In vitro sensitivity assays and clinical response to glucocorticoids in patients with inflammatory bowel disease☆

Joseph C. Maranvillea,b,c, Dejan Micicb, Stephen B. Hanauera,b, Anna Di Rienzoa,c,⁎, Sonia S. Kupferb,⁎⁎

a Committee on Clinical Pharmacology and Pharmacogenomics, The University of Chicago, Chicago, IL, USA
b Department of Medicine, The University of Chicago, Chicago, IL, USA
c Department of Human Genetics, The University of Chicago, Chicago, IL, USA

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Abstract

Background: Glucocorticoids (GCs) are steroid hormones used to induce remission in moderate-to-severe inflammatory bowel disease (IBD). A substantial fraction of patients do not respond to GC treatment and require alternate therapies or surgery. At present, non-response can only be assessed empirically by observing continued disease activity.

Methods: To identify potential biomarkers of GC response, we retrospectively identified and recruited 18 GC-responsive and 18 GC-nonresponsive IBD patients. This sample included 14 patients with ulcerative colitis (UC) and 22 patients with Crohn’s disease (CD), all previously treated with steroids. In peripheral blood mononuclear cells from each patient, we performed in vitro assays to measure GC inhibition of three different immune stimulants (phytohemagglutinin [PHA], α-CD3/α-CD28, and lipopolysaccharide [LPS]).

Results: In both diseases, we found that inhibition of PHA-mediated T cell proliferation was significantly associated with clinical GC response (P = 0.04). Inhibition of proliferation due to direct T cell receptor stimulation using α-CD3/α-CD28 was also significantly associated with clinical GC response in UC patients (P = 0.009), but not in CD patients (P = 0.78). Interestingly, inhibition of LPS-mediated cytokine secretion showed the strongest association with clinical GC response across both diseases (P = 0.005).

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⁎ Correspondence to: A. Di Rienzo, Department of Human Genetics, University of Chicago, 920 E. 58th Street, Rm. 424, Chicago, IL 60637, USA. Tel.: +1 773 834 1037; fax: +1 773 834 0505.

⁎⁎ Correspondence to: S. S. Kupfer, Department of Medicine, University of Chicago, 900 E. 57th Street, MB #9, Chicago, IL 60637, USA. Tel.: +1 773 834 8632; fax: +1 773 702 2281.

E-mail addresses: dirienzo@uchicago.edu (A. Di Rienzo), Sonia.Kupfer@uchospitals.edu (S.S. Kupfer).

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1. Introduction

Glucocorticoids (GCs) are steroid hormones that are widely used as therapeutic agents due to their potent anti-inflammatory and immunosuppressive effects. GCs play a critical role in the treatment of inflammatory bowel disease (IBD), including both ulcerative colitis (UC) and Crohn’s disease (CD), where they are used to induce remission in patients with moderate to severe disease. Although GCs have a long history of use, many patients do not adequately respond to treatment and require alternative medical therapies and/or surgery. A prospective cohort study following CD patients through a course of the synthetic GC prednisolone found that 20% of patients were GC resistant (no regression of symptoms) and 59% of patients required surgery within 1 month of initiating GC therapy. Interestingly, this figure is consistent with the rate of non-response in other immune-related diseases treated with GCs. These observations could reflect similar rates of intrinsic ‘GC resistance’ in the general population.

Several studies have shown that GC resistance is largely independent of clinical factors, such as disease severity. In one study the rate of GC resistance in severe disease (42%) was only slightly larger than that in moderate disease (35%). Few, if any, predictors of clinical response to GCs exist in IBD. Biomarkers that could identify GC response in patients before treatment would improve IBD management. For example, such biomarkers would allow patients to be treated with alternative medical therapies (e.g. biologics) or undergo more expeditious surgeries while avoiding the well-known and ubiquitous GC side effects associated with prolonged exposure.

While clinical responsiveness to GC therapy is poorly correlated with disease severity, it is correlated with in vitro measurements of GC sensitivity. GC inhibition of phytohemagglutinin (PHA)-induced T-cell proliferation has been correlated with GC response in severe UC as well as other diseases. Interestingly, all of these studies measured GC response in peripheral blood cells, rather than tissue from the disease site, suggesting that there is sufficient overlap in the mechanisms of GC nonresponsiveness between these cell types to make peripheral blood cells a suitable, and more accessible, proxy for the colon. Lee et al. directly stimulated the T cell receptor (TCR) with α-CD3/α-CD28 and found that T cells from steroid resistant UC patients showed less GC inhibition of proliferation compared to healthy controls. A similar result was observed in patients with uveitis, where in vitro measures of GC inhibition of T cell proliferation were estimated to have a 90% positive predictive value for clinical response. While these studies suggest that in vitro sensitivity assays could be useful predictors, replication of these results is needed to define the scope of their applicability across disease types.

Previous studies have focused on assays that measure GC inhibition of T cell proliferation, a key feature of the adaptive immune response. However, an assay based on response to bacteria and other aspects of the innate immune response may be more relevant for IBD as these processes play a critical role in pathogenesis. Pathway analyses based on results from genome-wide association studies have implicated genes involved in cytokine production and bacterial response in the pathogenesis of both CD and UC. Furthermore, a recent proteomic study of serum and tissue from mouse models and patients with IBD revealed protein signatures of active disease that were consistent with stimulation of an innate immune response. The aberrant response to bacteria by IBD patients is recapitulated by in vitro measures of response to bacterial molecules, such as lipopolysaccharide (LPS). Mazlam and Hodgson found that peripheral monocytes from patients with CD showed greater LPS induced secretion of pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β) relative to both healthy controls and patients with UC. These results raise the intriguing possibility that GC inhibition of LPS-induced cytokine secretion could be an additional in vitro predictor of GC response. Hew et al. found that GC inhibition of LPS stimulation was significantly different in patients with severe versus moderate asthma, suggesting that severe asthma reflected GC resistance. A single study has tested an LPS assay of GC response in IBD patients. This study found no association with clinical response comparing GC inhibition of LPS-induced cytokine secretion between GC-dependent CD patients, GC-responsive CD patients, and healthy controls. However, the investigators did not compare responsive patients to those who were non-responsive to GC treatment, instead comparing to patients who required continued GC treatment (i.e. GC-dependence).

Given limitations in previous studies, we sought to compare in vitro measures of GC inhibition using adaptive and innate immune stimulants in order to identify potential biomarkers of GC response in IBD patients and provide insights into the biological processes that underlie variation in clinical response to GCs. We collected peripheral blood mononuclear cells (PBMCs) from IBD patients, assessed retrospectively as responders or non-responders, and measured GC inhibition using three immune stimulants: PHA, αCD3/αCD28, and LPS. We found that clinical responders showed significantly greater GC inhibition of LPS-induced cytokine secretion compared to non-responders. We also found greater inhibition of PHA-stimulated proliferation in clinical responders. For GC inhibition of αCD3/αCD28 stimulated proliferation, we found a significant correlation with clinical response in UC, but not CD patients.
2. Materials and methods

2.1. Patients

Patients with confirmed diagnoses of UC or CD were enrolled through the University of Chicago IBD clinic between May 2012 and July 2013. All patients signed informed consent prior to inclusion. Electronic medical records were reviewed to identify 18 patients who were GC non-responsive (‘clinical non-responders’) and 18 who were GC responsive (‘clinical responders’). We defined clinical non-responders as patients who previously had received GC therapy, either PO prednisone or IV methylprednisolone, but continued to have symptoms as documented in the medical record and, as a result, were transitioned to an alternative medical therapy (i.e. a biologic or cyclosporine) or had surgery. We defined clinical responders as patients who had successfully responded to GCs in all recorded encounters, with at least one successful encounter described in the medical record. A successful response was defined as a case where the patient used to maintain remission.

Induction of remission by GCs. In these cases, biologics were used to maintain remission. The use of oral or intravenous corticosteroids within three months of recruitment was recorded. Clinical disease activity and recruitment was recorded. The need for 90-day follow-up. Concurrent medications included 5-ASA agents, immunomodulators (azathioprine, 6-mercaptopurine) or biologic agents (infliximab, adalimumab). We used a range of Dex concentrations, but found that inhibition at the highest tested concentration (1 μM) was especially informative for distinguishing clinical non-responders. Consequently, we used the same high concentration that had previously shown a correlation between inhibition of cell proliferation and with clinical response. In parallel, one cell aliquot was cultured in three replicates, but was not treated (blank). The final concentration of ETOH in Dex- and vehicle-treated (i.e. only ETOH) aliquots was 0.1 μl of ETOH per 1 ml of cell culture media; therefore, the effect of ETOH on cellular processes is expected to be negligible. For an additional 6 subjects (3 responders and 3 non-responders), three replicates of each of the following treatments were performed: no treatment (blank), 1 μM Dex + 5 μg/ml PHA, ETOH + 5 μg/ml PHA, 1 μM Dex + αCD28/αCD3, and ETOH + αCD28/αCD3. Similar studies in patients with UC used a range of Dex concentrations, but found that inhibition at the highest tested concentration (1 μM) was especially informative for distinguishing clinical non-responders. Consequently, we used the same high concentration that had previously shown a correlation between inhibition of cell proliferation and with clinical response. In parallel, one cell aliquot was cultured in three replicates, but was not treated (blank). The final concentration of ETOH in Dex- and vehicle-treated (i.e. only ETOH) aliquots was 0.1 μl of ETOH per 1 ml of cell culture media; therefore, the effect of ETOH on cellular processes is expected to be negligible. For an additional 6 subjects (3 responders and 3 non-responders), three replicates of each of the following treatments were performed: no treatment (blank), 1 μM Dex + 5 μg/ml PHA, ETOH + 5 μg/ml PHA. After 48 h of treatment, cell proliferation was measured by H3-thymidine incorporation, using standard protocols, at the Human Immunologic Monitoring Facility at the University of Chicago. Briefly, H3-thymidine was added for the last 6 h of the 48 h treatment period. Afterward, PBMCs were harvested onto glass-fiber filter paper and radiolabel was counted in a β-spectrometer in units of counts per minute (cpm). The median value was taken from across the three replicates.

2.4. Measuring in vitro inhibition of lymphocyte proliferation

PBMCs from each subject were grown in 96 well plates with 1 x 10^5 cells per well. For each of 30 subjects, three replicates of each of the following treatments were performed: 1 μM dexamethasone (Dex) + 5 μg/ml PHA, ETOH (vehicle for Dex) + 5 μg/ml PHA, 1 μM Dex + αCD28/αCD3, and ETOH + αCD28/αCD3. Similar studies in patients with UC used a range of Dex concentrations, but found that inhibition at the highest tested concentration (1 μM) was especially informative for distinguishing clinical non-responders. Consequently, we used the same high concentration that had previously shown a correlation between inhibition of cell proliferation and with clinical response. In parallel, one cell aliquot was cultured in three replicates, but was not treated (blank). The final concentration of ETOH in Dex- and vehicle-treated (i.e. only ETOH) aliquots was 0.1 μl of ETOH per 1 ml of cell culture media; therefore, the effect of ETOH on cellular processes is expected to be negligible. For an additional 6 subjects (3 responders and 3 non-responders), three replicates of each of the following treatments were performed: no treatment (blank), 1 μM Dex + 5 μg/ml PHA, ETOH + 5 μg/ml PHA. After 48 h of treatment, cell proliferation was measured by H3-thymidine incorporation, using standard protocols, at the Human Immunologic Monitoring Facility at the University of Chicago. Briefly, H3-thymidine was added for the last 6 h of the 48 h treatment period. Afterward, PBMCs were harvested onto glass-fiber filter paper and radiolabel was counted in a β-spectrometer in units of counts per minute (cpm). The median value was taken from across the three replicates.

2.5. Measuring in vitro inhibition of cytokine secretion

PBMCs from each of the 36 subjects were grown in 96-well plates with 1 x 10^5 cells per well. For each subject, three replicates of each of the following treatments were performed: 1 μM Dex + 1 μg/ml LPS, ETOH + 1 μg/ml LPS; a cultured but untreated aliquot was also collected in parallel (blank). As described for cellular proliferation assays, the concentration of ETOH in Dex- and vehicle-treated aliquots was 0.1 μl of ETOH per 1 ml of cell culture media. After 48 h of treatment, IL6 and IL1β levels were measured in the supernatant from each well using ELISAs, using Duo set ELISA kits from R&D Systems for IL1β (Cat# DY201) and IL6 (Cat# DY206), at the Human Immunologic Monitoring Facility at the University of Chicago. The median value was taken across the three replicates.
2.6. Statistical analysis

GC inhibition was summarized for each immune stimulant by taking the log₂-fold change (LFC) in either counts per million (for proliferation) or cytokine levels (ng/μl). All assays were performed in triplicate for 30 patients and in duplicate for 6 patients. We found that both proliferation and cytokine assays were highly reproducible. The median coefficient of variance (based on the log-normal distribution) across all conditions and assays was 0.097. Furthermore, 93% of assays showed a coefficient of variance less than 0.25 and 97% with CV less than 0.5. Using ANOVA, we found significant batch effects for LFC in cytokine secretion (IL1β: P = 2.3 × 10⁻⁷, IL6 P = 1.6 × 10⁻⁴). Consequently, we used linear regression to correct cytokine LFC estimates for batch. LFC for all assays were fit to a normal distribution across patients using quantile normalization. We used one-tailed t-tests to test for higher LFC (less sensitivity) in clinical non-responders for all three stimulants. To test for the effect of disease type on the association between LFC and clinical response, we fit a linear regression model where LFC was regressed on clinical response, disease type, and an interaction term to identify disease-specific associations. To directly compare the relative ability of different assays to distinguish clinical responders from non-responders, we fit a logistic model with probability of clinical non-response regressed on LFC measurements from multiple assays. All statistical analyses were performed in the R statistical package.

2.7. Ethical consideration

This study complied with the tenets of the Declaration of Helsinki. The study was reviewed and approved by an Institutional Review Board at the University of Chicago (IRB #12-1643). Informed consent was obtained from all individuals included in the study.

3. Results

3.1. Patient population

We recruited 36 outpatients from the IBD clinic at the University of Chicago. These patients included 18 clinical responders and 18 clinical non-responders, based on retrospective review of the medical record. This sample was comprised of both UC (n = 14) and CD (n = 22) patients. We found no significant differences between clinical responders and non-responders in gender, age, ethnicity, disease type, or current medications (see Table 1, P > 0.05). Clinical non-responders had significantly longer duration of disease at sampling (Table 1, P = 0.035) and were significantly more likely to have had IBD-related surgery (Table 1, P = 0.035). Neither of these covariates was associated with any of the in vitro measures of GC sensitivity described below (P > 0.1). Twenty-nine of 36 recruited patients were in partial or complete clinical remission at the time of study entry and 33 out of 36 had not had GC exposures within three months of recruitment (Table 1). Four patients required a change in clinical management at the time of recruitment. All patients were treated with oral GCs; additionally, we found 18 patients who had a record of IV GC exposure in the past. We found no significant differences between clinical responders and non-responders for these factors (Table 1, P > 0.1).

3.2. Lower inhibition of PHA-mediated lymphocyte proliferation in clinical non-responders

To characterize in vitro GC sensitivity, we stimulated PBMCs from each patient using the mitogen PHA. We then measured PHA-mediated cellular proliferation in the presence of GCs (Dex) or its vehicle (EtOH). We observed significantly higher levels of cellular proliferation (relative to untreated controls) in aliquots stimulated with PHA (median 13.1 fold increase, P = 9 × 10⁻²²). We found that PHA response was significantly higher in UC patients relative to patients with CD (P = 0.0014). PHA response was not significantly different between clinical responders and non-responders (P = 0.19).

We observed significantly less proliferation, relative to stimulated controls, in the presence of Dex (median 2.3 fold decrease, P = 4 × 10⁻⁹). We used the log₂-fold change in proliferation (Dex/vehicle) to summarize sensitivity for each individual (PHA–LFC). Consistent with previous observations in UC and other inflammatory diseases, we found that clinical responders showed significantly lower PHA–LFC compared to clinical non-responders, corresponding to greater GC inhibition of PHA-stimulated proliferation (Fig. 1, P = 0.04). In contrast, PHA–LFC was not significantly associated with age (P = 0.71), gender (P = 0.99), ethnicity (P = 0.13), disease type (P = 0.21), or current medication (P = 0.53). We used linear regression to test for differences between diseases in the association between PHA–LFC and clinical response. Specifically, we fit a linear model where PHA–LFC was regressed on clinical response status, disease status, and an interaction term. The interaction term represented a disease-specific association between clinical response and PHA–LFC. This analysis did not reveal differences in the strength of this association between diseases (interaction term P = 0.97, Supplementary Fig. 1A).

3.3. Association between inhibition of TCR stimulation and clinical response in ulcerative colitis

In parallel with PHA assays, we measured GC inhibition of direct stimulation of the TCR for 30 of the 36 subjects. Specifically, we stimulated PBMCs from each patient with αCD3/αCD28, which acts to cross-link the TCR and induce T cell proliferation, in aliquots treated with Dex or with vehicle. We found that αCD3/αCD28 caused a significant increase in cellular proliferation relative to aliquots with no treatment (median 13.4 fold increase, P = 9 × 10⁻¹⁹). The effects of αCD3/αCD28 on proliferation were very similar to PHA effects, in that fold increases were of similar magnitude and strongly correlated across patients (Fig. 2, r² = 0.82, P = 5 × 10⁻¹²). We also found that Dex-treated aliquots showed significantly less proliferation than vehicle-treated aliquots (median 1.3 fold decrease, P = 0.04). However, GC inhibition of stimulation by αCD3/αCD28 was significantly weaker than inhibition of PHA-mediated proliferation (P = 5 × 10⁻¹⁰). As we did for PHA, we used the log₂ fold change in proliferation (Dex/vehicle) to summarize sensitivity for each individual (TCR–LFC). We did not find significant
associations between TCR–LFC and age (P = 0.68), gender (P = 0.85), ethnicity (P = 0.27), disease type (P = 0.71), or current medication (P = 0.52).

In contrast to a previous study in UC patients, we did not find significant differences in TCR–LFC between clinical responders and non-responders (P = 0.3). This discrepancy could reflect the inclusion of patients with CD in our study. Although we found no significant differences in TCR–LFC between UC and CD patients when clinical response status was not considered, it is possible that TCR–LFC differs between clinical responders and non-responders only in patients with a particular disease. To test this hypothesis, we used linear regression to test for a disease-specific association between clinical response and TCR–LFC. We found a significant interaction between disease and clinical responder status for TCR–LFC (Fig. 2, P = 0.04), representing significantly less GC inhibition in non-responders only in UC patients. To test if this pattern reflected differences in treatment exposure between these diseases, we compared medications used at recruitment between CD and UC patients, but found no significant difference (P = 0.69, Supplementary Fig. 1B).

3.4. Lower inhibition of LPS-mediated cytokine secretion in clinical non-responders

To explore the role of innate immunity in GC resistance, we measured LPS-mediated secretion of the pro-inflammatory cytokines IL6 and IL1β in Dex- and vehicle-treated PBMCs from each patient. We found that LPS stimulation increased secretion of both cytokines (median 20 fold increase for IL-1β, P = 2 × 10⁻¹¹; median 15 fold increase for IL-6, P = 0.001). Dex treatment led to a decrease in secretion of both cytokines (median 4.2 fold decrease for both cytokines, for IL-1β P = 8 × 10⁻¹¹ and for IL-6 P = 1 × 10⁻¹²). We used the log₂ fold change in secretion (Dex/vehicle) to summarize sensitivity for each individual and each cytokine. Because inhibition of these cytokines was significantly correlated across individuals (r² = 0.2, P = 0.017), we used the mean log₂ fold change as a measure of overall GC inhibition of LPS stimulation (LPS–LFC). LPS–LFC was not significantly associated with the clinical covariates, including: age (P = 0.23), gender (P = 0.98), ethnicity (P = 0.18), disease type (P = 0.99), or current medication (P = 0.70). We found that clinical responders showed significantly greater GC inhibition of cytokine secretion compared to non-responders.

### Table 1 Patient characteristics. P-values shown in bold are significant at a threshold of < 0.05.

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<th>Responders</th>
<th>Non-responders</th>
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ᵃ Refers to active disease at time of blood sampling.
ᵇ Five patients had ileal involvement (3 responders and 2 non-responders) and 1 patient had jejuno-ileal involvement (non-responder).
ᶜ Three CD patients had fistulizing disease (1 responder and 2 non-responders).
secretion compared to clinical non-responders (Fig. 3A, $P = 0.005$). This represented a significant association between clinical response and IL-1β. LFC ($P = 0.012$) and a suggestive association with IL-6 LFC ($P = 0.21$). Using linear regression (as described for PHA–LFC and TCR–LFC), we did not find that the strength of this association differed between disease types (interaction term $P = 0.91$).

3.5. Association between PHA–LFC and clinical response reflects correlation with LPS–LFC

Despite very similar effects on T cell proliferation by PHA treatment and direct TCR stimulation, the PHA–LFC assay was significantly associated with clinical response in CD, while the TCR–LFC was not. Indeed, we found a very weak, and not significant, correlation between PHA–LFC and TCR–LFC across patients ($r = 0.32$, $P = 0.071$). In contrast, we found that LPS–LFC was positively correlated with PHA–LFC across patients (Fig. 3B, $r = 0.43$, $P = 0.036$), but not significantly correlated with TCR–LFC ($P = 0.62$, Supplementary Fig. 2). To explore the effect of this correlation on the association with clinical response, we fit a multivariate logistic regression model with both the PHA–LFC and LPS–LFC as explanatory variables. In this joint model, we found that the association with PHA–LFC was no longer significant ($P = 0.73$), but the association with LPS–LFC remained significant ($P = 0.02$). These results are consistent with a model where the association between PHA–LFC and clinical response reflects the correlation of this assay with LPS–LFC.

4. Discussion

Our goal was to compare GC-induced inhibition across types of immune responses (i.e., adaptive vs. innate) and across disease types (CD vs. UC). By performing inhibition assays of adaptive and innate immune response in the same set of CD and UC patients, we were able to show – for the first time – that inhibition of LPS-mediated cytokine secretion is more strongly associated with clinical response than either inhibition of either PHA-mediated or TCR-mediated T cell proliferation in both UC and CD. In contrast, GC-induced inhibition of the adaptive immune response was associated with clinical response only in UC patients. This is particularly interesting because both of the T cell proliferation assays had been associated with clinical response in previous studies, while an LPS-based assay had not.

Our results could reflect differences in the biology of these diseases. Specifically, while both innate and adaptive immune responses are involved in both diseases, the adaptive immune response may play a larger role than bacterial recognition in the pathogenesis of UC. This hypothesis is consistent with observations from genome-wide association studies that genetic variants that decrease activity of NOD2, which encodes a receptor involved in bacterial recognition, are associated with increased risk of CD, but with slightly decreased risk of UC. Furthermore, variants in the gene PTPN22, which encodes a regulator of T cell stimulation, are associated with increased risk of UC and decreased risk of CD.

In contrast to our results, Franchimont et al. did not find a significant association between GC inhibition of LPS-induced cytokine secretion and clinical response in CD patients. However, this study did not compare clinical responders to patients who were non-responsive to GC treatment. Instead, the authors focused on ‘GC dependent patients’ who required continued high-dose GC treatment to suppress disease activity. GC dependent patients are responsive to GCs (albeit at high doses) and, therefore, may be distinct from GC non-responsive patients. Furthermore, Franchimont et al. used a smaller sample size (7 GC-dependent and 12 GC-responsive patients); thus, the lack of a significant association with clinical response may be due to inadequate power. Alternatively, the discrepancy between these studies could indicate that GC-dependent patients are, generally, responsive to GCs, but unable to achieve remission for other reasons. For example, these patients may be non-responsive to maintenance therapies.
added as GC treatment was tapered (e.g. azathioprine). Response to these drugs is also variable across patients and a recent study has shown that this variation can be captured by in vitro measures of drug inhibition of TCR stimulation.30

We found that PHA–LFC was correlated with LPS–LFC across patients. Using multivariate logistic regression, we found that PHA–LFC was no longer associated with clinical response when we controlled for LPS–LFC. This suggests that both assays capture a biological process that is associated with clinical response, but that the LPS assay is a better measure as it shows a stronger association. For example, both assays may capture the ability of GC to inhibit aspects

Figure 2 Association between TCR–LFC (TCR–log fold change) and clinical response to GCs by disease. Boxplots show significant difference in the distribution of TCR–LFC by clinical response in patients with UC (P = 0.009, left panel), but not in patients with CD (P = 0.78, right panel). (R = responder, NR = non-responder). For UC patients, the median TCR–LFC for clinical non-responders is −0.26 (standard deviation = 0.05, range = [−0.33, −0.23]) in contrast to a median of −0.59 (standard deviation = 0.16, range = [−0.71, −0.29]) for clinical responders. For CD patients, the median TCR–LFC for clinical non-responders is −0.49 (standard deviation = 0.25, range = [−0.87, −0.05]) in contrast to a median of −0.37 (standard deviation = 0.34, range = [−0.62, 0.61]) for clinical responders.

Figure 3 LPS–LFC (lipopolysaccharide–log fold change) is associated with clinical response and correlated with PHA–LFC. A) Boxplot shows significant differences (P = 0.005) in LPS–LFC between clinical responders (R) and non-responders (NR). The median LPS–LFC for clinical non-responders is −2.03 (standard deviation = 0.47, range = [−2.57, −1.19]) in contrast to a median of −2.43 (standard deviation = 0.50, range = [−3.52, −1.74]) for clinical responders. B) Scatter plot shows significant correlation (r = 0.43, r² = 0.18, P = 0.036) between PHA–LFC (PHA–log fold change) and LPS–LFC. Patients with UC and CD are pooled in these plots.
of the innate immune response. Although PHA stimulation leads to T cell proliferation (a feature of the adaptive immune response), it may also stimulate aspects of the innate immune system. For example, PHA stimulation of T cell proliferation requires the presence of monocytes and also induces cytokine secretion. Furthermore, we previously found that genes differentially expressed in response to PHA stimulation were enriched for pathways related to innate immunity.

This study represents an important step towards understanding the biology of variation in GC response among IBD patients, strongly implicating GC inhibition of bacterial response. These results also support efforts to develop predictive biomarkers of therapeutic response in IBD. The LPS assay described here, and similar in vitro assays using other stimulants, could be used as predictive tools to guide treatment of IBD patients.

The strengths of this study are that we comprehensively assessed GC inhibition in the setting of adaptive and innate immune stimulants in parallel in the same set of IBD patients. We included patients with UC and CD allowing us to dissect differences in GC responsiveness between IBD sub-types. A possible limitation of our study is the retrospective ascertainment of clinical GC response, which may be influenced by unmeasured confounders. However, we note that responders and non-responders were carefully matched for clinical characteristics thereby minimizing confounding (Table 1). Furthermore, in contrast to expectations for a confounded study, the patterns we observed are consistent with those of previous smaller prospective studies performed in independent patient populations.

Additionally, the majority of the population was in complete or partial clinical remission without recent GC exposure, thus minimizing variability in disease management. Although we found significant differences in the mean in vitro sensitivity between clinical responders and non-responders, we also noted substantial overlap in the distribution of in vitro sensitivity between these groups. This indicates that these assays cannot predict clinical response with perfect accuracy, although the level of discrimination may be sufficient for clinical use. Prospective studies will be required to validate these assays for clinical prediction.

Conflict of Interest

The authors have no conflicts of interest to disclose.

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Appendix A. Supplementary data

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