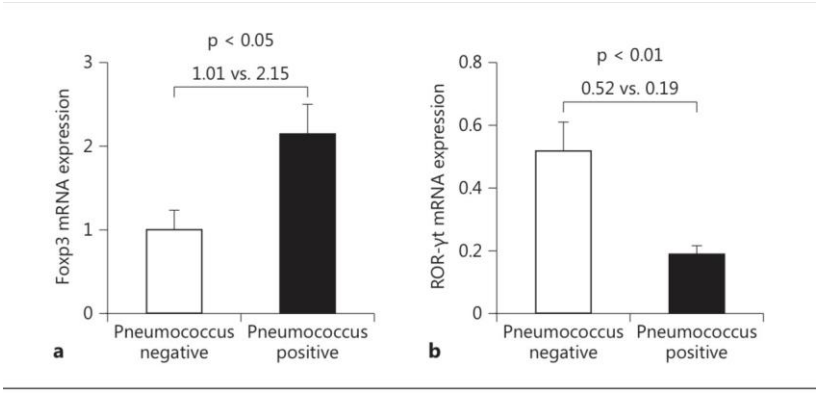

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Figure 5- In the pneumococcus-positive group, CCS stimulation resulted in the production of fewer Th17 cells and less ROR-t mRNA. a In both the pneumococcus-positive group (n = 10) and the pneumococcus-negative group (n = 10), there was an increase in Th17 Treg cells. b ROR-t mRNA levels increased in both the pneumococcus-positive (n = 8) and pneumococcus-negative (n = 8) groups

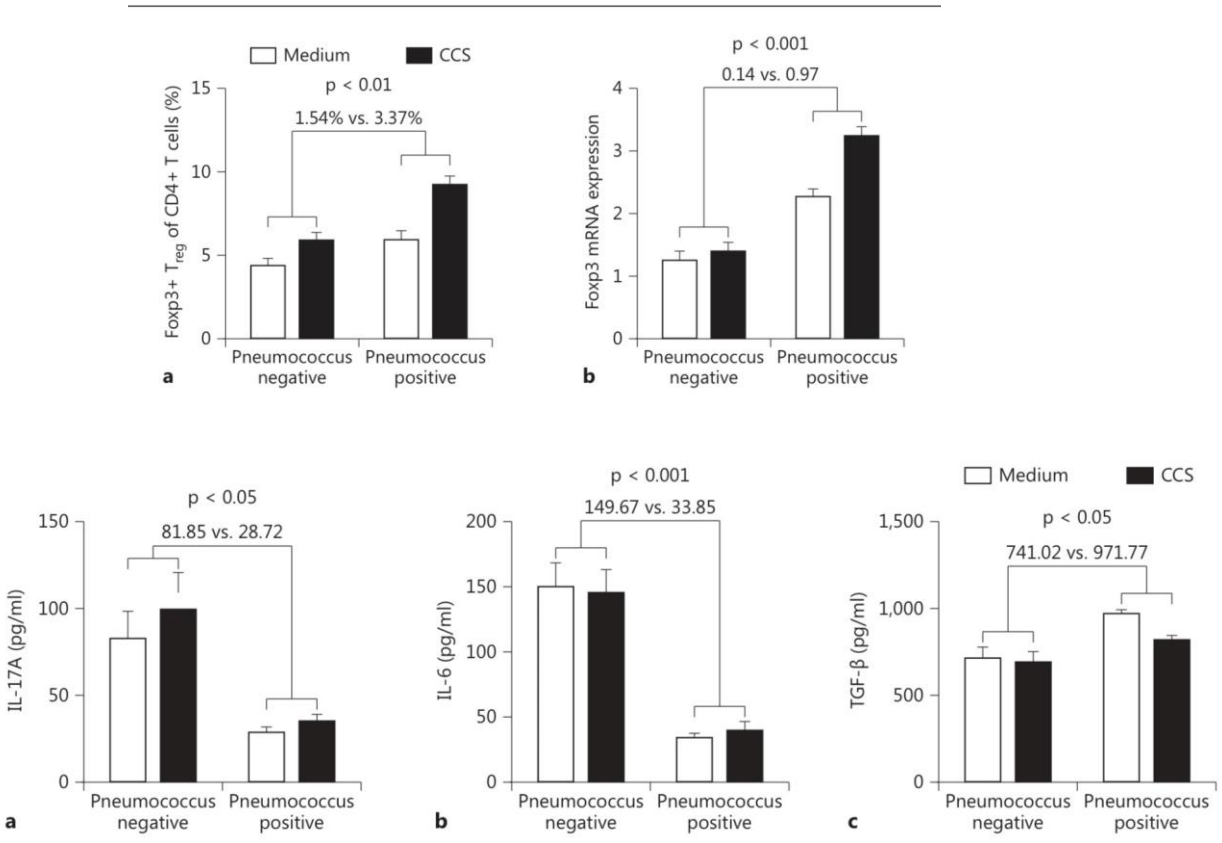

Figure 6- a IL-17A, b IL-6, and c TGF-β levels were stimulated by CCS in the pneumococcus-negative and pneumococcus-positive groups, respectively. Pneumococcus-negative groups in the control groups had

higher levels of IL-17A and IL-6 than pneumococcus-positive groups did, while pneumococcus-negative groups had lower levels of TGF- than pneumococcus-positive groups did. The levels of IL-17A, IL-6, and TGF-β varied similarly in the pneumococcus-positive and pneumococcus-negative groups after CCS stimulation (pneumococcus-negative group: n = 10; pneumococcus-positive group: n = 10).

DISCUSSION

Nearly 1 million people die worldwide each year from bacterial pneumonia, meningitis, and septicemia, all of which are caused by the pneumococcus S. pneumoniae. Although pneumococcus colonisation of the nasopharynx in children is prevalent, pneumococcus removal and shortening treatments are effective in preventing pneumococcus-related illnesses [3-5]. The causes of persistent pneumococcal carriage in some kids, however, remain unknown.

Here, we discuss a number of discoveries regarding the critical functions of adenoid Foxp3+ Treg cells and Th17 cells in pneumococcus carriage. First, we discovered that the patients in our study had a pneumococcal carriage rate of 32%, children aged 3-5 years had a carriage rate of 35.5%, and children aged 5-8 years had a carriage rate of 25%, all of which are compatible with epidemiological data [1]. The expression of Foxp3 mRNA and ROR-t mRNA further corroborated the elevation of Foxp3+ Treg cells and downregulation of Th17 cells in children who had pneumococcus (fig. 1, 2). (fig. 3) Third, after CCS stimulation, Foxp3+ Treg cell numbers increased considerably more in the pneumococcus-positive group than in the pneumococcus-negative group, while Th17 cell numbers increased less in the positive group compared to the negative group (fig. 4). These alterations were in line with the variations in Foxp3 and ROR-t mRNA levels (fig. 5). Finally, we showed that levels of IL-17A were higher in the pneumococcal-negative group, possibly reflecting the production of Th17 cells, and levels of IL-6 were higher in the pneumococcal- negative group, possibly reflecting the high proportion of Th17 cells among CD4+ adenoidal mononuclear cells.Pneumococcus-negative individuals had reduced TGF- levels, which, according to Ishigame et al. [12], may have contributed to the significant production of Th17 cells from CD4+ adenoidal mononuclear cells (fig. 6).

The thymus produces Foxp3+ Treg cells, and peripheral locations can also induce them from naive CD4+ T cells. It has been proposed that Foxp3+ Treg cells are significant immunoregulatory cells that can inhibit the amplifi- cation and activation of effector CD4+ T cells [14, 15]. In vivo, Foxp3+ Treg cells can be stimulated and multiplied in response to a variety of different infections. These pathogen-specific Foxp3+ Treg cells may reduce infection-induced immunopathology, but they may also increase pathogen persistence by suppressing protective immunity, which would promote chronic infections. By secreting certain cytokines (such as IL-17A, IL-17F, and IL-22) to stimulate neutrophil recruitment and the generation of antimicrobial peptides, Th17 cells play a crucial role in mucosal host defence.In animal models, IL-17A produced by Th17 cells was shown to play a protective effect for the host during infections and long-term S. pneumoniae carriage [17]. In IL-17 knockout mice, delayed clearance and persistent pneumococcal carriage were originally discovered [18, 19]. A recent study [20] showed that Th17 cells protect against pneumococcal carriage. In this study, we showed that Th17 cell numbers in pneumococcuspositive patients were significantly lower than those in the pneumococcus-negative group, while Foxp3+ Treg cell numbers in adenoidal tissues from pneumococcus-positive patients were significantly higher than those in adenoidal tissues from pneumococcus-negative patients.We also showed that Foxp3+ Treg and Th17 cell production may be induced in vitro by CCS stimulation.

Pneumococcus-negative patients showed a larger rise in Th17 cells, whereas the increase in Foxp3+ Treg cells in pneumococcus-positive patients was considerably bigger than that in pneumococcus-negative patients. These findings imply that excessive Foxp3+ Treg cell production in the adenoid may be linked to nasopharyngeal pneumococcal colonisation. The Th17 cell-inhibiting properties of these extra Foxp3+ Treg cells may delay the clearance of pneumococci or contribute to the persistence of pneumococcal carriage in children.This approach would be in line with the theory that excessively high Foxp3+ Treg cell production in the adenoid could be linked to pneumococcal carriage in the nasopharynx. The local cytokine milieu regulates the development of Foxp3+ Treg and Th17 cells from naive CD4+ T cells and is highly dependent on TGF- and IL-6. TGF- promotes the generation of immunosuppressive Treg cells in the absence of IL-6 [21, 22]. Immune homeostasis depends on the reciprocal balance between Foxp3+ Treg and Th17 cells; if improperly regulated, Th17 cells can promote pathogenic inflammation [23].In contrast, high concentrations of TGF- promote the differentiation of naive CD4+ T cells into Foxp3+ Treg cells by promoting the expression of Foxp3 and suppressing the expression of

ROR-t, while high concentrations of IL-6 promote the differentiation of naive CD4+ T cells into Th17 cells [12, 24]. It has been shown that pneumococci can cause airway epithelial cells to produce TGF-1. The development of Treg cells in the nasopharynx is then triggered by this TGF-ß1. By enhancing neutrophil flow into the nasopharynx and blocking TGF-1 signalling, pneumococci can be cleared [25]. In this study, we demonstrated that levels of IL-6 and IL-17 were considerably higher in a group of pneumococcus-negative patients than in a pneumococcus-positive patients.Furthermore, TGF- levels were lower in pneumococcus-negative individuals than in pneumococcus-positive patients. These findings imply that elevated TGF- levels in adenoidal tissue may promote the development of Foxp3+ Treg cells, which inhibit effector CD4+ Th17 cells.

In conclusion, we suggest that the presence of Foxp3+ Treg and Th17 cells in the adenoid and the release of cytokines like TGF- and IL-6 are strongly correlated with pneumococcal carriage in children. TGF- is a crucial cytokine in the development of Treg cells in the nasopharynx. However, an overabundance of Foxp3+ Treg cells may inhibit effector CD4+ Th17 cells, resulting in pneumococcal carriage over an extended period of time.

CONCLUSION

We have demonstrated that increasing the production of Foxp3+ Treg cells may suppress Th17 cells, resulting in reduced S. pneumoniae scavenging and consequent chronic pneumococcal carriage. A drawback of our study is that it only included a small number of patients, and we did not categorise the different strains of S. pneumoniae isolated from nasopharyngeal swabs. There will surely be additional possibilities to use our understanding of adaptive immunity to manage and ultimately prevent streptococcal pneumonia as our knowledge of the intricate interactions among Foxp3+ Treg cells, Th17 cells, and adenoid tissue grows.

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