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Reduced regulatory B cell subsets in mild to moderate hypertriglyceridemia: a preliminary study

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Abstract

Introduction: *This study evaluated the percentages of peripheral regulatory B cell subsets ($CD19^+CD24^+CD38^+$, $CD19^+CD24^+CD27^+$ and $CD19^+IL\text{-}10^+$) in patients with hypertriglyceridemia (HTG). In addition, interleukin (IL)-6, IL-10, and IL-1Ra levels were assessed. Finally, the impact of the saturated fatty acid palmitate on IL-10 production by B cells from HTG patients was examined.*

Material and methods: *The percentages of B cells were determined by flow cytometry. The levels of IL-6, IL-1Ra, and IL-10 were measured using ELISA assays.*

Results: *The percentages of $CD19^+CD24^+CD38^+$ and $CD19^+CD24^+CD27^+$ cells were significantly lower in patients with moderate HTG compared to individuals with a normal lipid (NL) profile. The percentage of $CD19^+IL\text{-}10^+$ cells was lower in both HTG and moderate HTG patients compared to the NL group. The percentage of $CD19^+CD24^+CD27^+$ cells was inversely associated with triglyceride (TG) levels and total cholesterol (TC). IL-10 production by B cells in the presence of palmitate was reduced in patients with moderate HTG compared to the NL group. Production of IL-6 by PBMC from HTG patients increased, while IL-10 production decreased; however, these changes did not reach statistical significance. HTG patients showed elevated serum levels of IL-1Ra compared to the NL group, and IL-1Ra levels were positively correlated with TG levels.*

Conclusions: *Patients with moderate HTG exhibit alterations in regulatory B cell phenotypes. A reduction in the percentage of $CD19^+CD24^+CD27^+$ regulatory B cells is associated with elevated TG levels.*

Key words: *hypertriglyceridemia, regulatory B cells, IL-10, palmitate, lipid metabolism.*

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Introduction

Hypertriglyceridemia (HTG) is a metabolic disorder characterized by elevated plasma triglyceride (TG) levels. It is commonly associated with visceral obesity and is a risk factor for cardiovascular diseases, triglyceride-induced pancreatitis, coronary artery disease, and cerebrovascular accidents [1-3]. Postprandial HTG is characterized by a prolonged elevation of postprandial triglyceride-rich lipoproteins (TRLs) [4] and inflammatory molecules such as intercellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM), and interleukin (IL)-8 [5], as well as increased leukocyte counts [6]. In individuals with abdominal obesity, postprandial HTG has been linked to increased IL-1β levels following high-fat meal consumption [7]. Hyperlipidemic mice exhibit elevated T helper type 1 (Th1) and Th17 responses [8, 9]. Moreover, individuals with elevated TG levels display higher concentrations of IL-6, tumor necrosis factor α (TNF-α), and monocyte chemoattractant protein-1 (MCP-1) compared to those

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with normal lipid profiles [10]. In the context of obesity, levels of IL-1Ra increase and contribute to development of insulin resistance (IR) [11, 12]. Furthermore, reduced levels of IL-10 have been associated with an increased body mass index (BMI) [13]. Free fatty acids (FFAs; the precursors of TG) such as palmitate are abundant in the circulation and have been implicated in inflammatory processes [14]. FFAs induce activation of the NOD-like receptor pyrin domain containing 3 (NLRP3) inflammasome [15]. In lipopolysaccharide-stimulated-macrophages, palmitate promotes IL-1β secretion and increases TNF-α production [16, 17]. In mononuclear cell cultures, palmitate induces IL-10 production through a pentraxin-3-dependent pathway [18]. Collectively, these findings suggest that dyslipidemia and lipid abnormalities are closely associated with a state of subclinical inflammation.

Regulatory B cells (Bregs), a subpopulation of B cells, suppress inflammatory responses mainly through IL-10 secretion. B10 cells predominantly exhibit the $CD19^{+}$-$CD24^{High}CD27^{+}$ phenotype [19, 20], and suppress TNF-α release from monocytes [21]. Another phenotype of Bregs, $CD19^{+}CD24^{High}CD38^{High}$ B cells [22], suppresses Th1 and Th17 responses [23] and can also induce the conversion of $CD4^{+}$ T cells into $Foxp3^{+}$ regulatory T cells [22, 24]. In diseases associated with high levels of cholesterol and TG in blood, it has been shown that B lymphocytes (B1a subtype) attenuate atherosclerotic lesions by secreting IgM, which is deposited in the lesions and reduces necrotic cores [25]. Inflammation plays an important role in atherosclerosis. Previous reports have identified a subtype of Bregs in mice with established atherosclerosis; these cells exhibit vascular-protective activity dependent on IL-10 [26]. In that study, it was proposed that the atherosclerotic microenvironment (increased expression of CD40L, IL-1β, IL-6) supports the differentiation of B cells into IL-10 producing Bregs in the lymph nodes of $ApoE^{-/-}$ mice [26]. In addition, higher proportions of B lymphocytes were associated with dyslipidemia, including increased TG, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C) levels, as well as decreased high-density lipoprotein cholesterol (HDL-C) levels, in patients with acute ischemic stroke [27]. Together, these results indicate a relationship between B-cell subtypes and lipid metabolism disorders.

In this study, we evaluated different Breg phenotypes ($CD19^{+}IL\text{-}10^{+}$, $CD19^{+}CD24^{+}CD27^{+}$, $CD19^{+}CD24^{+}CD38^{+}$) in patients with HTG. Additionally, we assessed IL-6 and IL-10 production by mononuclear cells from individuals with HTG, as well as serum levels of IL-1Ra as a surrogate marker of inflammation. We also analyzed the effect of palmitate on IL-10 production by B cells from individuals with a normal lipid (NL) profile and those with HTG. Finally, we assessed correlations between Breg percentages and various biochemical and anthropometric parameters.

Material and methods

Study population

Forty asymptomatic volunteers from the General Hospital Zona 1, Zacatecas, México, were selected as the study population. All participants were non-smokers and had no prior diagnosis of chronic diseases. They did not consume alcohol on a regular basis. Individuals under regular pharmacological treatment for metabolic disorders and/or hormonal treatment, those undergoing chemotherapy, as well as lactating women, were excluded. The biochemical and anthropometric characteristics of the participants are shown in Table 1. For the biochemical analysis, after a 12-hour overnight fast, early-morning blood samples were collected by peripheral venipuncture from each participant for biochemical screening tests. Levels of TC, HDL-C, LDL-C, and TG were measured using an autoanalyzer. Participants were classified into two groups – normal lipid (NL) and hypertriglyceridemia (HTG) – accord-

Table 1. Characteristics of study groups

Parameter	Normal lipid	HTG	Hypertriglyceridemia (HTG)	
			Mild	Moderate
n	22	18	9	9
Sex (M/F)	8/14	8/10	5/4	3/6
Age	39.05 ±3.010	42.72 ±2.699	42.11 ±3.557	43.33 ±4.269
BMI	27.15 ±1.438	30.38 ±1.014	29.27 ±1.306	31.48 ±1.534*
TG	103.0 ±5.487	205.8 ±8.980*	174.6 ±5.220*	237.1 ±8.466*
TC	160.4 ±5.726	201.0 ±5.168*	190.6 ±6.667*	211.4 ±6.475*
HDL-C	53.01 ±2.157	48.79 ±1.103	48.67 ±1.615	48.91 ±1.599
LDL-C	92.93 ±4.566	104.6 ±4.687	107.1 ±6.103	108.2 ±7.361

*Data are presented as mean ±SEM, *significant differences, $p < 0.05$ compared to the normal lipid group. Age (years), M – male, F – female, BMI – body mass index (kg/m^2), TG – triglycerides (mg/dl), TC – total cholesterol (mg/dl), HDL-C – high density lipoprotein cholesterol (mg/dl), LDL-C – low density lipoprotein cholesterol (mg/dl)*

ing to their lipid levels. The US guidelines of the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) were used to define hypertriglyceridemia. Furthermore, the HTG group was stratified by severity (mild and moderate) according to the NCEP-ATP III criteria. This research was approved by the Bioethics Committee of the National Scientific Research Commission of the Instituto Mexicano del Seguro Social, and the protocol was registered with the number: R-2014-785-080. All participants provided written informed consent, which complies with the requirements of the 1964 Declaration of Helsinki.

Peripheral blood mononuclear cell isolation

Peripheral blood mononuclear cells (PBMC) were isolated from 10 ml of fresh blood samples obtained by peripheral venipuncture. Blood samples were diluted in phosphate-buffered saline (PBS) solution and placed over a Ficoll-Histopaque gradient (Sigma-Aldrich, MA, USA). The PBMC layer was extracted and washed with PBS. The viability of the PBMC was evaluated using the trypan blue exclusion assay. A viability above 99% was considered acceptable.

Flow cytometry analysis

To evaluate the percentages of B cells ($CD19^+$) expressing CD24, CD27 and/or CD38 antigens, PBMC were incubated for 30 minutes at 4°C in the dark with anti-human CD19 conjugated with PECy7 (Miltenyi Biotec, Sunnyvale, CA, USA), anti-CD24 conjugated with Pacific Blue (Miltenyi Biotec), anti-human CD27 conjugated with allophycocyanin (APC) (Miltenyi Biotec), and anti-human CD38 conjugated with fluorescein isothiocyanate (FITC) (Miltenyi Biotec). Afterwards, cells were washed with PBS and fixed with 1% paraformaldehyde. Samples were acquired on a flow cytometer (FACS Canto II, BD Biosciences) and analyzed using the DIVA software. For detection of intracellular antigen, a specific IL-10 monoclonal antibody (eBioscience, San Diego, CA, USA) was used. A double labelling procedure was performed – first with anti-CD19-PECy7 monoclonal antibody (Miltenyi Biotec), followed by permeabilization/fixation using buffers supplied by eBioscience. Finally, cells were stained with anti-IL-10 conjugated with phycoerythrin (PE) (Miltenyi Biotec) and analyzed by flow cytometry. Results are expressed as the percentage of positive cells.

Preparation of palmitate solution

Palmitate (Sigma-Aldrich, Cat. No. P9767) was dissolved in methanol at a final concentration of 10 mM and then conjugated to fatty acid-free, globulin-free bovine serum albumin (BSA, Sigma-Aldrich, Cat. No. A8806). This particular BSA is tested by the manufacturer for low endotoxin content and is suitable for applications requiring minimal endotoxin interference. All solutions were prepared under sterile conditions using pyrogen-free water and handled aseptically. The final palmitate-BSA complex was sterile-filtered through 0.22 µm filters and stored at 4°C until use. As a control, cells were treated with a BSA solution alone (vehicle control), prepared under the same sterile conditions.

Cell cultures

Peripheral blood mononuclear cells were suspended in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 µg/ml streptomycin (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA), at a final concentration of 1×10^6 cells/ml. To evaluate IL-6 and IL-10 production by PBMC from HTG patients and NL controls, the cells were stimulated with the polyclonal stimulus, phytohemagglutinin (PHA, 5 µg/ml). Cultures were maintained for 18 hours at 37°C in a humidified incubator with 5% CO_2. Afterwards, supernatants were collected and stored at –70°C until analysis. To assess the effect of the saturated fatty acid (palmitic acid) on IL-10 production by B cells, 1×10^6 cells/ml were treated with 0.3 mM palmitate-BSA conjugated or with BSA as the vehicle control and stimulated with αCD40 monoclonal antibody (5C3; αCD40, 4 µg/ml) (eBioscience, San Diego, CA, USA). Cultures were incubated for 18 hours at 37°C in 5% CO_2 and humidity. Supernatants were then harvested and stored at –70°C until analysis.

Interleukin measurements in serum and culture supernatants

Levels of IL-6 and IL-10 were determined in the supernatants of PBMC cultures, while IL-1Ra was measured in participants' serum. Determination of these cytokines was carried out using quantitative enzyme-linked assays (ELISA) (eBioscience, San Diego, CA), performed according to the manufacturer's instructions.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 5.0 software (San Diego, CA, USA). Data distribution was assessed using the Kolmogorov-Smirnov test. Differences between groups in percentages of Bregs, IL-10 and IL-6 levels in supernatants, and serum IL-1Ra levels were evaluated by the Kruskal-Wallis test with Dunn's post-hoc test for pairwise comparisons. To examine associations between cell percentages and biochemical parameters, Spearman's rank correlation analysis was used. Correlation matrix plots were generated using the corrplot package in R. A p-value < 0.05 was considered statistically significant in all analyses.

Results

Comparison of laboratory findings between individuals with hypertriglyceridemia and controls

Based on the biochemical results, all volunteers were classified into four groups according to their fasting lipid levels: the Normal Lipid (NL) group (n = 22), consisting of individuals with no lipid abnormalities; the Hypertriglyceridemia (HTG) group (n = 18), including individuals with TG levels ≥ 150 mg/dl (1.70 mmol/l) and no other lipid abnormalities. This HTG group was further subdivided: the Borderline High Hypertriglyceridemia (mild HTG) group (n = 9), comprising individuals with TG levels between 150 and 199 mg/dl (1.70-2.24 mmol/l); and the High Hypertriglyceridemia (moderate HTG) group (n = 9), consisting of individuals with TG levels between 200 and 499 mg/dl (2.26-5.64 mmol/l), with no other lipid abnormalities. The demographic and biochemical characteristics of these groups, including mean age and BMI, are summarized in Table 1. No significant differences were found in age, HDL-C, or LDL-C levels among the groups. However, TG and TC levels were significantly higher in all HTG groups compared to the NL group. Additionally, BMI was significantly elevated only in the moderate HTG group compared to the NL group (Table 1). Individuals exhibiting HTG together with other lipid abnormalities – such as TC > 240 mg/dl (6.20 mmol/l), HDL-C < 40 mg/dl (1.3 mmol/l), or LDL-C > 100 mg/dl (2.6 mmol/l) – were excluded from the analysis.

Percentages of circulating Bregs are reduced in individuals with moderate hypertriglyceridemia

To assess Breg levels in individuals with HTG, we performed flow cytometry analysis using a previously described gating strategy (Fig. 1A). We observed significantly higher $CD19^+CD24^+CD38^+$ B cells in individuals with mild HTG compared to the NL group (mean = 43.85 vs. mean = 22.74 respectively, Fig. 1B). Interestingly, in individuals with moderate HTG, the percentage of these cells was significantly reduced compared to the mild HTG group (mean = 16.43 vs. mean = 43.85 respectively, Fig. 1B). Similarly, $CD19^+CD24^+CD27^+$ B cells were significantly lower in the moderate HTG group compared to both NL (mean = 29.93 vs. mean = 43.73) and mild HTG (mean = 29.93 vs. mean = 52.67) groups (Fig. 1C). Moreover, the percentage of IL-10-producing B cells ($CD19^+IL\text{-}10^+$) was lower in both HTG (mean = 3.48 vs. mean = 11.67) and moderate HTG (mean = 4.75 vs. mean = 11.67) groups compared to NL individuals (Fig. 1D).

Impaired IL-10 production and Breg dysfunction in individuals with hypertriglyceridemia

Because the HTG group showed reduced percentages of IL-10-producing B cells ($CD19^+IL\text{-}10^+$), we explored the effect of palmitic acid (a known proinflammatory lipid) on IL-10 production by B cells from individuals with moderate HTG and NL. CD40 is well known to be crucial for B cell activation; thus, PBMC from both NL and moderate HTG individuals were cultured in the presence of BSA-conjugated palmitate and then stimulated with an anti-CD40 monoclonal antibody (αCD40) to induce IL-10 production by B cells. We observed lower levels of IL-10 after activation of B cells from HTG patients in the presence of palmitate, compared with the NL group (mean = 16.74 vs. mean = 68.30 respectively, Fig. 2A). Furthermore, to examine the effect of HTG on soluble inflammatory cytokine production, we measured IL-10 and IL-6 levels in PBMC culture supernatants from HTG patients stimulated with PHA. The levels of IL-10 and IL-6 were similar between groups (Fig. 2B, C). We also measured serum levels of IL-1Ra as a surrogate marker for IL-1β; circulating IL-1Ra levels are more commonly detectable in inflammatory diseases than IL-1β [28, 29]. We observed that individuals with HTG (both mild and moderate) had higher IL-1Ra levels than the NL group (mean = 726.8 pg/ml vs. mean = 367.1 pg/ml; Fig. 2D). IL-1Ra levels correlated with serum TG levels (Fig. 2E).

Correlation analysis between Bregs and hypertriglyceridemia profile

Finally, we performed correlation analyses between B cell phenotypes and biochemical parameters of the individuals included in this study. We observed an inverse correlation between $CD19^+CD24^+CD27^+$ B cells and TG and TC levels. Additionally, TG levels were positively associated with TC and LDL-C serum levels. Finally, TC levels correlated directly with LDL-C levels (Fig. 3).

Discussion

In this study, we observed changes in the proportions of regulatory B cell phenotypes ($CD19^+CD24^+CD38^+$, $CD19^+CD24^+CD27^+$, and $CD19^+IL\text{-}10^+$) in individuals with HTG. An increase in $CD19^+CD24^+CD38^+$ and $CD19^+CD24^+CD27^+$ B cell phenotypes was observed in mild HTG. In line with this, an increase in B cells has been observed in patients after acute ischemic stroke (a condition in which dyslipidemia is a major etiological factor [27]), and this increase correlated with lipid metabolic abnormalities, including elevated TG levels [27]. More-

over, ischemic stroke is associated with increased susceptibility to infection, suggesting a kind of immunosuppression that can involve alterations in adaptive immune cells [30, 31]. In this context, elevated frequencies of $CD19^+$-$CD24^+CD27^+$ Bregs have been associated with inhibition of $CD4^+$ T cell proliferation and IFN-γ production [32]. In addition, increased $CD19^+CD24^+CD27^+$ Bregs have been linked to immunosuppression and poorer survival in patients with gastric cancer [32].

On the other hand, in the moderate HTG group, all Breg phenotypes tested here ($CD19^+CD24^+CD38^+$, $CD19^+$-$CD24^+CD27^+$, and $CD19^+$IL-10^+) were decreased. Evidence on Bregs in humans with HTG is scarce, but for other dyslipidemias such as atherosclerosis, alterations in both the percentages and activation of B cells have been described. In this context, IL-10, a cytokine produced by Bregs, has anti-atherogenic properties *via* modulation of the immune response [33]. Consistent with our results, previous research showed decreased percentages of IL-10^+ B cells in patients with atherosclerosis, which were negatively correlated with serum C-reactive protein (CRP) levels [34]. Diminished percentages of $CD40^+$ B cells have

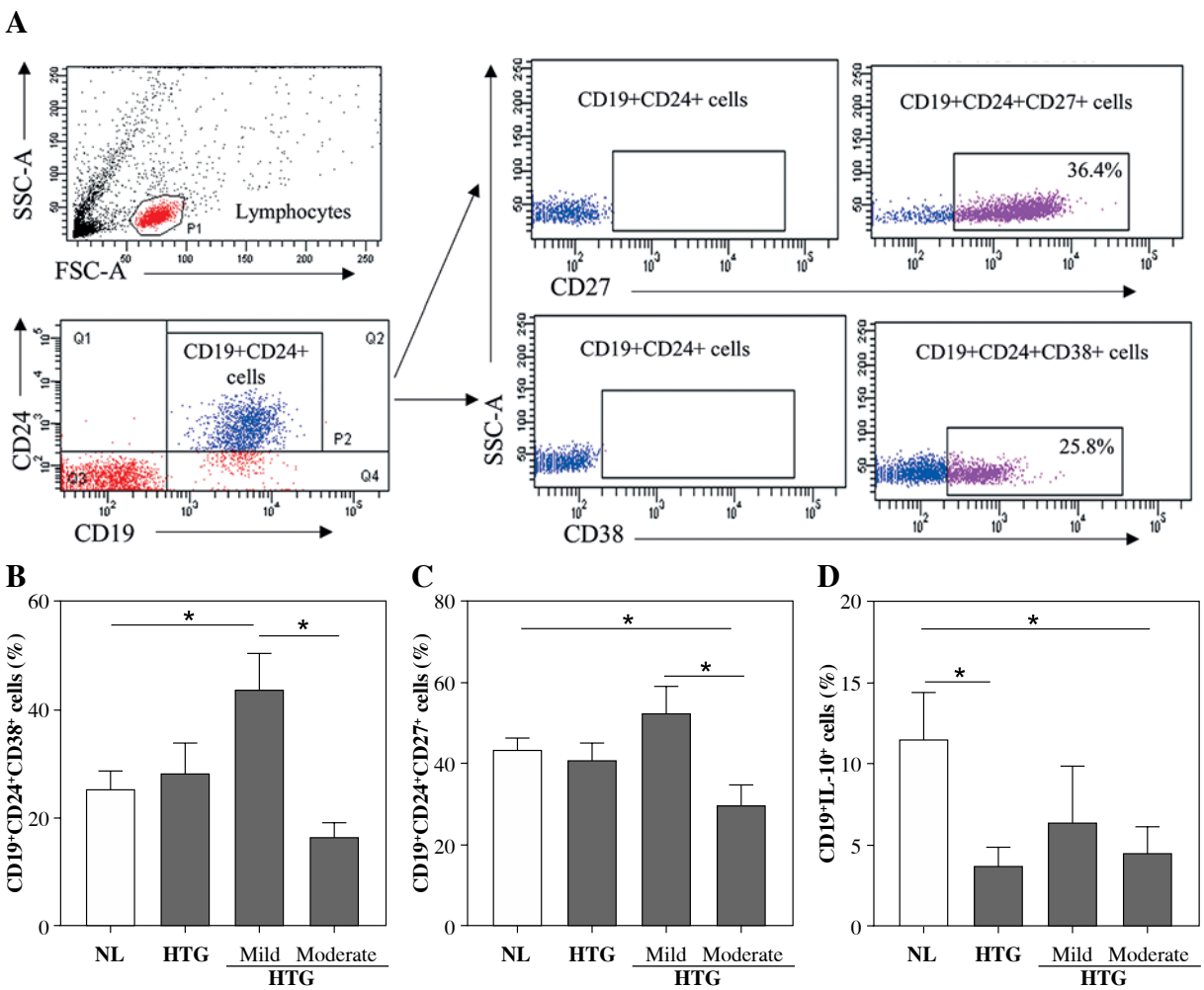

Fig. 1. Percentages of Bregs in individuals with hypertriglyceridemia. **A**) Representative dot plot showing the gating strategy used to determine the percentages of Bregs (the overall gating strategy is shown in Supplementary Figures 1 and 2). **B**) Percentages of $CD19^+CD24^+CD38^+$ and **C**) $CD19^+CD24^+CD27^+$ cells of peripheral blood mononuclear cells (PBMC) from individuals with normal lipid levels (NL; in white bars) and hypertriglyceridemia (HTG; mild and moderate, gray bars). **D**) Percentages of interleukin (IL)-10-producing $CD19^+$ cells ($CD19^+$IL-10^+) in PBMC from NL and HTG (mild and moderate) individuals treated with the polyclonal stimulus phytohemagglutinin (PHA) and cultured for 18 hours, as described in the Material and Methods section. The results show the percentage of positive cells. NL group $n = 22$, HTG group $n = 18$ (mild HTG, $n = 9$ and moderate HTG, $n = 9$) in all cases. Data are expressed as mean ± SEM. Statistical analysis was performed using the Kruskal-Wallis test. Statistically significant differences are indicated with asterisks (*$p < 0.05$)

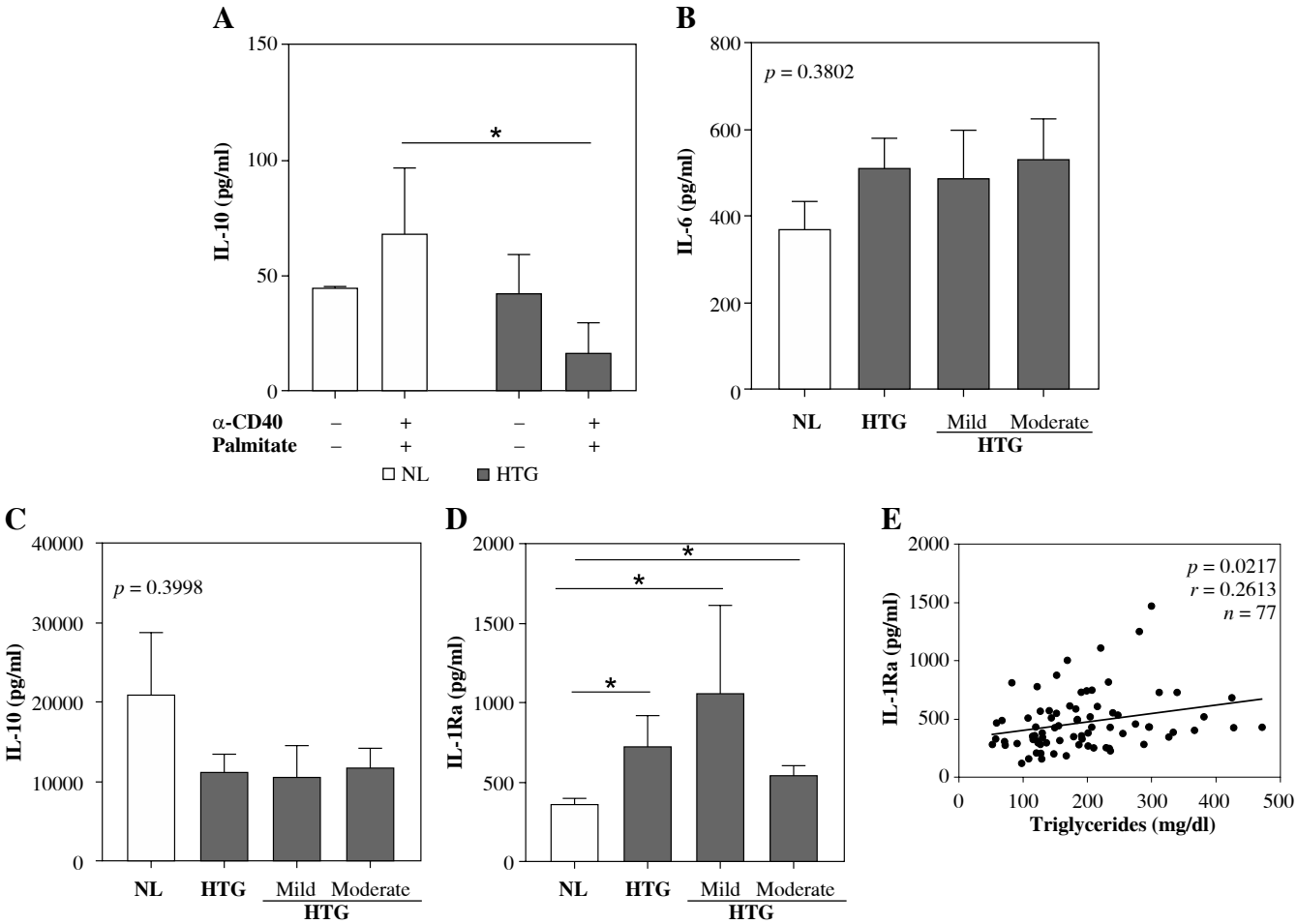

Fig. 2. Effect of palmitate on interleukin (IL)-10 production and levels of IL-10, IL-6 and IL-1Ra cytokines in individuals with hypertriglyceridemia. **A**) Levels of IL-10 were evaluated by ELISA in supernatants of peripheral blood mononuclear cell (PBMC) cultures ($n = 5$), stimulated with agonistic CD40 antibodies (αCD40) in the presence or absence of palmitate, from individuals with normal lipid (NL; in white bars) and hypertriglyceridemia (HTG; mild and moderate, gray bars). **B**) IL-6 and **C**) IL-10 levels in supernatants of PBMC cultures ($n = 5$ for both) from individuals with NL and HTG (mild and moderate) treated with phytohemagglutinin (PHA) and cultured for 18 hours, as measured by ELISA. **D**) Serum levels of IL-1Ra from individuals with NL ($n = 22$) and HTG ($n = 55$: mild and moderate), as measured by ELISA. Values represent mean ± SEM. Statistical analysis was performed using the Kruskal-Wallis test. Statistically significant differences are indicated with asterisks (*$p < 0.05$). **E**) Spearman correlation analysis between serum IL-1Ra and triglyceride levels, showing sample size (n), p-value, and correlation coefficient (r)

also been observed in patients who experienced complications related to atherosclerosis (e.g. myocardial infarction, stroke, or acute limb ischemia) compared to individuals with low cardiovascular risk, suggesting that $CD40^+$ B cells may be inversely related to atherosclerotic events [34].

Moreover, we observed reduced IL-10 levels when PBMC from HTG patients were stimulated with αCD40 in the presence of palmitate [35], suggesting that certain lipids may impair B cells' capacity to produce IL-10. Notably, our results showed no correlation between the percentages of $CD19^+IL\text{-}10^+$ B cells and serum TG levels. This may reflect the heterogeneity of circulating TG; for example, the glycerol backbone can be esterified with various fatty acids (e.g. oleic acid, linoleic acid), not only palmitic acid [36].

Additionally, we observed that only $CD19^+CD24^+CD27^+$ B cells were inversely correlated with circulating lipid levels (TG and total cholesterol). Previous reports showed that percentages of $CD19^+CD24^+CD27^+$ Bregs were inversely associated with TG levels [37]. Among human B cell subsets enriched for Bregs are $CD19^+CD24^+CD38^+$ and $CD19^+CD24^+CD27^+$; both produce large amounts of IL-10 upon activation but represent different differentiation stages. The $CD19^+CD24^+CD27^+$ subset cor-

responds to the memory B cell population, while $CD19^+CD24^+CD38^+$ cells are immature or transitional B cells [21, 38]. These Breg subpopulations have been implicated in the pathology of various diseases and appear to have distinct functional targets. For example, $CD19^+CD24^+CD38^+$ Bregs are reduced in rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), atopic dermatitis, and psoriasis [38-40]. Activating $CD19^+CD24^+CD38^+$ Bregs inhibit the differentiation of Th1 and Th17 cells while promoting the differentiation of $CD4^+CD25^+$ regulatory T cells [22]. The $CD19^+CD24^+CD27^+$ subset can suppress monocyte TNF-α production [21] and T $CD4^+$ cell IFN-γ production [32]. Alterations in the percentages and activity of the $CD19^+CD24^+CD27^+$ subset have been reported in RA [41] and in gastric cancer [32]. Together, we propose that these Breg phenotypes play distinct roles in hypertriglyceridemia. An increase in circulating lipids (triglycerides and cholesterol) may cause changes in the percentages and activity of peripheral Breg subsets. The significance of Breg changes in hypertriglyceridemia requires in-depth functional characterization to help identify therapeutic targets and support immune-interventional strategies for HTG patients.

On the other hand, serum levels of IL-1Ra were elevated in individuals with HTG, regardless of severity. A direct correlation between IL-1Ra and TG levels was also observed. It has been reported that both IL-1β and IL-1Ra are released following activation of P2X7 receptors by ATP [42]. IL-1Ra exerts anti-inflammatory effects by binding to interleukin-1 receptor type 1 (IL-1R1), thereby specifically inhibiting IL-1β signaling [43]. During inflammation, endogenous IL-1Ra is upregulated to counteract IL-1β activity, and elevated IL-1Ra levels are considered surrogate indicators of IL-1β-mediated processes. Consequently, elevated IL-1Ra may serve as a circulating marker for endogenous IL-1β or IL-1α activity, particularly because IL-1Ra is more abundant and more readily detectable in the circulation than IL-1β.

For its part, IL-1β has been shown to stimulate hepatic lipoprotein production, contributing to elevated serum TG levels [44]. In our study, we observed that IL-10 levels and the percentages of $CD19^+IL\text{-}10^+$ cells were reduced, while IL-1Ra levels were increased in HTG patients, regardless of HTG severity. Adipose tissue is a major source of systemic IL-1Ra, and serum IL-1Ra levels have been associated with HTG [45], supporting its relevance as a potential biomarker for metabolic disturbances [46]. These findings suggest that cytokines such as IL-1β and IL-10 play critical roles in the pathogenesis of HTG. IL-1β is a proinflammatory cytokine, while IL-10 has anti-inflammatory properties; therefore, an imbalance in their production may contribute to the low-grade systemic inflammation characteristic of HTG [47].

This study has certain limitations, including a relatively modest sample size, which reflects the challenge

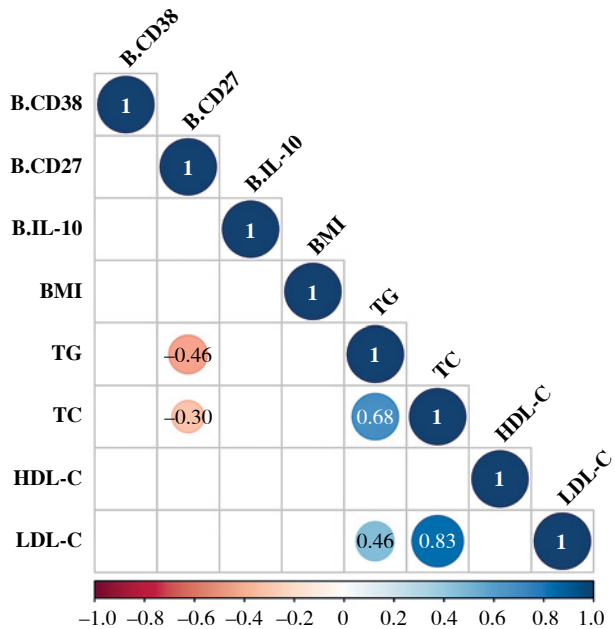

Fig. 3. Spearman correlation matrix of Breg phenotypes and biochemical parameters. The matrix includes the following variables: B.CD38 ($CD19^+CD24^+CD38^+$ B cells), B.CD27 ($CD19^+CD24^+CD27^+$ B cells), B.IL-10 ($CD19^+IL\text{-}10^+$ B cells) as percentages of each cell subset; all biochemical variables TG (triglycerides), TC (total cholesterol), HDL-C (high-density lipoprotein-cholesterol) and LDL-C (low-density lipoprotein-cholesterol) are in mg/dl; BMI (body mass index, kg/m^2). The numbers inside the circles (red or blue) represent the Spearman correlation coefficient (r) for each pair of variables. Statistically significant correlations ($p < 0.05$) are indicated by colored circles (red or blue) at the intersections of the variables. Red circles indicate an inverse/negative association, while blue circles indicate a direct/positive association. The horizontal scale and color bar at the bottom indicate the direction, negative (red) or positive (blue), of the correlation values

of identifying adults with isolated hypertriglyceridemia, as they more commonly exhibit mixed dyslipidemia. Additionally, it was not possible in this study to evaluate IL-10 expression in the $CD19^+CD24^+CD27^+$ and $CD19^+CD24^+CD38^+$ Breg phenotypes. Although both phenotypes produce IL-10, they represent distinct stages of differentiation and have different functional targets. Therefore, it is essential to determine the capacity of each phenotype to produce IL-10.

Future research should aim to expand the sample size and explore potential alterations in the suppressive activity of these Breg subsets in the context of hypertriglyceridemia. This would require a deeper understanding of the dynamic changes in Breg subpopulations, as well as the

identification of key pathogenic B cell subsets involved in lipid metabolism disorders. Furthermore, a more comprehensive investigation into the relationship between altered blood lipid profiles and disturbances in Bregs could prove valuable, as a higher proportion of B lymphocytes has been shown to possess elevated sensitivity and specificity in diagnosing atherosclerosis [27]. Finally, targeted therapies involving B cells with potentially regulatory functions may offer promising strategies for both the prevention and treatment of dyslipidemia and its associated complications, such as ischemic stroke.

Conclusions

Taken together, our data provide evidence that the percentages of regulatory B cells are altered in individuals with hypertriglyceridemia. In addition, our results show that palmitate can modulate IL-10 production by B cells. These results suggest that disturbances in lipid metabolism may affect the percentages of immune cells such as regulatory B lymphocytes, a concept that warrants further research.

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Disclosures

The study was approved by the Bioethics Committee of the National Scientific Research Commission of the Instituto Mexicano del Seguro Social (Approval No. R-2014-785-080).

The authors declare no conflict of interest.

Supplementary material is available on the journal's website.

References

1. Chait A, Feingold KR (2023): Approach to patients with hypertriglyceridemia. Best Pract Res Clin Endocrinol Metab 37: 101659.
2. Christian JB, Arondekar B, Buysman EK, et al. (2014): Determining triglyceride reductions needed for clinical impact in severe hypertriglyceridemia. Am J Med 127: 36-44.e1.
3. Miller M (1998): Is hypertriglyceridaemia an independent risk factor for coronary heart disease? The epidemiological evidence. Eur Heart J 19 Suppl H: H18.
4. Zhang T, Hou Y, Liu M, et al. (2023): Correlation between the levels of ANGPTL3, ANGPTL4, ANGPTL8 and postprandial triglyceride-rich lipoprotein (TRL). Diabetes Metab Syndr Obes 16: 3979-3993.
5. Esser D, van Dijk SJ, Oosterink E, et al. (2013): A high-fat SFA, MUFA, or n3 PUFA challenge affects the vascular response and initiates an activated state of cellular adherence in lean and obese middle-aged men. J Nutr 143: 843-851.
6. Huang ZS, Chien KL, Yang CY, et al. (2001): Peripheral differential leukocyte counts in humans vary with hyperlipidemia, smoking, and body mass index. Lipids 36: 237-245.
7. Devaraj S, Wang-Polagruto J, Polagruto J, et al. (2008): High-fat, energy-dense, fast-food-style breakfast results in an increase in oxidative stress in metabolic syndrome. Metabolism 57: 867-870.
8. Gao Q, Jiang Y, Ma T, et al. (2010): A critical function of Th17 proinflammatory cells in the development of atherosclerotic plaque in mice. J Immunol 185: 5820-5827.
9. Smith E, Prasad KM, Butcher M, et al. (2010): Blockade of interleukin-17A results in reduced atherosclerosis in apolipoprotein E-deficient mice. Circulation 121: 1746-1755.
10. Hong N, Lin Y, Ye Z, et al. (2022): The relationship between dyslipidemia and inflammation among adults in east coast China: A cross-sectional study. Front Immunol 13: 937201.
11. Somm E, Cettour-Rose P, Asensio C, et al. (2006): Interleukin-1 receptor antagonist is upregulated during diet-induced obesity and regulates insulin sensitivity in rodents. Diabetologia 49: 387-393.
12. Juge-Aubry CE, Somm E, Giusti V, et al. (2003): Adipose tissue is a major source of interleukin-1 receptor antagonist: upregulation in obesity and inflammation. Diabetes 52: 1104-1110.
13. Nishimura S, Manabe I, Takaki S, et al. (2013): Adipose natural regulatory B cells negatively control adipose tissue inflammation. Cell Metab 18: 759-766.
14. de Almeida IT, Cortez-Pinto H, Fidalgo G, et al. (2002): Plasma total and free fatty acids composition in human non-alcoholic steatohepatitis. Clin Nutr 21: 219-223.
15. Wen H, Gris D, Lei Y, et al. (2011): Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling. Nat Immunol 12: 408-415.
16. Prieur X, Mok CY, Velagapudi VR, et al. (2011): Differential lipid partitioning between adipocytes and tissue macrophages modulates macrophage lipotoxicity and M2/M1 polarization in obese mice. Diabetes 60: 797-809.
17. Haversen L, Danielsson KN, Fogelstrand L, Wiklund O (2009): Induction of proinflammatory cytokines by long-chain saturated fatty acids in human macrophages. Atherosclerosis 202: 382-393.
18. Slusher AL, Mischo AB, Acevedo EO (2016): Pentraxin 3 is an anti-inflammatory protein associated with lipid-induced interleukin 10 in vitro. Cytokine 86: 36-40.
19. Rosser EC, Mauri C (2015): Regulatory B cells: origin, phenotype, and function. Immunity 42: 607-612.
20. van der Vlugt LE, Zinsou JF, Ozir-Fazalalikhan A, et al. (2014): Interleukin 10 (IL-10)-producing CD1dhi regulatory B cells from Schistosoma haematobium-infected individuals induce IL-10-positive T cells and suppress effector T-cell cytokines. J Infect Dis 210: 1207-1216.
21. Iwata Y, Matsushita T, Horikawa M, et al. (2011): Characterization of a rare IL-10-competent B-cell subset in humans that parallels mouse regulatory B10 cells. Blood 117: 530-541.
22. Flores-Borja F, Bosma A, Ng D, et al. (2013): CD19+CD24hiCD38hi B cells maintain regulatory T cells while limiting TH1 and TH17 differentiation. Sci Transl Med 5: 173ra23.
23. Wei B, Deng Y, Huang Y, et al. (2019): IL-10-producing B cells attenuate cardiac inflammation by regulating Th1 and Th17 cells in acute viral myocarditis induced by coxsackie virus B3. Life Sci 235: 116838.

24. Mauri C, Gray D, Mushtaq N, Londei M (2003): Prevention of arthritis by interleukin 10-producing B cells. J Exp Med 197: 489-501.
25. Kyaw T, Tay C, Krishnamurthi S, et al. (2011): B1a B lymphocytes are atheroprotective by secreting natural IgM that increases IgM deposits and reduces necrotic cores in atherosclerotic lesions. Circ Res 109: 830-840.
26. Strom AC, Cross AJ, Cole JE, et al. (2015): B regulatory cells are increased in hypercholesterolaemic mice and protect from lesion development via IL-10. Thromb Haemost 114: 835-847.
27. Zhang Y, Jiang Y, Zou Y, et al. (2023): Peripheral blood CD19 positive B lymphocytes increase after ischemic stroke and correlate with carotid atherosclerosis. Front Neurol 14: 1308041.
28. Fischer E, Van Zee KJ, Marano MA, et al. (1992): Interleukin-1 receptor antagonist circulates in experimental inflammation and in human disease. Blood 79: 2196-2200.
29. Granowitz EV, Santos AA, Poutsiaka DD, et al. (1991): Production of interleukin-1-receptor antagonist during experimental endotoxaemia. Lancet 338: 1423-1424.
30. Wang H, Zhang S, Xie L, et al. (2023): Neuroinflammation and peripheral immunity: Focus on ischemic stroke. Int Immunopharmacol 120: 110332.
31. Westendorp WF, Dames C, Nederkoorn PJ, Meisel A (2022): Immunodepression, infections, and functional outcome in ischemic stroke. Stroke 53: 1438-1448.
32. Murakami Y, Saito H, Shimizu S, et al. (2019): Increased regulatory B cells are involved in immune evasion in patients with gastric cancer. Sci Rep 9: 13083.
33. Potteaux S, Esposito B, van Oostrom O, et al. (2004): Leukocyte-derived interleukin 10 is required for protection against atherosclerosis in low-density lipoprotein receptor knockout mice. Arterioscler Thromb Vasc Biol 24: 1474-1478.
34. Rincon-Arevalo H, Quintero JC, Fortich F, et al. (2020): Low frequency of IL-10(+) B cells in patients with atherosclerosis is related with inflammatory condition. Heliyon 6: e03441.
35. Shen P, Roch T, Lampropoulou V, et al. (2014): IL-35-producing B cells are critical regulators of immunity during autoimmune and infectious diseases. Nature 507: 366-370.
36. Ferramosca A, Zara V (2014): Modulation of hepatic steatosis by dietary fatty acids. World J Gastroenterol 20: 1746-1755.
37. Mendez-Frausto G, Romero-Aguilera G, Sanchez-Gutierrez R, et al. (2021): B regulatory cells associated with changes in biochemical and inflammatory parameters in normal-glycemic individuals, pre-diabetes and T2DM patients. Diabetes Res Clin Pract 173: 108692.
38. Blair PA, Norena LY, Flores-Borja F, et al. (2010): CD19(+) CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic Lupus Erythematosus patients. Immunity 32: 129-140.
39. Menon M, Blair PA, Isenberg DA, Mauri C (2016): A regulatory feedback between plasmacytoid dendritic cells and regulatory B cells is aberrant in systemic lupus erythematosus. Immunity 44: 683-697.
40. Hayashi M, Yanaba K, Umezawa Y, et al. (2016): IL-10-producing regulatory B cells are decreased in patients with psoriasis. J Dermatol Sci 81: 93-100.
41. Banko Z, Pozsgay J, Szili D, et al. (2017): Induction and differentiation of IL-10-producing regulatory B cells from healthy blood donors and rheumatoid arthritis patients. J Immunol 198: 1512-1520.
42. Wilson HL, Francis SE, Dower SK, Crossman DC (2004): Secretion of intracellular IL-1 receptor antagonist (type 1) is dependent on P2X7 receptor activation. J Immunol 173: 1202-1208.
43. Hannum CH, Wilcox CJ, Arend WP, et al. (1990): Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. Nature 1990;343:336.
44. Feingold KR, Soued M, Adi S, et al. (1991): Effect of interleukin-1 on lipid metabolism in the rat. Similarities to and differences from tumor necrosis factor. Arterioscler Thromb 11: 495-500.
45. Klemettila JP, Kampman O, Seppala N, et al. (2014): Cytokine and adipokine alterations in patients with schizophrenia treated with clozapine. Psychiatry Res 218: 277-283.
46. Luotola K (2022): IL-1 receptor antagonist (IL-1Ra) levels and management of metabolic disorders. Nutrients 14: 3422.
47. Majcher S, Ustianowski P, Tarnowski M, et al. (2021): IL-1beta and IL-10 gene polymorphisms in women with gestational diabetes. J Matern Fetal Neonatal Med 34: 3169-3174.