Prolonged Methylprednisolone Treatment Suppresses Systemic Inflammation in Patients with Unresolving Acute Respiratory Distress Syndrome

Evidence for Inadequate Endogenous Glucocorticoid Secretion and Inflammation-induced Immune Cell Resistance to Glucocorticoids

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Nuclear factor-κB (NF-κB) and glucocorticoid receptor-α (GR-α) have diametrically opposed functions in regulating inflammation. We investigated whether unresolving acute respiratory distress syndrome (ARDS) is associated with systemic inflammation-induced glucocorticoid resistance and whether prolonged methylprednisolone administration accelerates the suppression of systemic inflammatory indices and normalizes the sensitivity of the immune system to glucocorticoids. Patients enrolled into a randomized trial evaluating prolonged methylprednisolone administration in unresolving ARDS had serial plasma samples collected before and after randomization. In the plasma, we measured the concentrations of tumor necrosis factor-α (TNF-α), interleukins (IL) IL-1β and IL-6, adrenocorticotropic hormone (ACTH), and cortisol. The ability of patient plasma to influence the NF-κB and GR-signal transduction systems of normal peripheral blood leukocytes (PBL) was examined. Patients treated with methylprednisolone had progressive and sustained reductions of TNF-α, IL-1β, IL-6, ACTH, and cortisol concentrations over time. Normal PBL exposed to plasma samples collected during methylprednisolone exhibited significant progressive increases in all aspects of GR-mediated activity and significant reductions in NF-κB DNA-binding and transcription of TNF-α and IL-1β. These findings provide support for the presence of endogenous glucocorticoid inadequacy in the control of inflammation and systemic inflammation-induced peripheral glucocorticoid resistance in ARDS. Prolonged methylprednisolone administration accelerated the resolution of both systemic inflammation and peripheral acquired glucocorticoid resistance in ARDS.

Keywords: acute respiratory distress syndrome; glucocorticoid receptors; IκBα; methylprednisolone; nuclear factor-κB

Excessive systemic inflammation is the pathophysiologic hallmark of pulmonary and extrapulmonary organ dysfunction in patients with acute respiratory distress syndrome (ARDS) (1), a frequent cause of hypoxemic respiratory failure associated with a 40% to 60% mortality (2). It is now appreciated that systemic inflammation in ARDS is sustained over time and that the final outcome is affected by its degree and duration (1). Two cellular signaling pathways are central to the host inflammatory response, the stimulatory nuclear factor-κB (NF-κB) and the inhibitory glucocorticoid receptor-α (GR-α)-mediated signal transduction cascades (3).

NF-κB is a heterodimeric protein composed of the DNA-binding proteins p65 and p50 constitutively present in the cytoplasm in an inactive form stabilized by the binding to the inhibitory protein IκBα (4). Cellular activation by a multitude of adverse stimuli leads to phosphorylation and proteolytic degradation of IκBα (4). The liberated NF-κB then translocates into the nucleus and binds to promoter regions of target genes to initiate the transcription of multiple cytokines including tumor necrosis factor-α (TNF-α); the interleukins (IL) IL-1β, IL-2, IL-6; and chemokines such as IL-8, cell adhesion molecules, and inflammation-associated enzymes (4). Products of the genes that are stimulated by NF-κB activate this transcription factor. Thus, TNF-α and IL-1β both activate and are activated by NF-κB by forming a positive regulatory loop that amplifies and perpetuates inflammation (5).

Glucocorticoid hormones (GC), produced by the adrenal cortices, are the most important physiologic inhibitors of inflammation. GC exert most of their effects by activating ubiquitously distributed cytoplasmic heat shock protein–complexed glucocorticoid receptors (GR) with formation of GC—GR complexes (6). It is now appreciated that the GC—GR complexes modulate transcription in a hormone-dependent manner by binding to glucocorticoid response elements (GRE) in the promoters of glucocorticoid responsive genes and by interfering with the activity of other transcription factors such as NF-κB on genes regulated by these factors (7). GR-mediated transcriptional interference is achieved by five important mechanisms: (1) by physically interacting with the p65 subunit and formation of an inactive (GRα–NF-κB) complex (6); (2) by inducing the transcription of the inhibitory protein IκBα gene (6, 8, 9); (3) by blocking degradation of IκBα via enhanced synthesis of IL-10 (10); (4) by impairing TNF-α–induced degradation of IκBα (11); and (5) by competing for limited amounts of GRα coactivators such as CREB-binding protein and steroid receptor coactivator-1 (12).

Endogenous glucocorticoids are not always effective in suppressing life-threatening systemic inflammation, even though the degree of cortisolemia frequently correlates with severity of illness and mortality rate (13–16). Failure to suppress inflammation could be due to inadequacy of, and/or tissue resistance to, the concentrations and durations of endogenous glucocorticoid elevations, which allow the systemic inflammatory response to go awry (17). We have recently reported a significant physiologic and survival benefit when prolonged gluco-
corticoid treatment at moderate doses was administered late (9 ± 3 d) in the course of ARDS to patients failing to improve (18). We hypothesized that if endogenous glucocorticoid inadequacy and/or peripheral tissue resistance are important pathophyslogic factors in a dysregulated, protracted systemic inflammatory response in ARDS, then prolonged glucocorticoid therapy may be useful, not as an antiinflammatory treatment per se, but as hormonal supplementation necessary to compensate for the host’s inability to produce appropriately elevated amounts of cortisol for the degree of peripheral glucocorticoid resistance (19).

In this study, we tested the hypothesis that prolonged methylprednisolone versus placebo administration in patients with unresolving ARDS suppresses inflammation and/or corrects the glucocorticoid resistance of the inflammatory response of these patients. To test this hypothesis, we measured serially the plasma levels of TNF-α, IL-1β, IL-6, adrenocorticotropic hormone (ACTH), and cortisol in patients with unresolving ARDS treated with methylprednisolone or placebo and exposed a healthy volunteer’s peripheral blood leukocytes (PBL) to plasma samples from the patients. In the exposed cells, we measured upstream and downstream events associated with the NF-κB and glucocorticoid transduction cascades as they oppose each other’s actions.

METHODS

Patient Selection

The original study was conducted between October 1994 and November 1996 in the intensive care units of Baptist Memorial Medical Center and East hospitals, the Regional Medical Center, and the University of Tennessee Bowld Medical Center, all in Memphis, TN. The study protocol was approved by each institutional review board, and informed consent was obtained before enrollment. An active effort was made to identify and recruit eligible patients with ARDS. Patients at least 18 years old were eligible if they met previously described ARDS consensus criteria (20) and had a lung injury score (LIS) (21) ≥ 2.5. The ventilator management followed recently developed guidelines aimed at limiting plateau pressure to less than 35 cm H₂O (22). Positive end-expiratory pressure was increased by 3–5 cm H₂O to achieve the best lung compliance and oxygen saturation and to maintain FiO₂ less than or equal to 0.6. The presence or absence of improvement in lung function (as defined by LIS [21]) by Day 7 of ARDS treatment (18). Randomization was done on a 2:1 basis; 16 patients received methylprednisolone, and 8 received placebo. Data from 17 of the 24 randomized patients are reported in the present article. Serial blood samples were available for analysis in 17 patients, 6 of whom received placebo and served as control subjects. In 7 of the 24 randomized patients, blood samples were either not obtained (three patients) or were inadequate for serial measurements (four patients). Methylprednisolone or placebo was given daily as intravenous push every six hours (one-fourth of the daily dose) and changed to a single oral dose when oral intake was restored. If the patient was able to tolerate oral intake and had no obvious gastrointestinal dysfunction (i.e., diarrhea, etc.), we presumed that the gastrointestinal tract was functional. A loading dose of 2 mg/kg was followed by 2 mg/kg/day from Day 1 to 14, 1 mg/kg/day from Day 15 to 21, 0.5 mg/kg/day from Day 22 to 28, 0.25 mg/kg/day on Days 29 and 30, and 0.125 mg/kg/day on Days 31 and 32. During the study, components of the LIS (21), and multiple organ dysfunction syndrome score (23) were collected, and results are published elsewhere (24).

Table 1 shows clinical characteristics at the onset of ARDS. The 11 patients randomized to methylprednisolone improved LIS by study Day 10 and survived. In the placebo group, two patients improved LIS by study Day 10 and survived, whereas four failed to improve LIS. Two of the four nonimprovers died within seven days of randomization.

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<th>TABLE 1. CLINICAL VARIABLES AT THE ONSET OF ARDS</th>
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<td>Lung Injury Score†</td>
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* No significant difference was found between the two groups for any variable.
† APACHE III score at intensive care unit admission.
‡ ± SD.

Plasma Collection and Description of the Ex Vivo Model of ARDS

Blood samples were obtained on Days 1, 3, 5, 7, and 10 of ARDS, on the day of randomization (Day 0), and on Days 3 (+3), 5 (+5), 7 (+7), and 10 (+10) of treatment. All blood samples were obtained from a central venous line or an antecubital venipuncture, collected in a vacutainer tube containing ethylenediaminetetraacetic acid, placed immediately on ice after collection, and transported to the laboratory for immediate processing. A complete blood cell count with differential was determined using the Coulter A+7 diff Hematology Analyzer (Beckman-Coulter, Miami, FL). Blood samples were centrifuged at 500 × g for 10 minutes in a refrigerated centrifuge, and plasma was aspirated and aliquoted in plastic storage tubes. All samples were stored at −80°C until assay.

The ex vivo model of ARDS consisted of exposing PBL obtained from a single healthy volunteer to plasma samples obtained from patients with ARDS. Using the ex vivo model, we attempted to simulate the in vivo milieu, but we realize that there are limitations to the inferences that can be made by using this approach.

Laboratory Methodology

The laboratory methodology section is available in the online data supplement and includes the following: isolation of PBL and exposure to patients’ plasma; determination of plasma cytokine, ACTH, and cortisol concentrations; cellular fractionation and protein determination; electrophoretic mobility gel shift assay (EMSA); detection of NF-κB and GR-α binding to their response elements; quantification of EMSA; determination of NF-κB subunits, GR-α, and IκBα by Western blotting; and quantitative reverse transcription-polymerase chain reaction.

Statistical Analysis

This study has an unbalanced nested factorial design, with groups (methylprednisolone and placebo) as the main plot effect; patients were nested within groups; and time was cross-classified with the groups and patients. We made the assumption that the variances of continuous dependent variables on the assessment days were equal. If the variances were not equal or the distributions were positively skewed, data were transformed using natural logarithms. Comparisons were made both within and across groups. For comparisons within groups, the variance for each dependent variable was estimated by the pooled within-patient variance from data measured repeatedly over time. For comparisons across groups, that variance was estimated by the weighted average of the residual mean squares and the between-patient mean squares from repeated measures analysis of variance (25). Although constituents in plasma serially collected from patients were moderately and positively correlated, we assume that results from cell cultures were independent. For each group, partial correlation coefficients among selected intracellular markers, adjusted for repeated measurements on patients, were estimated. The assumptions necessary for this analysis are that (1) the markers are linearly associated over time and (2) the residuals are independently and identically distributed normally with variance $\sigma^2_p$. For all preplanned or a priori contrasts stipulated in the main hypotheses, a significance level of 0.05 was chosen for statistical significance. All hypothesis tests are two-tailed. Data were analyzed using the SAS statistical software package (SAS Institute, Inc., Cary, NC).
RESULTS

Cytokine Concentrations from Plasma of Randomized Patients
A total of 88 blood specimens was available for analysis, 58 from patients in the methylprednisolone group and 30 from patients in the placebo group. Figure 1 shows the patients’ plasma TNF-α, IL-1β, and IL-6 levels before and after randomization. On Day 1 of ARDS and up to randomization, plasma TNF-α, IL-1β, and IL-6 levels were similar in both groups. After randomization, plasma TNF-α, IL-1β, and IL-6 levels declined rapidly in the methylprednisolone group (on Days 5 to 7, p < 0.0001 for all three cytokines), whereas the control group had lesser reductions in plasma TNF-α and IL-6 and no reduction in plasma IL-1β. After randomization, the methylprednisolone group had significantly (p < 0.0001) lower plasma IL-1β concentrations than the control group for each recorded interval.

ACTH and Cortisol Concentrations from Plasma of Randomized Patients
Figure 2 shows plasma ACTH and cortisol concentrations in patients before and after randomization. On Day 1 of ARDS and up to randomization, plasma ACTH and cortisol levels were similar in both groups. After randomization, the methylprednisolone group had significant and sustained (on Days 5 to 7, p < 0.005 for both measurements) reductions in plasma ACTH and cortisol concentrations, whereas no reductions were observed in the control group.

NF-κB and Its Subunits from Cells of a Healthy Volunteer
Figure 3 shows NF-κB, p65 subunit, and p50 subunit binding to response elements before and after randomization. On Day 1 of ARDS, densities of NF-κB, p65 subunit, and p50 subunit were similar in cells exposed to plasma from both groups. From Day 1 of ARDS to randomization, densities of all three proteins increased significantly and similarly in cells exposed to plasma from both groups. After randomization, cells treated with plasma from the methylprednisolone group exhibited significant progressive reductions in NF-κB and its subunits (on Day 3, p < 0.02 for all three measurements), whereas no changes were observed in those exposed to plasma from the control group. After randomization, significant differences (p < 0.01) between the two groups were observed for NF-κB for each recorded interval.

Figure 1. Plasma TNF-α, IL-1β, and IL-6 concentrations before and after randomization. Plasma TNF-α (top), IL-1β (middle), and IL-6 (bottom) concentrations (mean ± SE) before and after randomization in the methylprednisolone group (open bar) and placebo (closed bar). p Values are taken from analyses of natural logarithms of cytokine values and reflect significances preplanned contrasts of each mean to the mean of the respective group observed on the day of randomization.
GR-α–Mediated Activity

Figure 4 shows cytoplasmic GR-α bound and unbound to NF-κB before and after randomization. On Day 1 of ARDS, cells exposed to plasma of both groups had similar densities of GR-α. After exposure to plasma obtained from Day 1 of ARDS up to randomization, the amounts of GR-α bound to NF-κB did not change, whereas the amounts of GR-α unbound decreased significantly. After randomization, exposure to plasma from the methylprednisolone group was associated with significant increases (on Day 3, p < 0.0001 for both measurements) in cytoplasmic GR-α bound and unbound, whereas lesser increases were observed in cells exposed to plasma from the control group. After randomization, a significant difference (p < 0.0001) between the two groups was observed for cytoplasmic GR-α bound and unbound to NF-κB for each recorded interval. Figure 5 shows cytoplasmic IκBα and GR-α binding to response elements. On Day 1 of ARDS and up to randomization, cells exposed to plasma from both groups had similar densities of cytoplasmic IκBα and GR-α bound to the response elements. After randomization, exposure to plasma from the methylprednisolone group was associated with significant increases (on Day 3, p < 0.0001 for both measurements) in cytoplasmic IκBα and GR-α bound to the response elements. In contrast, cells exposed to plasma from the control group had lesser increases in GRα bound to the response elements and significant reductions in cytoplasmic IκBα densities. After randomization, a significant difference (p < 0.0001) between the two groups was observed for cytoplasmic IκBα and GR-α binding to response elements for each recorded interval.

Cytokine Transcription

Figure 6 shows TNF-α, IL-1β, and IL-10 mRNA levels measured in media of cells exposed to plasma obtained before and after randomization. On Day 1 of ARDS and up to randomization, TNF-α, IL-1β, and IL-10 mRNA levels were similar in media of cells exposed to plasma from both groups. TNF-α and IL-1β mRNA were significantly lower after exposure to plasma from the methylprednisolone group after randomization (p < 0.0001 for both types of mRNA on each recording), whereas cells exposed to plasma from the control group after randomization had no reductions in TNF-α and IL-1β mRNA levels. Cells exposed to plasma from the methylprednisolone group after randomization had significant (p < 0.0001) and progressive increases in IL-10 mRNA levels, whereas no changes were observed in cells exposed to plasma from the placebo group after randomization until Day 10. After randomization, significant differences (p < 0.01) between the two groups were observed for IL-1β and IL-10 mRNA levels for each recorded interval.

Relations among Selected Variables after Randomization

Figure 7 depicts the relations on natural logarithmic scales between mean levels of nuclear NF-κB and nuclear GR-α (top) and between mean levels of nuclear NF-κB and cytoplasmic GR-α bound to NF-κB (bottom), a factor affecting the translocation of activated NF-κB to the nucleus. Untransformed means of these transcription factors were depicted separately in Figures 3 and 4. After natural logarithmic transformation and adjustment for repeated measurements, partial correlations among responses to plasma from the methylprednisolone group were −0.92 (p < 0.0001) both for nuclear NF-κB and nuclear GR-α and for nuclear NF-κB and cytoplasmic GR-α bound to NF-κB. For responses to plasma from the placebo group, no significant relationship was found between nuclear NF-κB and nuclear GR-α (r = 0.11; p = 0.70) or between NF-κB and cytoplasmic GR-α bound to NF-κB (r = 0.33; p = 0.23).

Figure 8 depicts the relations on natural logarithmic scales between mean levels of IκBα and factors affecting its formation (nuclear GR-α) and degradation (IL-10 mRNA and TNF-α.
mRNA). Untransformed means of these factors were depicted separately in Figures 5 and 6. After natural logarithmic transformation and adjustment for repeated measurements, partial correlations were \( r = 0.97 \) (\( p < 0.0001 \)) between I\( \kappa \)B and nuclear GR\( \alpha \), \( r = 0.98 \) (\( p < 0.0001 \)) between I\( \kappa \)B and IL-10 mRNA, and \( r = 0.95 \) (\( p < 0.0001 \)) between I\( \kappa \)B and TNF-\( \alpha \) mRNA. In contrast, for responses to plasma from the placebo group, the partial correlation coefficients were \( r = 0.73 \) (\( p = 0.003 \)) between I\( \kappa \)B and nuclear GR\( \alpha \), \( r = 0.85 \) (\( p < 0.0001 \)) between I\( \kappa \)B and IL-10 mRNA, and \( r = 0.27 \) (\( p = 0.33 \)) between I\( \kappa \)B and TNF-\( \alpha \) mRNA. Figure E1 in the web repository shows the EMSA of nuclear extract of NF-\( \kappa \)B over time from one patient randomized to methylprednisolone and from one patient randomized to placebo.

**DISCUSSION**

Patients treated with methylprednisolone had rapid, progressive, and sustained reductions in plasma TNF-\( \alpha \), IL-1\( \beta \), IL-6, ACTH, and cortisol concentrations over time. These were associated with parallel improvements in pulmonary and extra-pulmonary organ dysfunction scores (previously reported in reference [18]). Normal PBL exposed to plasma samples collected during methylprednisolone versus placebo treatment also exhibited rapid, progressive significant increases in GR-\( \alpha \)-mediated activities (GR-\( \alpha \) binding to NF-\( \kappa \)B, GR-\( \alpha \) binding to GRE DNA, stimulation of inhibitory protein I\( \kappa \)B, and stimulation of IL-10 transcription) and significant reductions in NF-\( \kappa \)B binding and transcription of TNF-\( \alpha \) and IL-1\( \beta \). These findings provide strong mechanistic evidence for the efficacy of prolonged methylprednisolone treatment in unresolving ARDS.

In the observation period before randomization, the biologic and physiologic characteristics of the methylprednisolone and placebo groups were similar. Patients had persistent elevations in plasma concentrations of inflammatory (TNF-\( \alpha \), IL-1\( \beta \), and IL-6) cytokines and hypothalamic-pituitary-adrenal (HPA)-axis (ACTH and cortisol) hormones and similar severity of organ dysfunction scores. We hypothesized that inadequate secretion of cortisol and/or immune tissue resistance to endogenous glucocorticoids might explain the observed failure to suppress inflammation in the presence of persistently elevated ACTH and cortisol concentrations. Because the GR ultimately controls GC-mediated activity, anything that affects its binding affinity, concentration, transport to the nucleus, interactions with GRE, or other relevant transcription
factors and coregulators can ultimately affect the response of cells to glucocorticoids (6). GR-mediated resistance was originally described as a primary inherited familial syndrome (26, 27) and was recently recognized as an acquired condition. Among others, acquired immune tissue-specific GR resistance has been described in patients with asthma (28–31), acquired immunodeficiency syndrome (32), and severe sepsis (33).

Recent in vitro studies have shown that cytokines may induce resistance to glucocorticoids by reducing GR-α binding affinity to cortisol and/or GRE (34–36). Such abnormalities of GR-α function were demonstrated in T cells incubated with a combination of IL-2 and IL-4 (35), IL-1, IL-6, and interferon-γ (34), or IL-13 (36). Glucocorticoid resistance was induced in a cytokine concentration-dependent fashion and was reversed...
by the removal of cytokines (35). GR-mediated resistance in the presence of systemic inflammation was also studied in experimental models of sepsis and sepsis-induced ARDS (33, 37, 38). In a sheep model of sepsis-induced ARDS, maximal binding capacity of GR decreased continuously after endotoxin infusion, whereas there was a marked elevation of cortisol concentrations (37). The reduced GR binding correlated negatively ($r = -0.87; p < 0.01$) with phospholipase A2 activity, a gene that is stimulated by NF-κB. In a rat model of septic shock, GR blockade by mifepristone (RU 486) exacerbated the physiologic and pathologic changes induced by endotoxemia (38). Phospholipase A2 activity in rats with 80% GR blockade was more marked than in those with 50% GR blockade (38). Monocytes of patients with sepsis developed near-total glucocorticoid resistance in vitro when cytokines, especially IL-2, were added (33).

Several inflammatory cytokines, including TNF-α, IL-1β, and IL-6, activate NF-κB (39). It has been proposed that when cytokine-activated NF-κB forms protein–protein complexes with activated GR-α, the availability and activity of effective GR-α molecules are reduced (6, 31). This functional reduction in GR-α availability is associated with decreased GR-α–GRE DNA binding and GC-mediated antiinflammatory activity (6, 31), and our findings support this hypothesis. The intracellular changes observed by exposing leukocytes of healthy volunteers to plasma samples collected before and after randomization included escalating increases in NF-κB–mediated activities (NF-κB DNA-binding, p50 and p65 DNA-binding, and transcription of TNF-α and IL-1β) and modest changes in GR-α–mediated activities. The reduction in cytoplasmic IkBα levels observed before randomization indicates that NF-κB–mediated IkBα degradation predominated over GR-α–mediated IkBα formation (40).

If acquired glucocorticoid receptor resistance played a role in the pathogenesis of unresolving ARDS, adequate hormonal supplementation should restore glucocorticoid antiinflammatory function, by decreasing the production of inflammatory cytokines, cytokine-driven HPA-axis activity, and cytokine-driven organ dysfunction. Indeed, after randomization, the biologic and physiologic characteristics of the two groups (methylprednisolone versus placebo) rapidly diverged. The responses observed during methylprednisolone administration support the concept of inflammation-dependent acquired glucocorticoid resistance in patients with ARDS. We found that the methylprednisolone-treated group had significant, progressive reductions in plasma concentrations of TNF-α, IL-1β, IL-6, ACTH,
and cortisol and a parallel improvement in severity of organ dysfunction scores (18). The biologic response observed in this study was similar to that of a prior uncontrolled report (41). In that study, methylprednisolone treatment was associated with significant and parallel reductions in plasma and bronchoalveolar lavage (BAL) TNF-α, IL-1β, IL-6, and IL-8 concentrations; LIS; and BAL indices of pulmonary vascular permeability (BAL albumin and percentage of polymorphonuclear cells) (41).

Our \textit{ex vivo} findings reflect the effects of a mixture of inflammatory cytokines and other factors and the variable methylprednisolone and cortisol concentrations in the plasma of our patients on normal nonactivated circulating blood leukocytes. The concentration of methylprednisolone in the plasma samples was unknown and may have varied during the study period. A prior study has shown similar changes in NF-κB activation in the peripheral monocytes of patients with septic shock (42) or with critical illness (43). These data suggest that the glucocorticoid resistance of the immune system of ARDS patients simply reflects the inflammatory state of the individual.

The extent to which methylprednisolone in the plasma has biased the magnitude of responses is unknown. We conducted an experiment to quantify this bias (data not reported). The amount of IL-8 released from fresh PBL incubated for four hours with plasma from a patient who received methylprednisolone was four times greater when the methylprednisolone had been removed by dialysis compared with undialyzed plasma. For TNF-α release, the effect of removing methylprednisolone was an increase of 1.6 times the amount compared with undialyzed plasma. Thus, the degree of bias probably varies greatly, depending on what intracellular or extracellular variable is selected. We point out that in the current report the results from PBL of a healthy volunteer are consistent with the outcomes of patients randomized in the clinical trial (18). However, we explicitly acknowledge this known source of bias on the magnitude, significance, and possibly the direction of the differences presented in this report.

Our current understanding of the physiologic antagonism between the NF-κB and GR-α cascades explains our findings on the exposure of normal leukocytes to plasma samples from patients receiving methylprednisolone or placebo. With methylprednisolone administration, the intracellular relations between the NF-κB and GR-α signaling pathways changed from an initial NF-κB–driven and GR-α–resistant state to a GR-α–driven and GR-α–sensitive one. In contrast, exposure to serial plasma samples collected during placebo administration demonstrated a significant—but lower (in comparison with the methylprednisolone group, \( p < 0.0001 \))—increase in GR-α–mediated activity over time, and persistently elevated NF-κB activity.

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