Fluctuations in Quality of Life and Immune Responses During Intravenous Immunoglobulin Infusion Cycles

Jordan Abbott (jordan.abbott@childrenscolorado.org)  
Children's Hospital Colorado  https://orcid.org/0000-0001-6334-5266

Sanny K Chan  
University of Colorado School of Medicine

Morgan MacBeth  
University of Colorado School of Medicine

James L Crooks  
University of Colorado School of Medicine

Catherine Hancock  
University of Colorado School of Medicine

Vijaya Knight  
University of Colorado School of Medicine

Erwin W Gelfand  
University of Colorado School of Medicine

Research Article

Keywords: IVIG, Immunoglobulin replacement therapy, quality of life, Regulatory T cell, cytokines, chemokines, wear-off

DOI: https://doi.org/10.21203/rs.3.rs-448987/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Despite adequate infection prophylaxis, variation in self-reported quality of life (QOL) throughout the intravenous immunoglobulin (IVIG) infusion cycle is a widely reported but infrequently studied phenomenon. To better understand this phenomenon, immunodeficiency subjects receiving replacement doses of IVIG were studied over 3 infusion cycles. Questionnaire data from 6 time points spread over 3 IVIG infusions cycles (infusion day and 7 days after each infusion) was collected in conjunction with monitoring the blood for number of regulatory T-cells (Treg) and levels of 40 secreted analytes: primarily cytokines and chemokines. At day 7, self-reported well-being increased, and self-reported fatigue decreased, reflecting an overall improvement in QOL 7 days after infusion. Over the same period, percentage of Treg cells in the blood increased (p<0.01). Multiple inflammatory chemokine and cytokine levels increased in the blood by 1 hour after infusion (CCL4 (MIP-1b), CCL3 (MIP-1a), CCL2 (MCP-1), TNF-α, granzyme B, IL-10, IL-1RA, IL-8, IL-6, GM-CSF, and IFN-γ). The largest changes occurred in subjects initiated on IVIG during the study. A significant decrease in IL-25 (IL-17E) following infusion was seen in a majority of intervals among subjects already receiving regular infusions prior to study entry. These findings confirm IVIG “wear off” and provide a basis for the phenomenon. Secondary findings including differences between IVIG-experienced and IVIG-naïve subjects and the novel finding of suppression of IL-25 levels in the blood following IVIG are identified as novel immunomodulatory aspects of IVIG infusion in immunodeficient patients worthy of further exploration.

Capsule Summary

IVIG "wear off" was assessed over the course of three infusion cycles by longitudinal analysis of blood analytes. Changes in levels of regulatory T cells and cytokines and chemokines were simultaneously assessed. IVIG infusion had cyclic effects on QOL, regulatory T cells, and cytokines/chemokines that warrant further investigation.

Introduction

Immunoglobulin (Ig) replacement either delivered intravenously (IVIG) or subcutaneously prevents infection in primary immunodeficiency diseases (PIDD) with defects in antibody production [1] and is used as an immunomodulatory and anti-inflammatory therapy in a variety of diseases [2]. Particularly in patients receiving IVIG for PIDD, it has been widely observed that patients experience a phenomenon of “wear-off” in quality of life or feelings of well-being following infusion [3]. Wear-off occurs in patients receiving IVIG every 3 to 4 weeks and is characterized by symptomatic improvement following infusion with subsequent deterioration at some time preceding the subsequent infusion, typically the preceding week.

Varied immune parameters fluctuate in response to IVIG. Blood Treg and inflammatory monocyte levels transiently increase in the immediate post-infusion period.[4,5] Chemokine and cytokine levels fluctuate at various time points following IVIG infusion.[6] Longer-term changes in other immune lineages have
also been associated with the initiation and continued use of IVIG infusion in immunoglobulin deficient populations.[7] These shifts suggest a dramatic interplay between IVIG and the immune system, one that provides rationale for its use in a variety of immunodeficient and immunodysregulatory conditions.

The implications of these transient immunologic alterations to clinical parameters have not been investigated extensively, particularly in relation to IVIG wear off. To address this knowledge gap, we sought to better understand the changes in immunologic parameters that occur over the time frame during which QOL changes. This study was initiated to specifically better characterize changes in either Treg numbers and/or blood cytokine and chemokine levels that occur around the times when IVIG wear off are typically reported. In this cohort of immunodeficient patients, IVIG wear-off was indeed a common phenomenon, and both Tregs and numerous chemokines and cytokines fluctuated in amount over the time period when IVIG-wear off occurred.

Methods

Study Design

Subjects were monitored over the course of 3 infusion cycles beginning with a visit on the day of the infusion and followed by a visit 7 days after the infusion (day 7). In total, there were 6 study visits. Blood was drawn on infusion days both before (pre-infusion) and 1 hour after infusion completion (post-infusion). On day 7, blood was drawn once. Questionnaires were administered on the day of infusion and on day 7 (Fig 1). This sequence was repeated 3 times for a total of 3 cycles.

Subjects

Subject data are presented in Table 1. The study was approved by the National Jewish Health IRB. Twenty-one subjects signed an IRB-approved consent form to participate in the study. One subject failed to meet enrollment criteria due to concurrent use of tocilizumab and was removed. Two subjects were enrolled but were lost to follow up prior to completing study visits on at least 2 consecutive intervals. In total, eighteen subjects with a clinical and laboratory diagnosis requiring replacement immunoglobulin therapy were enrolled and completed the study with at least 2 evaluable intervals: 15 subjects completed 6 visits, 1 subject completed 5 visits, and 2 subjects completed 4 visits. The mean age at enrollment was 50.1 years ± 17.8 years. Twelve subjects (66.7%) were female. The diagnoses included: autosomal dominant hyper IgE Syndrome (n=1), combined immunodeficiency (n=1), common variable immunodeficiency (CVID) (n=10), X-linked agammaglobulinemia (XLA) (n=3), and undefined hypogammaglobulinemia (n=3). The mean IVIG replacement dose was 494 ± 110 mg/kg. All subjects received IVIG at 4-week intervals through the duration of the study. Three subjects initiated IVIG during the study (naïve group, mean age 66.3 ± 10.5 years, all female). Twelve subjects received Gamunex-C (Grifols) and 6 subjects received Privigen (CSL Behring).

Table 1
### Patient Demographics.

| Subject | Age (yr) | IVIG Indication                  | Sex     | IVIG Dose (g/kg) | IVIG Naïve? | # Study Visits |
|---------|----------|----------------------------------|---------|------------------|-------------|----------------|
| 01      | 67       | Hypogammaglobulinemia            | Female  | 0.42             | no          | 7              |
| 02      | 52       | Combined immunodeficiency        | Male    | 0.59             | no          | 7              |
| 03      | 72       | Hypogammaglobulinemia            | Female  | 0.36             | no          | 7              |
| 04      | 61       | XLA                              | Male    | 0.63             | no          | 7              |
| 05      | 18       | AD Hyper IgE Syndrome            | Female  | 0.73             | no          | 4              |
| 06      | 51       | CVID                             | Female  | 0.48             | no          | 7              |
| 07      | 60       | Immunodeficiency                 | Female  | 0.49             | no          | 7              |
| 08      | 48       | CVID                             | Male    | 0.47             | no          | 6              |
| 09      | 24       | XLA                              | Male    | 0.66             | no          | 1              |
| 10      | 68       | CVID                             | Female  | 0.39             | no          | 7              |
| 11      | 52       | CVID                             | Female  | 0.37             | yes         | 6              |
| 12      | 60       | CVID                             | Female  | 0.51             | no          | 7              |
| 13      | 33       | XLA                              | Male    | 0.50             | no          | 7              |
| 14      | 32       | CVID                             | Female  | 0.72             | no          | 7              |
| 15      | 31       | CVID                             | Female  | 0.39             | no          | 7              |
| 16      | 33       | CVID                             | Male    | 0.40             | no          | 7              |
| 17      | 20       | XLA                              | Male    | 0.54             | no          | 5              |
| 18      | 77       | CVID                             | Female  | 0.38             | yes         | 7              |
| 19      | 57       | CVID                             | Female  | 0.53             | no          | 6              |
| 20      | 70       | Hypogammaglobulinemia            | Female  | 0.50             | yes         | 4              |
| 21      | 58       | CVID                             | Male    | 0.47             | yes         | 2              |

### Questionnaires

Subjects were administered two QOL questionnaires on the infusion day and day 7: a modified SF-12 that was adjusted to better capture perceived health over the week prior to administration of the questionnaire.
and a visual analog scale (VAS) (Fig S1). Questionnaires were administered once on each visit day.

**Measurement of T\textsubscript{reg} Levels**

Peripheral blood mononuclear cells (PBMC) were separated from heparinized whole blood using density gradient centrifugation and incubated with 20 mcL each of CD4-FITC, CD25-PE and CD3-PerCP-Cy5.5 (all purchased from BD Biosciences, San Jose, CA) for 20 minutes. The cells were permeabilized and stained with 20 mcL of anti-FoxP3-Alexa647 (BD Biosciences) for 30 minutes. Regulatory T cells were identified as CD3+CD4+CD25\textsuperscript{hi}FoxP3+ lymphocytes, gating initially on lymphocytes based on forward- and side-scatter, and were reported as a percentage of CD3+CD4+ T cells.

**Measurement of Blood Cytokines and Chemokines**

Serum analytes were measured using the Luminex Human XL Cytokine Discovery Kit (R&D Systems) run on the MAGPIX CCD imaging system (Luminex Corp.). Standard curves were generated using a 5-point weighted logistic model using xPONENT 4.2 software. For the mixed model analysis, values below the limit of detection (LOD) were removed. For the remainder of analysis, the lower of either LOD/2 or (lowest measured value/2) were used.

**Statistical Analysis**

All analyses were performed in the R language (version 3.6.0) [8]. For ordinal questionnaire data, relationships between infusion day and day 7 pre-infusion were analyzed using the clmm function from the ordinal package (version 2019.4-25) [9] with random intercept by subject, probit link, and equidistant cut-points, though similar results were found with the lmer function with Gaussian response. VAS scores and T\textsubscript{reg} numbers were analyzed using the lmer function from the lme4 package (version 1.1-21) [10] with random intercept by subject. Log-transformed cytokine and chemokine concentrations were analyzed using the lmec function in the lmec package (version 1.0) [11] with random intercept by subject and left censoring of concentrations below the assay limit of detection (LOD). The Benjamini-Hochberg procedure was used to adjust p-values to control the rate of false discoveries among the cytokines and chemokines [12]. Figures were produced with the following R packages: ggplot2, corrplot, and ggpubr.

In post-hoc analysis, VAS score was normalized to the subject mean. Kruskal-Wallis test was used to assess correlation between VAS and SF-12 responses. For cytokine analysis, concentrations below the LOD were substituted with the lesser of LOD/2 or the lowest detected value divided by 2 which varied by cytokine/chemokine. Cytokine and chemokine concentrations were log-transformed prior to analysis.

**Results**
Self-reported QOL increases following IVIG infusion

Self-reported quality of life was assessed on the day of infusion — the presumed QOL nadir — and at day 7, when the positive effect of IVIG was likely to have onset and unlikely to have diminished (Fig 1). Changes over this interval were modeled with random intercept by subject to control for inter-subject variation. Under this model, the overall VAS score increased (improved) at day 7 (p=0.0003, Table 2, Fig 2a). In the modified SF-12 questionnaire, fatigue (question 5) and general assessment of health for the prior week (question 2) decreased (improved) over this same period (p=0.03, p=0.03, Table 2, Fig 2c & 2d). We then tested for correlation between the VAS and individual items from the modified SF-12. Changes in VAS score were significantly correlated with modified SF-12 question 2 (health for the prior week) on day 7 (Kruskal Wallis, p=0.001, Fig 2b) but did not correlate with question 5 (fatigue) either at day 7 or as the difference in fatigue scores between infusion day and day 7 (p=0.13, p=0.32, Fig S2). These findings support the concept of IVIG “wear-off”. VAS scores were consistently lower on the day of infusion compared to 7 days later. Question 2 of the modified SF-12 confirms this observation, as on day 7, subjects reported their health was worse 7 days prior to questionnaire administration. In addition, fatigue over the preceding week decreased from infusion day to day 7; however, fatigue scores did not correlate with the VAS score or day 7 report of prior weeks. Therefore, the questionnaires captured two independent quality of life measures – general health and fatigue – that changed from infusion day to day 7.

| Measure                              | Coefficients | p-values | CI_2.5     | CI_97.5     |
|--------------------------------------|--------------|----------|------------|------------|
| Weekly Health (1-5 scale, 1 is high) | -0.5088      | 0.0295   | -0.9552    | -0.0624    |
| Suffer from fatigue?                 | -0.4840      | 0.0320   | -0.9154    | -0.0527    |
| VAS                                  | 4.6440       | 0.0003   | 2.1439     | 7.1441     |

Treg numbers increase following IVIG infusion

Treg numbers were measured at 3 time points during the cycle: before infusion, one hour after infusion completion, and 7 days after the infusion. There were no significant changes in numbers between the pre-infusion and post-infusion blood draw; however, levels increased at the visit 7 days following the infusion (p=0.01, Fig 3 and Fig S3A). We tested whether Treg levels on infusion day, day 7, or change from pre-infusion to day 7 correlated with changes in VAS score or fatigue score over the same time interval. There was no correlation between Treg levels and changes in VAS score or changes in fatigue score (Fig S3B & S3C), suggesting either the absence of a relationship between increases in Treg numbers and the assessed QOL measures, or insufficient numbers of measurements to identify such a relationship.
Changes in serum cytokine and chemokine levels during the infusion cycle

Multiple changes in cytokine and chemokine levels occurred between the pre- and 1h post-infusion blood draws. CCL2, CCL3, CCL4, TNF-a, granzyme B, IL-10, IL-1RA, IFN-g, IL-8, and CCL20 increased while IL-25 decreased (Fig S8). After stratification of naïve vs. IVIG-experienced subjects, (Fig 4) additional significant increases in IL-6 and GM-CSF and significant decreases in EGF and CD40L were seen in IVIG-naïve subjects. In IVIG-experienced subjects, CCL3, CCL4, and granzyme B were the only significant cytokine increases, with increases in CCL2, TNF-a, IL-10, IL-1RA, IFN-g, IL-8, and CCL20 no longer attaining significance. Interestingly, the decrease in IL-25 level identified in the non-stratified subject sample was entirely attributable to the non-naïve group and was not detected in IVIG-naïve subjects (Fig 4). Thus, IVIG infusion in IVIG-naïve subjects was rapidly followed by an increase in a subset of inflammatory cytokines in the blood. In IVIG-experienced subjects, a partially overlapping set of changes was identified with similar but lower magnitude changes in CCL3, CCL4, and granzyme B, and a distinct decrease in IL-25 that was not detected in the naïve subjects.

To determine whether these changes represented large changes in few subjects or whether these changes were widespread, a direction of change was assigned for each interval and the proportions of direction of change were examined (Fig 5). CCL3, CCL4, granzyme B, TNF-a, and IFN-g increased for the majority of pre-to-post infusion intervals, whereas IL-10, CCL2, IL-6, GM-CSF, IL-8, and IL-1RA were increased in only the minority. Among the cytokines found to decrease significantly, CD40 ligand, IL-25, and EGF decreased for the majority of pre-to-post infusion intervals (Fig 5, Fig S4, Fig S5, Fig S6). These findings suggest that increases in the levels of CCL3, CCL4, granzyme B, TNF-a, and IFN-g are typical changes in response to IVIG infusion, as are decreases in CD40 ligand, IL-25, and EGF.

Comparing pre-infusion to day 7, no changes in cytokine levels reached statistical significance of adjusted p-value < 0.05; however, IL-25 trended towards a decrease during the interval (p = 0.051). As IL-25 was decreased immediately after infusion, we further determined that the log-fold change in IL-25 between the pre-infusion and post-infusion time point was not different than the pre-infusion and day 7 time points (paired T-test, p=0.46, Fig S7). Therefore, in the IVIG-experienced group, IL-25 levels decrease immediately following IVIG infusion and frequently remain low at least 7 days post-infusion. IL-25 decreases occurred in 55% of pre-to-post intervals and 58% of pre-to-day-7 intervals and increases occurred in 30% of both intervals. Thus, IVIG infusions are frequently associated with immediate and prolonged decreases in IL-25 in a subset of subjects.

Correlation analysis links clusters of cytokines

To better understand the relationship between cytokines, we calculated correlation coefficients for changes in cytokine/chemokine levels before and immediately after infusion. In IVIG-experienced subjects, CCL2, CCL3, CCL4, TNFa, IL-8, IL-1RA, granzyme B, and IL10 were highly correlated (Fig 6). This
suggests that these cytokines were secreted by the same cell or at least were induced in response to the same stimulus. IL-25 was not correlated with this group of cytokines, indicating that decreases in IL-25 occurred independently of increases in the identified inflammatory cytokines and chemokines.

Relationships between cytokine changes and QOL measures

Changes in cytokine levels were tested for relationships with either the change in VAS or fatigue score from infusion day to day 7. IVIG-naïve subjects were not evaluated independently as the number of measurements was inadequate to power such an analysis. No linear relationship was seen between change in VAS or change in fatigue score and any of the cytokines or chemokines that were significantly changed in the study. Since the largest changes in cytokine levels were detected in the small number (3) of IVIG-naïve subjects, this result was not surprising but warrants further analysis in a larger cohort.

Discussion

The objective of this study was to better understand the IVIG “wear off” phenomenon by characterizing changes in Tregs, cytokines, and chemokines in blood samples drawn around the times when wear off occurs. Despite the small sample size, we identified several aspects. We confirmed that following infusion of replacement doses of IVIG there is a decline in quality of life in a subset of patients over time. We showed that Treg levels increase over 7 days following IVIG infusion. We showed that several serum cytokines and chemokine levels change immediately and at the day 7 time point. We performed an analysis to determine if these blood changes correlated with changes in QOL measures but were unable to identify any clear relationship. In this patient cohort, IVIG improved measures of general health and fatigue while simultaneously affecting Treg, chemokine, and cytokine levels, but the relationship between these cytokine changes and self-perceived quality of life remains to be defined.

The “wear off” phenomenon has been reported in multiple contexts[3,13,14], and patients with a primary immune deficiency disease often report a significant diminished quality of life compared to their “normal” counterparts. In this study, 50% of all intervals between day 7 and the next infusion were associated with a decrease in VAS score. These intervals where worsening occurred involved a majority of patients (12 of 17, 70%) with evaluable intervals. Rojavin et al. reported “wear off” in 43% of patients on a 4-week cycle but only in 10% of all cycles [3]. Our approach was different in that they analyzed QOL changes between week 2 and the last week of dosing after decreases in QOL occurs which may account for some of the differences in the two studies. Additional variation could arise from differences in the patient population, length of IVIG treatment, and geography.

The impact of IVIG infusion on T\textsubscript{reg} cells has been studied extensively in the context of inflammatory disease and less so in primary immunodeficiency. The proportion of Tregs increased following high-dose IVIG-treatment in patients with vasculitis[15,16], Guillain-Barre syndrome[17], and Kawasaki disease[18].
Likewise, a prior report indicated an increased percentage of CD4 T<sub>reg</sub> 30 minutes following replacement-dose IVIG infusion in CVID patients [4]. While we did not confirm that T<sub>reg</sub> levels as a percentage of CD4 T-cells increase immediately following infusion, we saw a significant increase at the day 7 in patients receiving replacement-dose IVIG. This change in Treg proportion did not appear to be associated with QOL changes over the measured interval, suggesting that in this cohort, Tregs were not involved in the process that drives improved QOL over the week following infusion.

This study aimed to identify associations between cytokine changes and variation in QOL through the IVIG cycle. Despite identifying quite large immediate cytokines changes, we did not find associations between these changes and the reported QOL improvements. There a multiple likely reasons for this. First, most cytokine changes were predominantly the result of the few IVIG-naïve subjects, whereas the magnitude of cytokine changes in the IVIG-experienced group was of much lower magnitude. Second, the low number of enrolled subjects restricts the power of the study to identify subtle correlations missed here. Third, it is possible that timing of this relationship differs from that designed in the study, in that additional analyte changes may be occurring between the infusion and day 7. In light of these deficiencies, it is impossible to rule out the possibility that a relationship between these cytokine changes and QOL changes was missed.

Despite not finding cytokine-QOL associations, this study identified important and potentially relevant changes in cytokine levels that follow IVIG infusion in patients with hypogammaglobulinemia. It has previously been reported that inflammatory cytokines such as TNF-α, IL-6, IL-1RA, and IL-8 levels increased following IVIG infusion in similar patient populations;[6,19] however, we have shown that these changes predominate in IVIG-naïve subjects, suggesting an adaptation in experienced subjects that diminishes these responses. At least part of this process remains active in IVIG-experienced subjects, as evidenced by the statistically significant increases in CCL3, CCL4, and granzyme B that persist, albeit at a lower level. The timing of this adaptation has not yet been studied but is likely greater than 3 months, as dramatic shifts in cytokines were still observable during the third infusion in the IVIG-naïve group. Another novel finding was the decrease in serum IL-25 level detected both after infusion and 7 days later in a subset of IVIG-experienced subjects. IL-25, among other functions, amplifies atopic inflammation and is produced by various cell types including epithelial cells, Th2 cells, alveolar macrophages, mast cells, basophils, and eosinophils can produce IL-25.[20] The attenuation of levels of this cytokine provides a partial rationale for the clinical efficacy of IVIG in allergic disease.[21]

In summary, in this study, the QOL "wear off effect" following IVIG infusion was confirmed, and although several potentially-relevant factors were identified, a clear role for these factors in this effect remain elusive. In this cohort, increases in Treg levels and various serum chemokines and cytokines did not correlate with reported improvement and subsequent deterioration in QOL throughout the IVIG cycle. Nonetheless, novel findings regarding replacement-dose IVIG infusions were discovered particularly relating to the differential response of IVIG-naïve vs. IVIG-experienced subjects. This constellation of findings provides a framework for future work exploring the non-infectious physiologic alterations
induced by IVIG infusions and how they relate to the feeling of well-being among the highly burdened PIDD patient population.

**Declarations**

**Acknowledgements**

We are grateful to the patients who participated in the study.

**Authorship Contributions**

JKA and EWG conceived of the study; JKA, SKC, EWG, and KH coordinated the study; MM and VK performed experiments; JKA, SKC, and EWG analyzed the data; and JKA, SKC, and EWG wrote and edited the manuscript.

**Funding**

This work was carried out under an unrestricted grant from CSL Behring SKC, JKA and EWG. JKA receives funding from NIH K08AI141734.

**Disclosure of Conflicts of Interests**

This work was carried out under an unrestricted grant from CSL Behring to Sanny Chan, Jordan Abbott and Erwin Gelfand.

**References**

1. Orange J, Hossny E, Weiler C. Use of intravenous immunoglobulin in human disease: a review of evidence by members of the Primary Immunodeficiency Committee of the American Academy of. Journal of Allergy and Clinical Immunology. 2006;

2. Gelfand EW. Intravenous Immune Globulin in Autoimmune and Inflammatory Diseases. New England Journal of Medicine. 2012;367:2015–25.

3. Rojavin MA, Hubsch A, Lawo J-P. Quantitative Evidence of Wear-Off Effect at the End of the Intravenous IgG (IVIG) Dosing Cycle in Primary Immunodeficiency. Journal of clinical immunology. J Clin Immunol; 2016;36:210–9.

4. Kasztalska K, Ciebiada M, Cebula-Obrzut B, Górski P. Intravenous immunoglobulin replacement therapy in the treatment of patients with common variable immunodeficiency disease: an open-label prospective study. Clinical drug investigation. Clin Drug Investig; 2011;31:299–307.
5. Siedlar M, Strach M, Bukowska-Strakova K, Lenart M, Szafierska A, Węglarczyk K, et al. Preparations of intravenous immunoglobulins diminish the number and proinflammatory response of CD14+CD16++ monocytes in common variable immunodeficiency (CVID) patients. Clinical Immunology. 2011;139:122–32.

6. Ibáñez C, Suñé P, Fierro A, Rodríguez S, López M, Alvarez A, et al. Modulating effects of intravenous immunoglobulins on serum cytokine levels in patients with primary hypogammaglobulinemia. BioDrugs. 2005;19:59–65.

7. Quinti I, Mitrevski M. Modulatory Effects of Antibody Replacement Therapy to Innate and Adaptive Immune Cells. Front Immunol. 2017;8:697.

8. R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available online at https://www.R-project.org/. [Internet]. Available from: https://www.r-project.org/

9. Christensen RHB (2019). "ordinal—Regression Models for Ordinal Data ." R package version 2019.12-10. https://CRAN.R-project.org/package=ordinal. [Internet]. [cited 2020 Jun 16]. Available from: https://cran.r-project.org/web/packages/ordinal/citation.html

10. Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software. 2015;67:1–48.

11. Vaida F, Liu L. Package ‘ lme4 ‘. R. 2015;1–6.

12. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society: Series B (Methodological). John Wiley & Sons, Ltd; 1995;57:289–300.

13. Rider NL, Kutac C, Hajjar J, Scalchunes C, Seeborg FO, Boyle M, et al. Health-Related Quality of Life in Adult Patients with Common Variable Immunodeficiency Disorders and Impact of Treatment. Journal of clinical immunology. J Clin Immunol; 2017;37:461–75.

14. Misbah SA. Effective dosing strategies for therapeutic immunoglobulin: managing wear-off effects in antibody replacement to immunomodulation. Clinical and experimental immunology. Clin Exp Immunol; 2014;178 Suppl 1:70–1.

15. Bayry J, Mouthon L, Kaveri SV. Intravenous immunoglobulin expands regulatory T cells in autoimmune rheumatic disease. J Rheumatol. 2012;39:450–1.

16. Tsurikisawa N, Saito H, Oshikata C, Tsuburai T, Akiyama K. High-dose intravenous immunoglobulin treatment increases regulatory T cells in patients with eosinophilic granulomatosis with polyangiitis. J Rheumatol. 2012;39:1019–25.

17. Maddur MS, Trinath J, Rabin M, Bolgert F, Guy M, Vallat J-M, et al. Intravenous immunoglobulin-mediated expansion of regulatory T cells in autoimmune patients is associated with increased prostaglandin E2 levels in the circulation. Cell Mol Immunol. 2015;12:650–2.

18. Olivito B, Taddio A, Simonini G, Massai C, Ciullini S, Gamberini E, et al. Defective FOXP3 expression in patients with acute Kawasaki disease and restoration by intravenous immunoglobulin therapy. Clin Exp Rheumatol. 2010;28:93–7.
19. Aukrust P, Frøland SS, Liabakk NB, Müller F, Nordøy I, Haug C, et al. Release of cytokines, soluble cytokine receptors, and interleukin-1 receptor antagonist after intravenous immunoglobulin administration in vivo. Blood. 1994;84:2136–43.

20. Roan F, Obata-Ninomiya K, Ziegler SF. Epithelial cell-derived cytokines: more than just signaling the alarm. J Clin Invest. 2019;129:1441–51.

21. Rabinovitch N, Gelfand EW, Leung DY. The role of immunoglobulin therapy in allergic diseases. Allergy. 1999;54:662–8.

Figures

Fig 1

![Diagram showing cycle 1, 2, and 3 with questionnaire and blood draw](image-url)
Outline of study visits. Subjects participated in three identical cycles. Each cycle consisted of an infusion-day visit and a day-7 visit (2 visits per cycle). Questionnaires were completed once per study visit day for a total of 6 time points. Blood was drawn for Treg levels and blood analytes 3 times per cycle, twice on the infusion day (before infusion and 1 hour after completion) and once at the day-7 visit.

**Figure 2**

A. Paired boxplot representing the change in normalized VAS score from infusion day to the day-7 visit. The box borders represent the first and third quartiles with the whiskers extending to 1.5*(interquartile range). Median is represented by a horizontal line. VAS scores were normalized to Z-scores by subtracting the mean for each subject and dividing by the standard deviation. B. Boxplot of response to question 2 from the modified SF-12: “Compared to the week before, how would you rate your health in general now?” vs. change in normalized VAS score between infusion day and day 7. Box features as in 2A with mean scores are marked by a short horizontal bar. Patients who reported no improvement (#3) or worsening (#4) of general health had lower mean normalized VAS scores at day 7 and patients who reported improvement (#1 or #2) had higher mean normalized VAS scores. C. Paired boxplot representing change in weekly-health score
(question 2) between infusion day and day 7. Scores were normalized to the mean for each subject. D. Paired boxplot representing change in fatigue scores (question 5) between infusion day and day 7. Fatigue scores were normalized to the mean for each subject. Infusion day scores are enriched for positive values (more fatigue) and day 7 scores are enriched for negative scores (less fatigue).

**Figure 3**

Proportion of T cells with Treg Phenotype Increases from Pre-Infusion to Day 7. Paired boxplot representing the change in Tregs as a percentage of CD4+ T cells from the pre-infusion blood draw to day 7. P-value is derived from the random effects model of the relationship between day 7 and the pre-infusion draw.
Figure 4

Statistically-significant Cytokine Changes Between the Pre-infusion and Post-infusion Blood Draws Plot of the mean (dot) and confidence interval (whiskers) for the coefficients of the pre-infusion to post-infusion interval. Subjects were separated by whether IVIG treatment was initiated during the study (naïve) or if the subject was already receiving IVIG regularly (non-naïve). P-values for coefficients were adjusted by the Benjamini-Hochberg method. Adjusted p-values < 0.05 are highlighted in red.
Figure 5

Proportions of Intervals Where Cytokines Change Varies Between Cytokines

Stacked bar graph for each cytokine found to change between the pre- and post-infusion measurements. Direction of change is signified by depth of color as shown in the figure legend. "Up" signifies a greater than 10% increase and "down" signifies a greater than 10% decrease.
Figure 6

Correlations of Cytokines that Significantly Change Between the Pre-infusion and Post-infusion Blood Draws Pearson's correlation between cytokine pairs is indicated by color and size of dots. The larger and deeper colored dots indicate a higher r value. The analytes were hierarchically clustered, and the black boxes correspond to clusters generated at a level of 6 clusters.

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.

- figureS1.pdf
- Suppmentaryfig28.pdf