Fc-gamma receptor expression and cytokine responses to intravenous human immunoglobulin in whole blood from non-pregnant and pregnant women and newborns

Stephania Vázquez-Rodríguez[1,2,B,C,F], Lourdes A. Arriaga-Pizano[3,4,C,D,F], Ismael Mancilla-Herrera[5,A,C,D,F], Jessica Prieto-Chávez[4,2,B,F], Roberto Arizmendi-Villanueva[6,B,F], Rafael Torres-Rosas[3,D–F], Ana Flisser[7,D,F], Ethel García-Latorre[2,D,F], Arturo Cérbulo-Vázquez[8,A,C,D,F]

[1] Department of Physiology and Cellular Development, National Institute of Perinatology (INPer), Mexico City, Mexico
[2] Department of Immunology, National School of Biomedical Sciences, Mexico City, Mexico
[3] School of Dentistry, Benito Juárez Autonomous University of Oaxaca, Mexico
[4] Medical Research Unit in Immunochemistry, Specialty Hospital "Dr. Bernardo Sepúlveda Gutiérrez", Mexico City, Mexico
[5] Department of Infectious Diseases and Immunology, National Institute of Perinatology (INPer), Mexico City, Mexico
[6] Women's Hospital, Secretariat of Health, Mexico City, Mexico
[7] Faculty of Medicine, Department of Microbiology and Parasitology, National Autonomous University of Mexico (UNAM), Mexico City, Mexico
[8] Genomic Medicine Service, General Hospital of Mexico "Dr. Eduardo Liceaga", Mexico City, Mexico

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online) *Adv Clin Exp Med*. 2024;33(10):1115–1122

Address for correspondence
Arturo Cérbulo-Vázquez
E-mail: cerbulo@hotmail.com

Funding sources
This work was supported by CONACYT SALUD-2010-C01-141102, National Scholarship CONACYT (grant No. 289859).

Conflict of interest
None declared

Received on May 2, 2023
Reviewed on June 20, 2023
Accepted on October 26, 2023

Published online on January 5, 2024

Cite as
Vázquez-Rodríguez S, Arriaga-Pizano L, Mancilla-Herrera I, et al. Fc-gamma receptor expression and cytokine responses to intravenous human immunoglobulin in whole blood from non-pregnant and pregnant women and newborns. *Adv Clin Exp Med*. 2024;33(10):1115–1122. doi:10.17219/acem/174566

DOI
10.17219/acem/174566

Copyright
Copyright by Author(s)
This is an article distributed under the terms of the Creative Commons Attribution 3.0 Unported (CC BY 3.0) (https://creativecommons.org/licenses/by/3.0/)

Abstract

Background. Intravenous immunoglobulin (IVIG) can suppress the inflammatory response in adults, but its role in pregnant women and newborns is poorly studied. While the adult immune system is considered mature, it is immature in neonates and suppressed in pregnancy. Since the immune response differs in these 3 groups, the use of IVIG could differentially modulate the immune response.

Objectives. We aimed to explore the effect of IVIG on myeloid blood cells from non-pregnant women, pregnant women and newborns.

Materials and methods. Whole blood from healthy donors was incubated with lipopolysaccharide (LPS) and/or IVIG. After 0 h, 24 h and 48 h of culture, Fc-gamma receptor (CD16, CD32 and CD64) expression, monocyte and neutrophil bacterial phagocytosis, and cytokine and chemokine concentrations were determined in the supernatant.

Results. The baseline expression of monocyte CD16 was higher in newborns than in adult women, but the expression of CD32 and CD64 was similar between groups. Furthermore, LPS and IVIG stimulation, together or separately, did not change Fc-gamma receptor expression in monocytes or neutrophils and did not modify their phagocytosis capacity. On the other hand, IVIG did not downregulate the proinflammatory cytokine response induced by LPS in any group. Interestingly, IVIG induced a strong interleukin 8 (IL-8) response in neonates but not in non-pregnant or pregnant women.

Conclusions. Our results show that IVIG did not induce changes in Fc-gamma receptor expression, phagocytic ability, or the cytokine response to LPS in blood cells from neonates, non-pregnant or pregnant women. However, IVIG induced a strong IL-8 response in neonates that could improve immunity.

Key words: IVIG, Fc-gamma receptors, maternal-newborn response

Background

Intravenous human immunoglobulin (IVIG) is a serum-based polyclonal pharmaceutical preparation used as a medical treatment for immunodeficiencies and an immunomodulator treatment in patients with autoimmunity or infectious diseases.[1] Furthermore, IVIG has been used in pregnant women to treat fetal-neonatal alloimmune thrombocytopenia, antiphospholipid syndrome and recurrent pregnancy loss.[2,3] However, the ex vivo effects of IVIG on healthy pregnant women and newborns have been poorly analyzed.

Non-pregnant women, pregnant women and newborns have different immune responses. Adult female immunity is mature, pregnant women express tolerogenicity that suppresses the immune response to the fetus, while newborn immunity is immature. In addition, pregnant women and the fetus share multiple mechanisms that regulate the immune system and develop immunological tolerance during pregnancy.[4,5]

Usually, a lower immune response is detected in newborns when comparing adult and newborn immune mechanisms. However, the newborn is exposed to a different hormonal environment than non-pregnant women. Pregnancy appears to be useful for contrasting the immune response in non-pregnant women and newborns because pregnant women express unique tolerogenic and hormonal characteristics that they share with newborns.[6–9] Furthermore, non-pregnant women have a level of immune maturity shown by pregnant women when facing immunoregulatory challenges such as those invoked by IVIG.

The immunoregulatory effects of IVIG involve several mechanisms, such as inhibiting lymphocyte proliferative responses, limiting the inflammatory cytokine response, and apoptosis induction, among others.[10–12] Most of the mechanisms invoked by IVIG depend on binding to Fc-gamma receptors (FcγRs), expressed on most leukocytes.[12] Three FcγR have been described: FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16).[13] CD64 is a high-affinity receptor for monomeric IgG, while CD32 and CD16 are low-affinity receptors for IgG. Monocytes constitutively express CD64, macrophages, neutrophils express CD32, and neutrophils and natural killer (NK) cells express CD16.[13] Upon binding of IgG to the FcγR, leukocytes mediate cytokine synthesis and phagocytosis.[12] However, FcγR expression is different in adults and newborns, with monocyte CD16 and CD32 expression lower in adults than in newborns, while CD64 expression is higher in granulocytes in newborns than in adults.[14] In mice, the cellular expression of FcγR changes after IVIG treatment.[15,16]

Objectives

We used an ex vivo model to explore the effects of IVIG on neonatal monocytes and granulocytes, and compare it with the non-pregnant and pregnant response. Whole blood (WB) was stimulated with lipopolysaccharide (LPS), and the effect of IVIG on the expression of FcγRs, monocyte and granulocyte phagocytic capacity as well as the proinflammatory response were analyzed.

Materials and methods

Patients and sample collection

The study included 3 groups: non-pregnant women (NP, n = 18), prepartum pregnant women (P, n = 15) and newborns (N, n = 18). All adult donors were healthy women (25 ±5 years old) at the time of blood collection, and patients with comorbidities such as overweight, obesity, diabetes, hypertension, autoimmunity, and immunosuppression were excluded. All babies were born at term (37–40.3 weeks of gestation) and had healthy anthropomorphic parameters. The Research Committee reviewed and approved our study (Project: HM: INV/2015:2020), which was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Peripheral blood (6 mL) was collected by venipuncture from NP and P participants, while umbilical cord blood (UCB) was collected by arterial umbilical venipuncture (n = 18) after placenta delivery. Blood samples were collected using Vacutainer® plastic sodium heparin tubes (Cat. No. 367876, BD Biosciences, Franklin Lakes, USA). Both UCB and peripheral blood were processed immediately after collection.

Cell culture

Whole blood (1 mL) was incubated in 24-well culture plates (cat. No. 13485; Costar, New York, USA), either alone or with 10 ng/mL of *Escherichia coli* O55: B5 LPS (cat. No. L2880; Sigma Aldrich, St. Louis, USA), 10 mg/mL of IVIG (cat. No. 5240IgG6; Kedrigamma Kedrion Group, Barga, Italy), or LPS/IVIG (both 10 ng/mL) for 0 h, 24 h and 48 h at 37°C with 5% CO_2.

Evaluation of Fcγ receptor expression in monocytes and neutrophils

After cell culture, 50 µL of WB was mixed with antibodies, including CD45-PacificOrange (cat. No. MHCD4530; Invitrogen, Waltham, USA), CD14-PE/Cy7 (cat. No. 301804; BioLegend, Santa Clara, USA), CD16-APC/Cy7 (cat. No. 302018; BioLegend), CD32-PE (cat. No. 303206; BioLegend), and CD64-APC (cat. No. 305014; BioLegend). Appropriate compensation was performed, and isotype controls were used for each antibody. After 15 min of incubation, erythrolysis was performed using FACS™ Lysing Solution (cat. No. 349202; BD Biosciences). The samples were washed twice with ×1 phosphate-buffered saline (PBS) (1,500 rpm, 5 min, 4°C) and resuspended

in 100 µL of PBS. Thirty thousand single leukocytes were acquired on a FACS Aria II flow cytometer (BD Biosciences), and FCS files were analyzed using Infinicyt v. 1.8 software (Cytognos, Salamanca, Spain). The leukocytes were single cells (FSC-A compared to FSC-H plot), CD45-positive, and with typical size and complexity (side scatter (SSC) compared to CD45 plot). Monocytes were SSC^{mid} forward scatter (FSC)$^{mid}CD45^{+}CD14^{+}$, and neutrophils were $SSC^{mid}FSC^{mid}CD45^{+}CD16^{+}$ cells. The percentage of CD64-, CD32- and CD16-positive cells and the mean fluorescence intensity (MFI) were calculated.

Phagocytosis assay

After cell culture, the samples were tested for phagocytosis according to the manufacturer's instructions (pHrodoTM Green *Escherichia coli* BioParticle Kit, cat. No. P35381; Invitrogen). Briefly, WB was precultured alone or with IVIG, LPS or both, and then incubated with pHrodo-conjugated opsonized *E. coli* bioparticles at 37°C or on ice for 15 min. Leukocytes were immunophenotyped using anti-CD45-PacificOrange (cat. No. MHCD4530; Invitrogen), CD14-PE/Cy7 (cat. No. 301804; BioLegend) and CD16-APC/Cy7 (cat. No. 302018; BioLegend). The cells were lysed, washed and counted, their viability was assessed, and they were resuspended in a wash buffer for acquisition on an Aria II BD cytometer. Phagocytosis was evaluated as the percentage of bacteria-positive monocytes or neutrophils, while the MFI value represented the presence of bacteria within a cellular acidic compartment. The nucleated phagocytes were discriminated using the SSC and FSC parameters, with neutrophils defined by the $SSC\text{-}A^{med}FSC\text{-}H^{med}CD45^{+}CD14^{+}$ phenotype.

Cytokine quantification

After culture, plasma cytokine quantification (interleukin (IL)-8, tumor necrosis factor alpha (TNF-α), IL-1β, IL-6, IL-10, and IL-12) was performed according to the manufacturer's instructions (LEGENDplex human inflammation kit, cat. No. 740808; BioLegend). Data were acquired with a FACS Aria III BD cytometer. Logarithmic transformed data were used to construct standard curves fitted to 10 discrete points using a 4-parameter logistic model. Concentrations were calculated using interpolations of the corresponding reference curves.

Statistical analyses

Data analysis employed IBM SPSS Statistics for Windows v. 25.0 (IBM Corp., Armonk, USA) or GraphPad Prism 7.0 (GraphPad Software, San Diego, USA). Results are expressed as mean ± standard error (M ±SE) or mean ± standard deviation (M ±SD). Fc-gamma receptors and phagocytosis data were analyzed using a bootstrap repeated measures analysis of variance (RM-ANOVA), while cytokine kinetics analysis used two-way RM-ANOVA with Tukey´s multiple comparisons. The 95% confidence interval (95% CI) was calculated for each test, and $p < 0.05$ was considered statistically significant. Supplementary data contain the normality tests, bootstrap and Tukey's multiple comparisons analysis for conditions analyzed.

Results

Fc-gamma receptor expression in monocytes and granulocytes

The P blood samples contained the lowest percentage of monocytes (2.2 ±1.2%), which was lower than in N patients (8.9 ±4.4%, p = 0.001). Also, pregnant women had the highest percentage of granulocytes (69.2 ±6.1%), significantly more than N patients (40.9 ±10.4%, p = 0.001). Since the monocyte and granulocyte percentages differed between P and N, we calculated if the expression of FcγR per cell was different between the groups and calculated MFI/% for CD16, CD32 and CD64. Table 1 shows the index for each Fc-gamma receptor (FcγR) on monocytes and neutrophils.

At the beginning of the kinetics assessment, N showed the highest CD16 index in monocytes, with a statistical difference between the NP and N CD16 index (282 ±199, 95% CI: –109.4–674.5 compared to 2003.7 ±199; 95% CI: 1,611–2,395). After 24 h and 48 h of culture, the CD16 index decreased for each group, though only the N CD16 index decreased from 0 h to 48 h (2004 ±199; 95% CI: 1611–2,395 compared to 221.1 ±141.1; 95% CI: –56–498.3).

Treatment with IVIG, LPS or IVIG+LPS did not induce significant changes in the CD16 index during kinetics. Generally, the neutrophil CD16 index was similar in NP and P, and remained almost unchanged with or without IVIG or LPS. Meanwhile, the CD16 index in N neutrophils remained unchanged when WB was cultured alone. In contrast, IVIG, LPS and IVIG+LPS decreased the CD16 index in N after 48 h of culture (525 ±199; 95% CI: 133.2–917.3 compared to 273.8 ±141.1; 95% CI: –3.3–551; 241.6 ±141.1; 95% CI: –35.5–518.8; and 280.8 ±141.1, 95% CI: 3.6–558, respectively).

The monocyte CD32 index was similar between times and groups throughout, with only IVIG causing a significant change in the CD32 index between 24 h and 48 h in NP women (77.8 ±261; 95% CI: –434.8–590.6 compared to 345.6 ±261.1; 95% CI: –167–858.4). Also, the neutrophil CD32 index was changed by IVIG between 24 h and 48 h (111.3 ±261.1; 95% CI: –401.4–624 compared to 346.4 ±261.1; 95% CI: –166.2–859.2). Furthermore, N had the lowest CD32 index after 48 h of culture with IVIG, with the response lower than P and NP (194.6 ±182, 95% CI: –163.1–552.5 or 43.7 ±257.7; 95% CI: –462.3–549.8 compared to 346.4 ±261.1; 95% CI: –166.2–859.2, respectively).

Table 1. FcγR index on monocytes or neutrophils of non-pregnant women, pregnant women and newborns

Index	Hours								
	0			24			48		
	NP	P	N	NP	P	N	NP	P	N
CD16 MFI/% monocytes									
WB alone	282 ±275	549 ±350	1920 ±2427	47 ±28	342 ±248	319 ±298	80 ±35	125 ±35	526 ±437
+IVIG	282 ±275	549 ±350	1920 ±2427	862	296 ±122	167 ±112	134 ±96	132 ±70	358 ±195
+LPS	282 ±275	549 ±350	1920 ±2427	124	416 ±434	312 ±222	141 ±107	126 ±109	270 ±202
++IVIG+LPS	282 ±275	549 ±350	1920 ±2427	124	416 ±434	312 ±222	96 ±52	99 ±51	383 ±447
CD32 MFI/% monocytes									
WB alone	230 ±137	122 ±55	139 ±46	310 ±32	239 ±55	227 ±159	349 ±282	200 ±30	344 ±297
+IVIG	230 ±137	122 ±55	139 ±46	77 ±22	182 ±42	127 ±62	345 ±221	202 ±102	201 ±118
+LPS	230 ±137	122 ±55	139 ±46	198 ±161	192 ±106	211 ±137	216 ±148	144 ±45	183 ±147
+IVIG+LPS	230 ±137	122 ±55	139 ±46	198 ±161	192 ±32	211 ±137	197 ±102	160 ±9	172 ±106
CD64 MFI/% monocytes									
WB alone	232 ±60	208 ±110	135 ±26	225 ±49	349 ±91	164 ±103	300 ±103	191 ±53	155 ±76
+IVIG	232 ±60	208 ±110	135 ±26	276 ±164	270 ±68	150 ±34.99	281 ±187	204 ±21	110 ±13
+LPS	232 ±60	208 ±110	135 ±26	432 ±254	317 ±123	248 ±150	399 ±218	197 ±43	208 ±123
+IVIG+LPS	232 ±60	208 ±110	135 ±26	432 ±60	317 ±123	155 ±76	399 ±102	250 ±26	133 ±66
CD16 MFI/% neutrophils									
WB alone	386 ±447	432 ±276	351 ±337	784 ±739	444 ±235	369 ±302	273 ±276	328 ±211	324 ±253
+IVIG	386 ±447	432 ±276	351 ±337	409 ±109	392 ±267	431 ±313	312 ±269	341 ±211	324 ±238
+LPS	386 ±447	432 ±276	351 ±337	405 ±459	355 ±235	403 ±274	248 ±266	307 ±213	306 ±243
+IVIG+LPS	386 ±447	432 ±276	351 ±337	405 ±459	355 ±235	403 ±274	332 ±210	374 ±240	229 ±180
CD32 MFI/% neutrophils									
WB alone	83 ±24	145 ±81	89 ±10	129 ±90	88 ±41	80 ±36	216 ±173	63 ±26	120 ±88
+IVIG	83 ±24	145 ±81	89 ±10	111 ±90	37 ±6	45 ±7	346 ±263	43 ±7	42 ±15
+LPS	83 ±24	145 ±81	89 ±10	104 ±67	264 ±314	101 ±53	143 ±91	75 ±18	90 ±35
+IVIG+LPS	83 ±24	145 ±81	89 ±10	104 ±67	264 ±314	101 ±53	132 ±70	119 ±97	60 ±33
CD64 MFI/% neutrophils									
WB alone	100 ±71	159 ±49	93 ±27	133 ±89	148 ±24	190 ±128	177 ±227	346 ±320	164 ±106
+IVIG	100 ±71	159 ±49	93 ±27	141 ±111	184 ±84	141 ±67	151 ±182	169 ±51	187 ±119
+LPS	100 ±71	159 ±49	93 ±27	134 ±84	167 ±71	165 ±77	123 ±103	246 ±123	177 ±127
+IVIG+LPS	100 ±71	159 ±49	93 ±27	134 ±84	167 ±71	165 ±77	165 ±88	283 ±182	127 ±89

Data show the mean ± standard deviation (M ±SD) for each FcγR index on monocytes or neutrophils. Non-pregnant women (NP, n = 5), pregnant (P, n = 3) and newborns (N, n = 5). Differences among groups were calculated using a bootstrap repeated measures analysis of variance (RM ANOVA). The whole bootstrap RM ANOVA is shown in Supplementary Table 1. WB – whole blood; IVIG – intravenous immunoglobulin; LPS – lipopolysaccharide.

The monocyte CD64 index showed some differences between NP and P, with P monocytes from WB cultured alone having a lower CD64 index after 48 h than at 24 h of culture (191.3 ±257.7; 95% CI: –314.7–697.4 compared to 208.5 ±257; 95% CI: –297.5–714.6). Meanwhile, LPS led to a higher CD64 index after 24 h compared to 0 h of culture in the NP group (432.9 ±202.2; 95% CI: 35.7–830 compared to 232.9 ±199.6; 95% CI: –159–624.9). In contrast, N monocytes did not show statistical differences in the CD64 index. However, a lower CD64 index was detected in N monocytes than NP monocytes after 48 h of culturing WB alone (221.3 141.1; 95% CI: –55.8–498.5 compared to 300.8 ±202.2; 95% CI: –96.3–697.9), and with IVIG (146.8 ±141.1; 95% CI: –130.3–424 compared to 281.1 ±202.2; –95% CI: 116–678.3), LPS (130.3 ±141.1; 95% CI: –146.8–407.5 compared to 399.4 ±202; 95% CI: 2.2–796.6) or IVIG+LPS (146.8 ±141.1; 95% CI: –130.3–424 compared to 399.6 ±202.2; 95% CI: 2.4–796). These differences were not observed in the CD64 index of neutrophils, with only minor changes detected.

Effect of intravenous immunoglobulin on phagocytosis

Since IVIG can opsonize antigens or interact with FcγRs to enhance or reduce the phagocytic capacity of blood cells, we performed an ex vivo phagocytosis assay. We observed that monocytes from the NP and P groups had a similar percentage of cells that phagocytosed bacteria. On the contrary,

Table 2. Comparison of pHrodo *Escherichia coli* phagocytosis in monocytes and neutrophils of non-pregnant women, pregnant women and newborns in a 24-h culture

Phagocytosis	Hours					
	0			24		
	NP	P	N	NP	P	N
	% monocytes bacteria-positive					
IVIG	76.1 ±2.5	87.3 ±2.8	76 ±11.3	82.9 ±8.0	78.2 ±2.6	37.4 ±3.2
LPS	76.1 ±2.5	87.3 ±2.8	76 ±11.3	68.7 ±10.0	84.1 ±4.5	34.5 ±9
IVIG+LPS	76.1 ±2.5	87.3 ±2.8	76 ±11.3	74.3 ±1.5	88 ±1.0	38.5 ±4.5
	% neutrophils bacteria-positive					
WB alone	82.8 ±7.9	93.3 ±3.2	93.7 ±4.0	78.5 ±5.7	93.2 ±1.9	49.1 ±29.4
IVIG	82.8 ±7.9	93.3 ±3.2	93.7 ±4.0	51.2 ±15.9	92.5 ±3.5	54.8 ±9.6
LPS	82.8 ±7.9	93.3 ±3.2	93.7 ±4.0	52.8 ±14.1	94.9 ±6.2	51.4 ±4.3
IVIG+LPS	82.8 ±7.9	93.3 ±3.2	93.7 ±4.0	74.7 ±0.6	93.9 ±6.9	53.8 ±3.2

Data show the mean ± standard deviation (M ±SD) of percentage in monocytes and neutrophils. Non-pregnant (NP, n = 3), pregnant (P, n = 3) and newborns (N, n = 3). Differences between times and groups were calculated using a bootstrap repeated measures analysis of variance (RM ANOVA). The whole bootstrap RM ANOVA is shown in Supplementary Table 2. WB – whole blood ; IVIG – intravenous immunoglobulin; LPS – lipopolysaccharide.

the percentage of phagocytic monocytes in N was lower than in NP or P (74.5 ±1,076.6; 95% CI: –2,049.9–2,198.9 compared to 90.8 ±1,076.6; 95% CI: –2,033.5–2,215.2, or 93.8 ±1076.6; 95% CI: –2,030.6–2,218.2, respectively). Interestingly, culture conditions (IVIG, LPS, or IVIG+LPS) or 24 h with stimulation did not induce changes in the percentage of phagocytic monocytes in NP or P women, but the phagocytic ability of N was severely compromised after 24 h of culture, independently of stimulus.

Regarding neutrophils, their intrinsic ability to phagocytose bacteria was reduced in N compared to NP and P women but did not change by stimulus or incubation time (Table 2). In addition to evaluating the percentage of cells that phagocytosed bacteria, we quantified the relative amounts of intracellular bacteria. According to the reduced percentage of monocytes that phagocytosed bacteria at 24 h of incubation, intracellular bacteria was only reduced in N, but not in NP or P women. For neutrophils, the number of phagocytosed bacteria was similar among the 3 groups based on stimulus and incubation time (Supplementary Table 2).

Cytokine response to intravenous immunoglobulin

Figure 1 shows the cytokine response to IVIG, LPS and IVIG+LPS challenge in WB cultures of adults and newborns. As expected, all groups had a cytokine response after the LPS stimulus, and the IL-1β, IL-6 and IL-8 concentrations were higher than in the WB cultured alone. However, the IL-6 response was lower in P than in NP (Fig. 1D, $p < 0.001$) and N (Fig. 1F, $p < 0.001$) after 12 h and 24 h of LPS challenge (Fig. 1D–F, $p < 0.001$ for both). Furthermore, IVIG alone did not evoke an IL-1 or IL-6 in response in NP or P women, but it did for IL-8 in N after 12 h and 24 h of culture (Fig. 1I, both $p < 0.001$). The IVIG+LPS challenge led to similar IL-1β, IL-6 and IL-8 kinetics in adults and newborns. In addition, the cytokine response elicited by IVIG+LPS was similar to the cytokine response to LPS alone in all groups.

Discussion

Intravenous immunoglobulin is an immunomodulator used to treat various diseases. However, since non-pregnant women, pregnant women and newborns have different regulatory and ontogeny statuses, the IVIG immunomodulation mechanism could also differ in each group. The current work presents the basal differences between groups and the effect of IVIG on myeloid cells under LPS stimulus in NP, P and N.

Since IVIG can stimulate the synthesis of cytokines and promote phagocytosis, among other effects, we explored whether it could change the expression of FcγRs (CD16, CD32 and CD64) over 24 h. Before the stimulus, consistent with other reports, our data showed differential expression of FcγRs at baseline in adults and newborns (Table 1),[14] but when used alone or in combination, IVIG only reduced monocyte and neutrophil CD16 expression in newborns after 24 h. Furthermore, CD16 only reached statistical significance in newborns, even though adults and newborns tended to express lower levels. These results suggest that adults and newborns could express differential biological activity elicited by IVIG through varied CD16 expression.

Among FcγRs, the CD32b isoform (FcγRIIb) expresses immunoreceptor tyrosine-based inhibitory motifs[13,17] and could support an inhibitory IVIG-mediated response. Intravenous immunoglobulin has been reported to increase CD32b expression in myeloid cells, maintaining

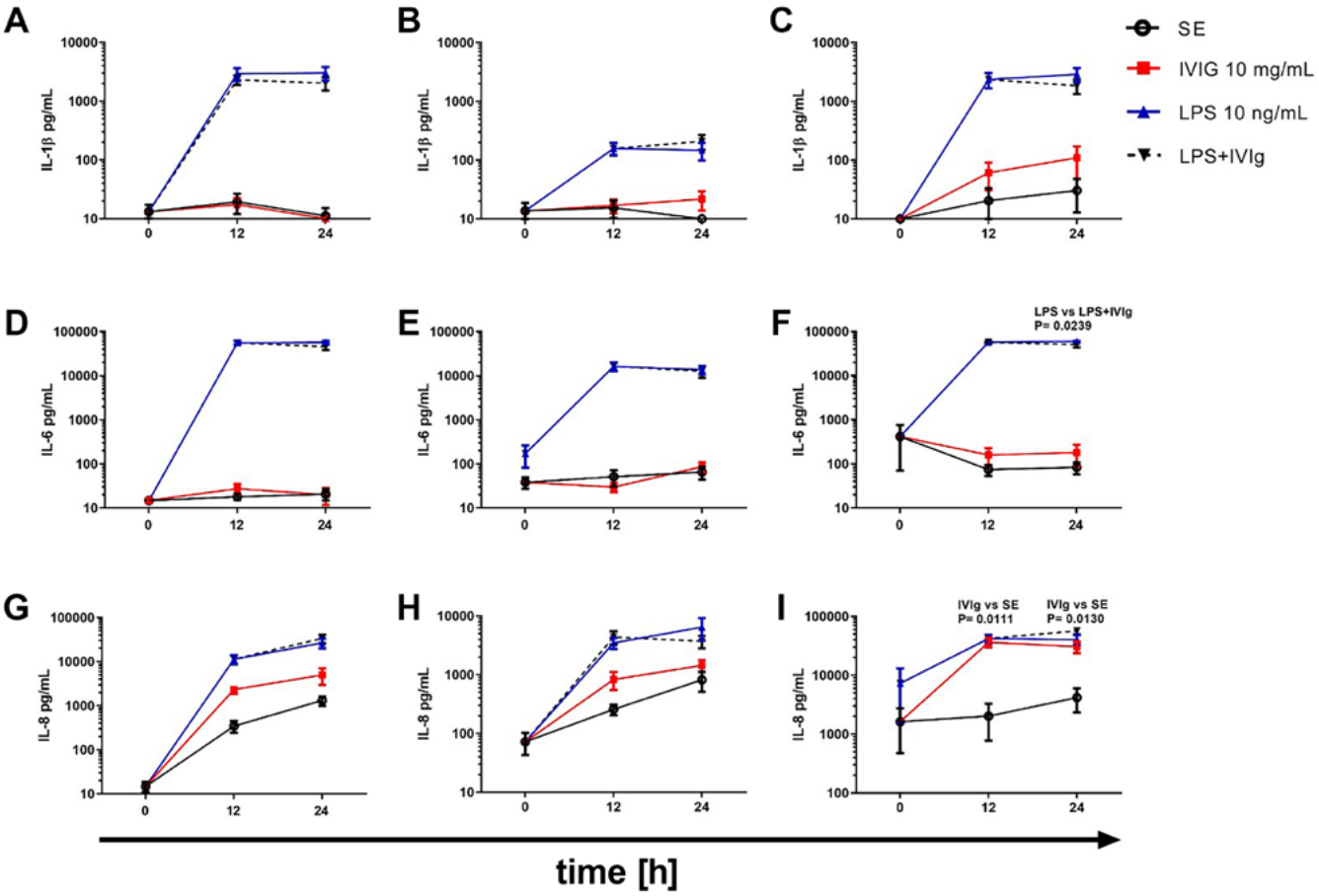

Fig. 1. Cytokine response after lipopolysaccharide (LPS) and intravenous immunoglobulin (IVIG) challenge. Whole blood was cultured for 0 h, 12 h or 24 h in the presence or absence of LPS and IVIG, after which the plasma was collected, and cytokine levels were determined. Ten pairs of mothers and neonates are shown, including non-pregnant women (NP, n = 9), pregnant women (P, n = 9) and neonates (N, n = 9). Results are expressed as mean ± standard deviation (M ±SD) with 95% confidence interval (95% CI) and were analyzed with two-way analysis of variance (ANOVA) with Tukey´s multiple comparisons and significance set at $p < 0.05$

an anti-inflammatory response.[10,12,18] Therefore, we analyzed the CD32 index in monocytes and neutrophils in response to IVIG, but its expression was similar in P and N, with an increased index only observed in NP women (Supplementary Table 1). The study did not analyze the expression of CD32b. As such, future studies must be conducted to determine whether IVIG could overexpress CD32b.

Kozicky et al.[19] reported that IVIG skews monocytes to an anti-inflammatory response. Even when the expression level of FcγR is similar among non-pregnant women, pregnant women and newborns, the transduction signal induced by IVIG after engaging the FcγR could be differential and lead to an inflammatory or anti-inflammatory response. Regarding CD64 expression, Maeda et al.[14] reported that neonatal granulocytes expressed higher levels of CD64, and Luppi et al.[20] and Davis et al.[21] showed that CD64 expression in polymorphonuclear cells gradually increased in the 3rd trimester of pregnancy. Our data show that the monocyte CD64 index was consistently higher in non-pregnant women than in newborns (Table 1), though this was not the case for the neutrophil CD64 index, which remained similar between groups and over time (Table 1 and Supplementary Table 1). In summary, FcγR expression in non-pregnant or pregnant women and newborns was differential, and IVIG only increased the monocyte and neutrophil CD32 index in non-pregnant women.

Phagocytosis could be regulated by IVIG through FcγR. However, contrary to Gille et al.,[22] we did not observe an increase in phagocytosis after IVIG treatment. Mononuclear cells were used in the study by Gille et al. (in vitro model), while we used WB (ex vivo model). We argue that our ex vivo model is closer to the response that could be observed in vivo. Our findings showed that both adult groups (non-pregnant and pregnant) expressed more than 80% of pHrodo *E. coli*-positive monocytes after 24 h in culture. In contrast, only 75% of neonatal monocytes had this ability at the beginning of the culture, which was less than 20% after 24 h. Such a functional limitation in newborns could be due to the physiologic leukocytosis. Despite this condition, IVIG did not change the response to phagocytosis in adults or newborns. Monocytes and neutrophils are fundamental phagocytic cells in peripheral blood that support many aspects of inflammatory immune responses.[23] In particular, they produce large amounts of IL-1β, IL-6 and IL-8.[24–26]

Intravenous immunoglobulin could limit the production of inflammatory cytokines, such as TNF-α, IL-1β and

IL-6 in patients with sepsis.[27–29] However, some studies found that IVIG could have inhibitory or enhancing effects on IL-6 concentration.[30,31] Our results showed that IVIG increased IL-8 concentration in newborns but not adults, suggesting that it may be especially important for modulating the inflammatory response in newborns. Interestingly, we observed a low IL-8 response in the mother, indicating that the pregnant state could modulate the effects of IVIG. Such an IL-8 response could promote neutrophil migration and support inflammation in newborns; however, limited phagocytosis by neutrophils could limit the effect in the neonate. On the other hand, higher production of IL-8 in newborns could compensate for the lower phagocytic capacity.

Although IVIG has been used in adults and newborns with sepsis,[18,32,33] contradictory results have been reported. Indeed, since IVIG did not improve fatal outcomes in newborns, its use is not recommended in this group.[34,35] In contrast, some authors report prevention of early sepsis through IVIG treatment,[36] though its use does not prevent fatal outcomes. More studies are needed to determine if the clinical condition improves with this immunomodulatory drug. Furthermore, it should be elucidated if IVIG could substantially support the treatment of newborns.

Limitations

Our study had several limitations, such as small sample sizes and short-term kinetics. Nonetheless, the study showed differential IL-8 responses between groups, indicating that IVIG could up-regulate different mechanisms in adults and newborns. We infer that the hormonal status in pregnant women and newborns is similar since steroids can pass through the placenta, as are some of the characteristic tolerogenic and immunoregulatory responses. However, we did not analyze the hormonal status in any of the groups and cannot determine if they regulate the response to IVIG.

Our model was limited to the UCB response after birth, and newborn peripheral blood can express different responses. In addition, the response to IVIG can vary in newborns if they have an infection at birth. More studies are needed to determine the responses to IVIG in adults and newborns.

Conclusions

Intravenous immunoglobulin induced a strong IL-8 response in the cord blood of newborns that could lead to improved immunity.

Supplementary data

The Supplementary materials are available at https://doi.org/10.5281/zenodo.10407063. The package includes the following files:

Supplementary Table 1. FcγR Index on monocytes or neutrophils of non-pregnant women, pregnant women and neonates during 48 h of culture, and the bootstrap RM ANOVA test for Table 1.

Supplementary Table 2. Mean fluorescence intensity of bacteria phagocyted by monocytes or neutrophils of non-pregnant women, pregnant women and neonates, and the bootstrap RM ANOVA test for Table 2.

PROIVIg Cytokine Normality test dic2023. Excel archive with the normality test of cytokine.

PROIVIg cytokine Tukey's test dic2023. Excel archive with the Tukey test of cytokine.

PROIVIg Fcgamma R monocyte normality test dic2023. Excel archive with the normality test of FcgammaR on monocyte.

PROIVIg Fcgamma R PMN normality test dic2023. Excel archive with the normality test of FcgammaR on PMN.

PROIVIg phagocytosis monocyte normality test dic2023. Excel archive with the normality test of phagocytosis on Monocyte.

PROIVIg phagocytosis PMN normality test dic2023. Excel archive with the Normality test of phagocytosis on PMN.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Stephania Vázquez-Rodríguez https://orcid.org/0000-0002-7736-8634
Lourdes A. Arriaga-Pizano https://orcid.org/0000-0003-4433-2106
Ismael Mancilla-Herrera https://orcid.org/0000-0001-8195-8082
Jessica Prieto-Chávez https://orcid.org/0000-0002-0720-4271
Roberto Arizmendi-Villanueva https://orcid.org/0000-0002-4317-9309
Rafael Torres-Rosas https://orcid.org/0000-0002-5934-003X
Ana Flisser https://orcid.org/0000-0002-1744-8480
Ethel García-Latorre https://orcid.org/0000-0002-0223-4033
Arturo Cérbulo-Vázquez https://orcid.org/0000-0002-4267-3479

References

1. Shankar-Hari M, Spencer J, Sewell WA, Rowan KM, Singer M. Bench-to-bedside review: Immunoglobulin therapy for sepsis – biological plausibility from a critical care perspective. *Crit Care*. 2012;16(2):206. doi:10.1186/cc10597
2. Yang X, Meng T. Is there a role of intravenous immunoglobulin in immunologic recurrent pregnancy loss? *J Immunol Res*. 2020;2020:6672865. doi:10.1155/2020/6672865
3. Branch DW, Porter TF, Paidas MJ, Belfort MA, Gonik B. Obstetric uses of intravenous immunoglobulin: Successes, failures, and promises. *J Allergy Clin Immunol*. 2001;108(4 Suppl):S133–S138. doi:10.1067/mai.2001.117821
4. Rendell V, Bath NM, Brennan TV. Medawar's paradox and immune mechanisms of fetomaternal tolerance. *OBM Transplant*. 2020;4(1):26. doi:10.21926/obm.transplant.2001104

5. PrabhuDas M, Bonney E, Caron K, et al. Immune mechanisms at the maternal–fetal interface: Perspectives and challenges. *Nat Immunol*. 2015;16(4):328–334. doi:10.1038/ni.3131
6. Walsh SW, Stanczyk FZ, Novy MJ. Daily hormonal changes in the maternal, fetal, and amniotic fluid compartments before parturition in a primate species. *J Clin Endocrinol Metab*. 1984;58(4):629–639. doi:10.1210/jcem-58-4-629
7. Walsh SW, Ducsay CA, Novy MJ. Circadian hormonal interactions among the mother, fetus, and amniotic fluid. *Am J Obstet Gynecol*. 1984;150(6):745–753. doi:10.1016/0002-9378(84)90679-3
8. Raghupathy R, Szekeres-Bartho J. Progesterone: A unique hormone with immunomodulatory roles in pregnancy. *Int J Mol Sci*. 2022; 23(3):1333. doi:10.3390/ijms23031333
9. Piccinni MP, Raghupathy R, Saito S, Szekeres-Bartho J. Cytokines, hormones and cellular regulatory mechanisms favoring successful reproduction. *Front Immunol*. 2021;12:717808. doi:10.3389/fimmu.2021.717808
10. Tha-In T, Bayry J, Metselaar HJ, Kaveri SV, Kwekkeboom J. Modulation of the cellular immune system by intravenous immunoglobulin. *Trends Immunol*. 2008;29(12):608–615. doi:10.1016/j.it.2008.08.004
11. Gelfand EW. Intravenous immune globulin in autoimmune and inflammatory diseases. *N Engl J Med*. 2012;367(21):2015–2025. doi:10.1056/NEJMra1009433
12. Nagelkerke SQ, Kuijpers TW. Immunomodulation by IVIg and the role of Fc-gamma receptors: Classic mechanisms of action after all? *Front Immunol*. 2015;5:674. doi:10.3389/fimmu.2014.00674
13. Rosales C. Fcgamma receptor heterogeneity in leukocyte functional responses. *Front Immunol*. 2017;8:280. doi:10.3389/fimmu.2017.00280
14. Maeda M, Van Schie RCAA, Yüksel B, et al. Differential expression of Fc receptors for IgG by monocytes and granulocytes from neonates and adults. *Clin Exp Immunol*. 1996;103(2):343–347. doi:10.1046/j.1365-2249.1996.d01-615.x
15. Flores-Mejía LA, Cabrera-Rivera GL, Ferat-Osorio E, et al. Function is dissociated from activation-related immunophenotype on phagocytes from patients with SIRS/sepsis syndrome. *Shock*. 2019;52(5): e68–e75. doi:10.1097/SHK.0000000000001314
16. Samuelsson A, Towers TL, Ravetch JV. Anti-inflammatory activity of IVIG mediated through the inhibitory Fc receptor. *Science*. 2001; 291(5503):484–486. doi:10.1126/science.291.5503.484
17. Smith KGC, Clatworthy MR. FcγRIIB in autoimmunity and infection: Evolutionary and therapeutic implications. *Nat Rev Immunol*. 2010; 10(5):328–343. doi:10.1038/nri2762
18. Schwab I, Nimmerjahn F. Intravenous immunoglobulin therapy: How does IgG modulate the immune system? *Nat Rev Immunol*. 2013;13(3): 176–189. doi:10.1038/nri3401
19. Kozicky LK, Menzies SC, Zhao ZY, et al. IVIg and LPS co-stimulation induces IL-10 production by human monocytes, which is compromised by an FcγRIIA disease-associated gene variant. *Front Immunol*. 2018;9:2676. doi:10.3389/fimmu.2018.02676
20. Luppi P, Haluszczak C, Betters D, Richard CAH, Trucco M, DeLoia JA. Monocytes are progressively activated in the circulation of pregnant women. *J Leukoc Biol*. 2002;72(5):874–884. PMID:12429709.
21. Davis D, Kaufmann R, Moticka EJ. Nonspecific immunity in pregnancy: Monocyte surface Fcγ receptor expression and function. *J Reprod Immunol*. 1998;40(2):119–128. doi:10.1016/S0165-0378(98)00076-X
22. Gille C, Dreschers S, Spring B, et al. Differential modulation of cord blood and peripheral blood monocytes by intravenous immunoglobulin. *Cytometry B Clin Cytom*. 2012;82(1):26–34. doi:10.1002/cyto.b.20609
23. Venet F, Monneret G. Advances in the understanding and treatment of sepsis-induced immunosuppression. *Nat Rev Nephrol*. 2018;14(2): 121–137. doi:10.1038/nrneph.2017.165
24. Kwiatkowska K, Ciesielska A. Lipid-mediated regulation of pro-inflammatory responses induced by lipopolysaccharide. *Postepy Biochem*. 2018;64(3):175–182. doi:10.18388/pb.2018_129
25. Geng Y, Zhang B, Lotz M. Protein tyrosine kinase activation is required for lipopolysaccharide induction of cytokines in human blood monocytes. *J Immunol*. 1993;151(12):6692–6700. PMID:8258685.
26. Lakshmikanth CL, Jacob SP, Chaithra VH, De Castro-Faria-Neto HC, Marathe GK. Sepsis: In search of cure. *Inflamm Res*. 2016;65(8):587–602. doi:10.1007/s00011-016-0937-y
27. Domínguez-Soto Á, Simón-Fuentes M, De Las Casas-Engel M, et al. IVIg promote cross-tolerance against inflammatory stimuli in vitro and in vivo. *J Immunol*. 2018;201(1):41–52. doi:10.4049/jimmunol.1701093
28. Murakami K, Suzuki C, Kobayashi F, et al. Intravenous immunoglobulin preparation attenuates LPS-induced production of pro-inflammatory cytokines in human monocytic cells by modulating TLR4-mediated signaling pathways. *Naunyn Schmiedebergs Arch Pharmacol*. 2012;385(9):891–898. doi:10.1007/s00210-012-0765-8
29. Kasztalska K, Ciebiada M, Górski P. Mechanism of action of immunoglobulin applied intravenously [in Polish]. *Pol Merkur Lekarski*. 2010; 29(172):263–268.
30. Aukrust P, Müller F, Frøland SS. Elevated serum levels of interleukin-4 and interleukin-6 in patients with common variable immunodeficiency (CVI) are associated with chronic immune activation and low numbers of $CD4^+$ lymphocytes. *Clin Immunol Immunopathol*. 1994;70(3):217–224. doi:10.1006/clin.1994.1032
31. Ling ZD, Yeoh E, Webb BT, Farrell K, Doucette J, Matheson DS. Intravenous immunoglobulin induces interferon-gamma and interleukin-6 in vivo. *J Clin Immunol*. 1993;13(5):302–309. doi:10.1007/BF00920238
32. Hamano N, Nishi K, Onose A, et al. Efficacy of single-dose intravenous immunoglobulin administration for severe sepsis and septic shock. *J Intensive Care*. 2013;1(1):4. doi:10.1186/2052-0492-1-4
33. Nimmerjahn F, Ravetch JV. Anti-inflammatory actions of intravenous immunoglobulin. *Annu Rev Immunol*. 2008;26:513–533. doi:10.1146/annurev.immunol.26.021607.090232
34. Ohlsson A, Lacy JB. Intravenous immunoglobulin for suspected or proven infection in neonates. *Cochrane Database Syst Rev*. 2015;(3): CD001239. doi:10.1002/14651858.CD001239.pub5
35. INIS Collaborative Group; Brocklehurst P, Farrell B, King A, et al. Treatment of neonatal sepsis with intravenous immune globulin. *N Engl J Med*. 2011;365(13):1201–1211. doi:10.1056/NEJMoa1100441
36. Jenson HB, Pollock BH. Meta-analyses of the effectiveness of intravenous immune globulin for prevention and treatment of neonatal sepsis. *Pediatrics*. 1997;99(2):E2. doi:10.1542/peds.99.2.e2