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High density lipoprotein inhibited group II innate lymphoid cells proliferation and function in allergic rhinitis

Shengli Gao[†], Qingxiang Zeng[†], Yinhui Zeng[†], Yiquan Tang and Wenlong Liu^{*}

Abstract

Background: More and more studies had suggested that dyslipidemia was closely related to allergic diseases. High density lipoprotein (HDL) often plays anti-inflammatory and anti-oxidative roles by suppressing immune cell chemotaxis and activation. We aimed to explore the role of HDL in the regulation of group II innate lymphoid cells (ILC2) in allergic rhinitis (AR).

Methods: The blood lipid levels and their correlation with symptom scores of 20 AR subjects and 20 controls were analyzed. Purified ILC2 were stimulated by HDL and cytokines production were examined by enzyme-linked immunosorbent assay (ELISA) and flow cytometry. The mRNA levels of GATA binding protein 3 (GATA3) and retinoid-related orphan receptor α (ROR α) expressed by ILC2 were detected using reverse transcription polymerase chain reaction (RT-PCR).

Results: HDL level was significantly lower in AR than controls and correlated with the symptom scores. The serum HDL levels were negatively related to the increased number of ILC2, IL-5⁺ ILC2, and IL-13⁺ ILC2 in AR patients. HDL decreased the number of ILC2 and type II cytokines levels significantly by inhibiting expression of GATA3 and ROR α .

Conclusions: Our data provide preliminary evidence that HDL may play a negative role in ILC2 inflammation in AR, suggesting that HDL may serve as promising treatment target in AR.

Keywords: HDL, Allergic rhinitis, ILC2

Introduction

Allergic rhinitis (AR), featured with allergic inflammation of the upper airway, impairs the quality of life of more than 10% world population [1]. Traditionally, AR is considered as eosinophilic and augmented type 2 immune inflammation [2].

Accumulating evidence confirmed the correlation between dyslipidemia and allergic diseases, such as AR, [3–5] asthma, [4, 6] and atopic dermatitis (AD). Dyslipidemia regulates both innate and adaptive immune reaction by releasing pro-inflammatory cytokines, which contribute to the activation and polarization of T-helper 2 (Th-2) and Th17 as well as inhibiting interleukin-10 (IL-10) cytokine production [7].

HDL is composed of several apolipoproteins, such as apoA-I, apoA-II, apoCs, apoE, serum amyloid A (SAA). HDL often plays anti-inflammatory [8] and antioxidant roles by suppressing immune cell chemotaxis and activation [9, 10]. Trakaki's study found that AR significantly altered the composition and function

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of HDL, suggesting a new correlation between HDL metabolism and allergy [11].

Group II innate lymphoid cells (ILC2) are a group of cells lacking markers of T cell, B cell, natural killer cell, et al. ILC2 can initiate allergic inflammation by producing Th2 cytokines after stimulation of IL-33, IL-25 or thymic stromal lymphopoietin (TSLP). Several studies had shown that the frequency of ILC2 elevated significantly in AR patients. However, the interaction between HDL and ILC2 was not understood.

In this study, we aimed to assess (1) the correlation between HDL and the proliferation and function of ILC2, (2) the regulation of HDL on ILC2 by cell culture.

Methods

Patients

A total of 40 subjects (>18 years old) included 20 AR and 20 controls were enrolled in our study. The inclusion criteria for AR patients included: (1) confirmed diagnosis of AR as described by the Allergic Rhinitis and its Impact on Asthma (ARIA) guideline, (2) at least 1 year history, (3) typical symptoms such as sneezing, blocked nose, itchy nose and runny nose, (4) allergic to at least one inhalant allergen (dust mites, pets, molds, cockroach, etc.) confirmed by specific IgE measurement (Phadia ImmunoCAP, Sweden). The exclusion criteria included: (1) the presence of other allergic diseases, such as asthma, dermatitis, (2) acute or chronic rhinosinusitis, (3) pregnancy or breastfeeding, (4) previous treatment with immunotherapy, (5) with immunologic disease, tumors, or chronic infection, (6) use of systemic corticosteroids or anti-histamine drugs in the past 2 weeks, and (7) with diabetes, hypertension, myxedema, hypothyroidism, obesity, liver and kidney diseases and other diseases which may affect blood lipid levels. Normal subjects had no history of allergy and positive allergen test. The exclusion criteria for controls were similar to that of AR. Our study obtained approval and written informed consent from local ethics committee boards.

Total nasal symptom scores

The nasal symptoms severity questionnaire was completed. The nasal symptoms including sneezing, runny nose, itchy nose, and blocked nose were rated between 0 and 3 scale (0, none; 1, mild; 2, moderate; and 3, severe).

Blood lipid measurement

Venous fasting blood were obtained for assaying serum total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein, and triglyceride (TG) levels.

The blood lipids were determined using a Cobas Integra 400 (Roche, Switzerland).

Effects of HDL on ILCs

Peripheral blood mononuclear cells (PBMCs) were purified using density-gradient centrifugation from of AR and controls. PBMCs (1.0×10^6 cells/mL) were incubated in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum and treated by human HDL-Dil (Biotrend, FL) for 24 h at 37 °C.

For determination of ILC2s, lineage markers-FITC, FcεRI-APC, CD45-APC/Cy7, CRTH2-PE, CD127-PE-Cy7 antibody (BD Bioscience, NJ) were used for staining. Lin⁻ FcεRI CD45⁺ CRTH2⁺CD127⁺ cells were defined as ILC2s. The proliferation of ILC2 was determined using tritiated thymidine incorporation.

Real-time PCR

Total RNA was extracted from stimulated PBMCs by TRIzol (Invitrogen, US). RNA was reverse-transcribed for cDNA synthesis. PCR reaction was performed using real-time PCR detection system (BioRad). The relative levels of target genes was normalized to GAPDH housekeeping gene using 2-ΔΔCt method. The primers were listed as follows: GATA3 sense, 5'-GCGGGCTCT ATCACAAAATGA-3', antisense, 5'-GCTCTCCTG GCTGCAGACAGC-3'; RORα sense, 5-AAGGAGCCA GAAGGATGAAC-3', antisense, 5'-GGAACA ACA GACGCCAGTAAG-3'; GAPDH sense, 5'- AGCCAC ATCGCTCAGACAC-3', antisense, 5'- GCCCAATAC GACCAAATCC -3'.

Enzyme-linked immunosorbent assay (ELISA)

The concentration of IL-5, IL-13 were examined using ELISA kits (R&D systems, USA). The sensitivity of cytokines was: IL-5, 3.9 pg/mL, IL-13, 125 pg/mL.

Statistical analysis

Prism (GraphPad Software 8.0) were used for analysis with *P* values lower than 0.05 as statistically significant in all analyses. The Kruskal–Wallis H test or nonparametric Mann–Whitney U test was done. Spearman rank was done for correlation analysis.

Results

Blood lipids levels

The demographic information of subjects is summarized in Table 1. The levels of LDL, TC, TG between control and AR group had no significant difference, whereas the HDL level in AR group was significantly lower compared with control group.

Table 1 Demographic characteristic of AR patients and normal controls

Groups	AR	Control
Number	20	20
Age (years)	24.6 ± 8.8	27.1 ± 9.2
BMI	21.3 ± 3.5	21.9 ± 3.8
Sex (Male:Female)	12:8	11:9
TC (mg/dl)	151.09 ± 36.78	128.45 ± 27.33
LDL (mg/dl)	67.17 ± 19.65	75.31 ± 18.11
HDL (mg/dl)	31.18 ± 6.52*	68.75 ± 16.29
TG (mg/dl)	78.24 ± 19.15	83.26 ± 11.45

Compared with control group, $P < 0.05$

AR allergic rhinitis, TC total cholesterol, LDL low-density lipoprotein cholesterol, HDL high-density lipoprotein, TG triglyceride

Moreover, the HDL level was significantly correlated with the symptom scores of AR.

Correlation between HDL and ILC2

The number of ILC2, IL-5⁺ ILC2, and IL-13⁺ ILC2 cells were significantly higher in AR than controls (Fig. 1). The serum HDL concentration was negatively correlated with the number of ILC2, IL-5⁺ ILC2, IL-13⁺ ILC2 (Fig. 1). However, serum TC, TG and LDL

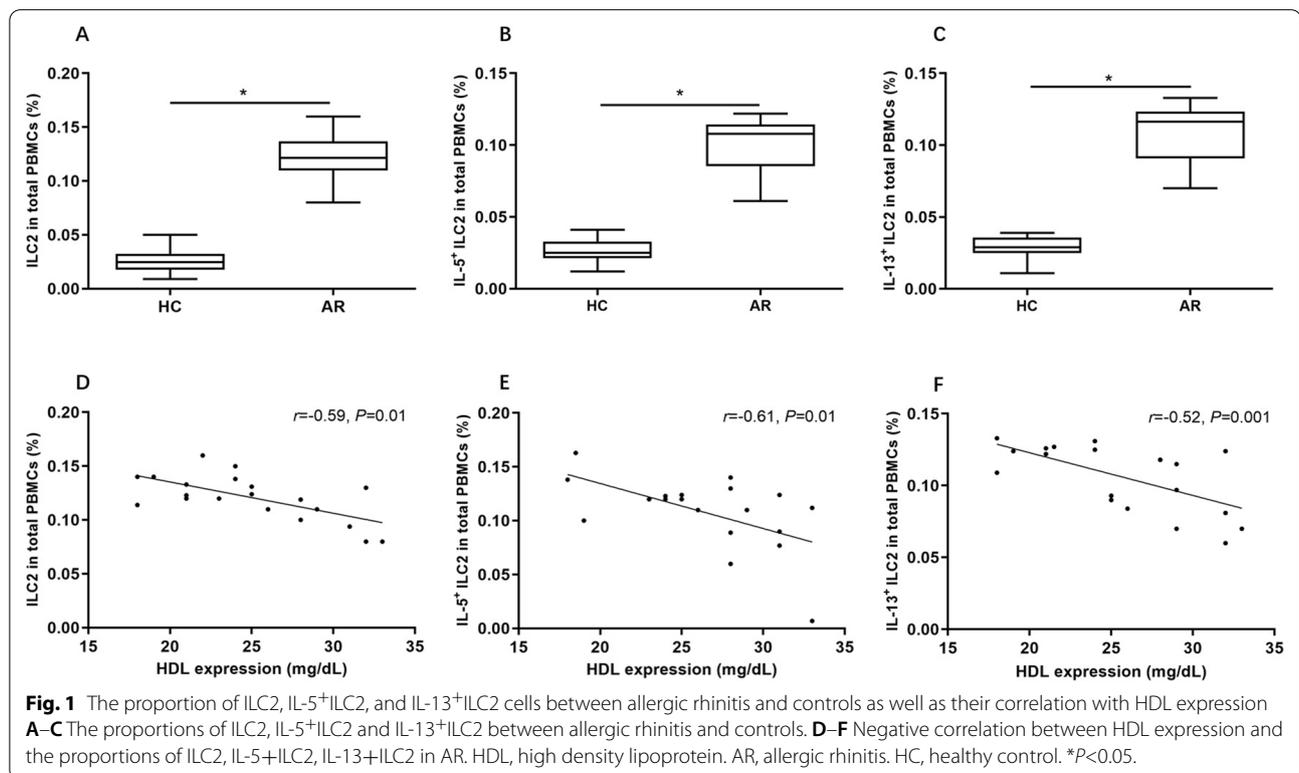
were not correlated with the number of ILC2, IL-5⁺ ILC2, and IL-13⁺ ILC2 cells.

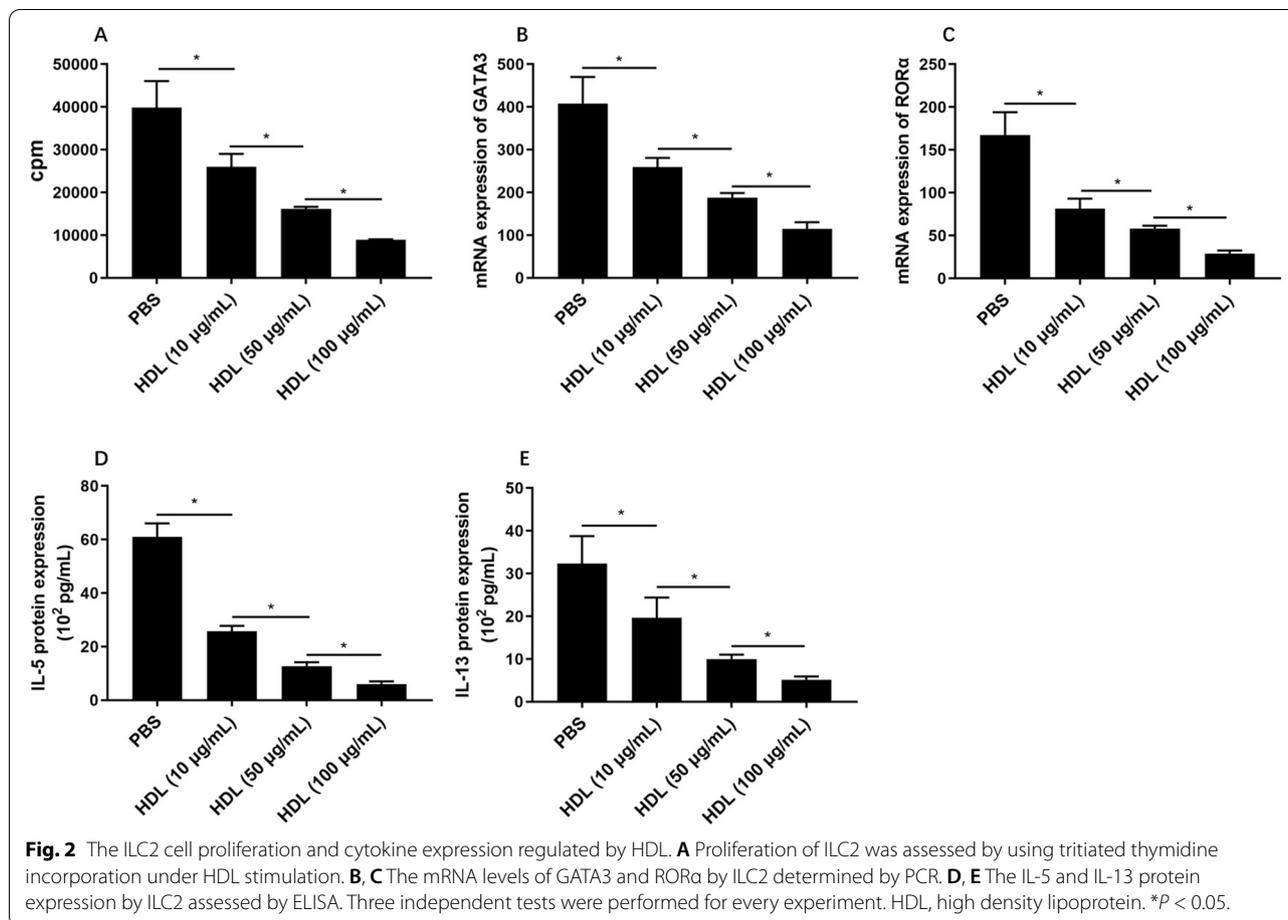
ILC2s inhibited by HDL

HDL decreased the number of ILC2 significantly when they were cocultured together accompanied by down-regulating mRNA level of GATA3 and ROR α by ILC2 (Fig. 2). The protein levels of IL-5 and IL-13 were inhibited after HDL treatment (Fig. 2).

Discussion

Over last years, the prevalence of obesity, allergic sensitization and atopic diseases increased significantly [12]. Accumulating evidence suggested that metabolism is correlated with chronic airway inflammation [13]. More and more studies have concentrated on the correlation of between allergic diseases and various lipids. For example, HDL-C has an anti-inflammatory effect by regulating T-cell activation [14, 15]. A meta-analysis including ten studies showed elevated LDL level and decreased HDL levels in asthma subjects compared with controls [6]. Vinding et al.'s [4] study found that LDL and TG levels were correlated with the occurrence of asthma, airway obstruction and aeroallergen sensitization. Recently, hypercholesterolemia has been associated with Th2-oriented inflammation in allergic





diseases, such as asthma and related disorders [16–18]. However, the role of dyslipidemia in the regulation of ILC2 mediated inflammation was not reported in the previous studies.

Consistent with previous study, our study also confirmed that.

Down-regulated HDL level in AR children was correlated with disease severity [19, 20]. Moreover, HDL was related to the proliferation and function of ILC2, suggesting that HDL is engaged in the regulation of ILC2.

Next, we purified and cultured ILC2 to investigate the role of HDL. We found that HDL inhibited the proliferation and type II cytokine production by ILC2, which was regulated by GATA3 and ROR α pathway. These data confirmed that HDL regulated ILC2 directly. Similarly, Li's study demonstrated that coculture of ox-LDL and peripheral blood mononuclear cells (PBMC) contributed to increased number of ILC1s and decreased number of ILC2s in a dose-dependent manner [21]. Moreover, their studies showed that ILC1s and ILC2s were more susceptible to ox-LDL-mediated alterations in

ACI patients compared with controls [21]. These results suggested that lipids may play different roles in different backgrounds.

Our study had following limitations. First, the sample size was limited. Therefore, large sample and multi-center study was needed in future. Secondly, we did not introduce mice model in this study to prove our in vitro results. Thirdly, the effect of HDL on ILC2 in obese AR patients was needed in future study. Fourth, ILC2 are believed to have a role in homeostasis of adipose tissue. Whether the response of ILC2 to HDL is just a reflection of this property and how HDL exert its effect on ILC2 at different site of body needs animal studies to explore, especially HDL gene related Knockout mice.

In summary, our study provided preliminary evidence for the first time that HDL inhibited the proliferation and function of ILC2 from peripheral blood in AR by in vitro studies, further in vivo studies were needed to explore the effect of HDL on ILC2 as well as the causal relationship between allergic inflammation and metabolic disorders.

Abbreviations

HDL: High density lipoprotein; ILC2: Group II innate lymphoid cells; AR: Allergic rhinitis; ELISA: Enzyme-linked immunosorbent assay; GATA3: GATA binding protein 3; ROR α : Retinoid-related orphan receptor α ; RT-PCR: Reverse transcription polymerase chain reaction; AD: Atopic dermatitis; Th-2: T-helper 2; IL-10: Interleukin-10; SAA: Serum amyloid A; TSLP: Thymic stromal lymphopoietin; TC: Total cholesterol; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; TG: Triglyceride; PBMCs: Peripheral blood mononuclear cells.

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Author contributions

Conception and design of the research: SG, WL; acquisition of data: QZ, YZ; analysis and interpretation of data: SG, QZ; statistical analysis: YZ, YT; obtaining funding: WL; drafting the manuscript: SG, WL; revision of manuscript for important intellectual content: QZ, YZ and YT. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

The authors have read and agreed to the contents of the manuscript and agreed to publish it.

Competing interests

The authors declare that they have no competing interests.

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