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In Vitro–Administered Dexamethasone Suppresses T Cell Function With Reversal by Interleukin-7 in Coronavirus Disease 2019

OBJECTIVES: Corticosteroid therapy has become standard of care therapy for hospitalized patients infected with the severe acute respiratory syndrome coronavirus-2 global pandemic-causing virus. Whereas systemic inflammation is a notably important feature in coronavirus disease 2019 pathogenesis, adaptive immune suppression and the inability to eradicate effectively the virus remain significant factors as well. We sought to evaluate the in vitro effects of dexamethasone phosphate on T cell function in peripheral blood mononuclear cells derived from patients with acute, severe, and moderate coronavirus disease 2019.

DESIGN: Prospective in vitro laboratory study.

SETTING: Coronavirus disease 2019-specific medical wards and ICUs at a single-center, quaternary-care academic hospital between October 1, 2020, and November 15, 2020.

PATIENTS: Eleven patients diagnosed with coronavirus disease 2019 admitted to either the ICU or hospital coronavirus disease 2019 unit. Three patients had received at least one dose of dexamethasone prior to enrollment.

INTERVENTIONS: Fresh whole blood was collected, and peripheral blood mononuclear cells were immediately isolated and plated onto pre-coated enzyme-linked immunospot plates for detection of interferon- γ production. Samples were incubated with CD3/CD28 antibodies alone and with three concentrations of dexamethasone. These conditions were also stimulated with recombinant human interleukin-7. Following overnight incubation, the plates were washed and stained for analysis using Cellular Technology Limited ImmunoSpot S6 universal analyzer (ImmunoSpot by Cellular Technology Limited, Cleveland, OH).

MEASUREMENTS AND MAIN RESULTS: Functional cytokine production was assessed by quantitation of cell spot number and total well intensity after calculation for each enzyme-linked immunospot well using the Cellular Technology Limited ImmunoSpot Version 7.0 professional software (CTL Analyzers, Shaker Heights, OH). Comparisons were made using *t* test and using a nonparametric analysis of variance Friedman test. The number of functional T cells producing interferon- γ and the intensity of the response decrease significantly with exposure to 1.2- μ g/mL dexamethasone. About 0.12 μ g/mL does not significantly affect the functional immune response on enzyme-linked immunospot. Interleukin-7 increases the overall number of activated T cells, including those exposed to dexamethasone.

CONCLUSIONS: Further evaluation of the effect of immunomodulatory therapies is warranted in coronavirus disease 2019. A refined functional,

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precision medicine approach that evaluates the cellular immune function of individual patients with coronavirus disease 2019 is needed to better define which therapies could have benefit or cause harm for specific patients.

KEY WORDS: coronavirus disease 2019; corticosteroids; cytokine storm; dexamethasone; immunosuppression

The ongoing coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus-2 has resulted in over 2 million deaths worldwide. Although a mechanistic understanding of the disease remains broadly unclear, perturbations in host immunity, injury to respiratory endothelium, and alterations in hemostasis are hallmarks of disease severity. Numerous pharmacologic therapies targeting the viral replication mechanics, inflammatory cascade, complement system, coagulation cascade, and the host immune response have been tested in clinical trials but demonstrated limited efficacy. No silver bullet to cure critically ill patients and thereby quell the global effects of the pandemic has been revealed; however, corticosteroids have demonstrated improvements in survival, presumably through suppression of a “cytokine storm” and its pathologic effects. Administration of dexamethasone is currently recommended for use in COVID-19 acute respiratory distress syndrome (ARDS) patients by leading authorities (1–3). Administration of dexamethasone is currently recommended for use in COVID-19 ARDS patients by leading authorities (4).

While the anti-inflammatory effects of dexamethasone have potential benefit in reducing cytokine production and edema, corticosteroids suppress a number of critical cellular immune functions that could impair viral clearance and lead to secondary infections (5). Corticosteroids, like dexamethasone, decrease B cell production of immunoglobulins and induce T cell apoptosis, two immune cellular effects that would be counterproductive in COVID-19 (6).

Although cytokine-mediated hyperinflammation may lead to mortality in COVID-19, many groups, including our own, have demonstrated that COVID-19 is less a disorder of hyperinflammation than the one characterized by immunosuppression (7–9). Additionally, there exist reports in the literature of corticosteroids in severe COVID-19 patients with ARDS,

demonstrating an increased 28-day mortality rate (3). Given the heterogeneity of the immune response in patients with COVID-19 and the potential and deleterious effects of using a glucocorticoid in patients with existing immune suppression, we investigated the effect of dexamethasone on T cell function in blood from hospitalized COVID-19 patients.

MATERIAL AND METHODS

In a cohort of 11 patients admitted to an academic quaternary care ICU or COVID-19 hospital unit, we obtained the first blood sample within 72 hours from admission. Three of the 11 patients received dexamethasone 6 mg prior to blood sample draw. Two of the patients admitted to the ICU subsequently died. Patient characteristics between \pm in vivo dexamethasone are shown in Table 1. We evaluated adaptive immune function using the enzyme-linked immunospot (ELISpot) assay to quantitate blood mononuclear cell interferon (IFN)- γ production after CD3/CD28 stimulation from 11 hospitalized COVID-19 patients. We mimicked in vitro dexamethasone administration to the standard 6-mg dexamethasone dose being used in patients with COVID-19.

Given this typical daily dose of 6 mg, and an expected peak plasma concentration of approximately 1.5 μ g/mL and volume of distribution of 648 mL/kg, dexamethasone concentrations of 0.12, 1.20, and 12.0 μ g/mL were tested after CD3/CD28 stimulation in ICU (**Fig. 1A**) and non-ICU patients (**Fig. 1B**) (10). Patients that received dexamethasone are shown in green. Representative ELISpot figures are shown in **Figure 1D**. Samples were compared in ICU or non-ICU patients by analysis of variance (ANOVA) in three separate doses against CD3/CD28 stimulated positive control cells using the GraphPad Prism Version 9.0 software (GraphPad, San Diego, CA).

RESULTS

Dexamethasone produced in patients a dose-dependent decrease in T cell IFN- γ production with a 30% (ICU) and 49% (non-ICU) reduction in the number of IFN- γ secreting cells, and 61% (ICU) and 58% (non-ICU), respectively, decrease in IFN- γ production (measured by ELISpot total well intensity), in the 1.20-mg/mL concentration (most closely approximating the 6-mg equivalent in patients) (**Fig. 1B**; $p < 0.05$;

TABLE 1. Patient Demographics and Clinical Information

Demographics	Received Dexamethasone (<i>n</i> = 3)	Did Not Receive Dexamethasone (<i>n</i> = 8)
Age, mean (range)	42.6 (21.6–55.5)	47.9 (19.3–69.6)
Sex, <i>n</i> (%)		
Female	0 (0)	3 (37.5)
Male	3 (100)	5 (62.5)
Race, <i>n</i> (%)		
African American	0 (0)	7 (87.5)
White	3 (100)	1 (12.5)
Body mass index, mean (range)	43.3 (29–68.7)	32.3 (21.7–40.5)
ICU admission, <i>n</i> (%)		
Yes	2 (66.7)	3 (37.5)
No	1 (33.3)	5 (62.5)
Mortality status, <i>n</i> (%)		
Alive	2 (66.7)	7 (87.5)
Deceased	1 (33.3)	1 (12.5)

all comparisons). Importantly, when coincubated with both dexamethasone and interleukin (IL)-7, a potent T cell stimulant, T cell function was restored in the aggregate of ICU and non-ICU patients (**Fig. 1C**). IL-7 has previously been shown to be safely administered and to reverse profound lymphopenia in critically ill patients with COVID-19 and could function as an adjunct to corticosteroid therapy (11).

DISCUSSION

COVID-19 has demonstrated an elusive yet heterogeneous immune phenotype across all patients (7–9). These data make a compelling argument for using a precision medicine approach to the immune endotypes in COVID-19 patients when considering treatments such as corticosteroids. Undeniably, increased severity of illness (ICU vs non-ICU patients) demonstrated, in the absence of corticosteroids, significant immune suppression. However, the effect was dramatically worsened by increasing doses of in vitro administration of dexamethasone, especially in non-ICU, less severe patients. Likewise, IL-7 restoration of T

cell IFN- γ production after coincubation with dexamethasone may show a promising therapy for some patients that have T cell exhaustion and concomitant “cytokine storm.”

The strengths of our study include a younger population that may not exhibit immunosenescence as seen with older patients (mean age in this study of 42.6 vs 47.9 yr \pm dexamethasone), differing severity of illnesses (ICU vs non-ICU), and evaluation of dexamethasone dose response. Nonetheless, our findings (while hypothesis generating) should be taken with caution as they only represent in vitro findings. A before and after T cell IFN- γ production evaluation in patients receiving standard of care dexamethasone would best delineate the true in vivo effects of dexamethasone in this population.

CONCLUSION

Invariably, application of a therapy such as dexamethasone may be beneficial to some patients and harmful in others. We recommend further evaluation with a refined functional, precision medicine

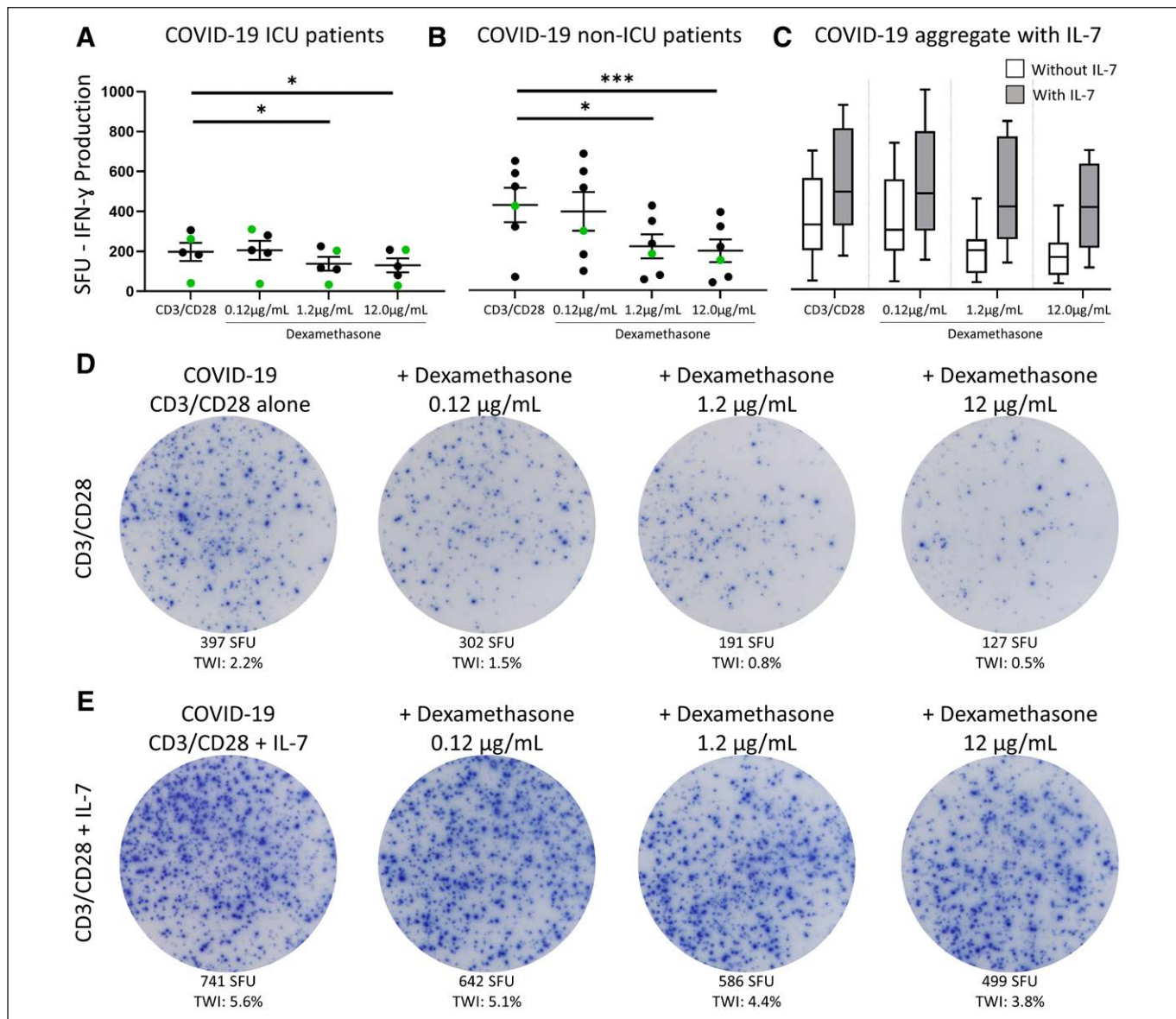


Figure 1. Effect of dexamethasone on ex vivo interferon (IFN)- γ production in patients with coronavirus disease 2019 (COVID-19). Dot plots demonstrate dose decreased number of T cells secreting IFN- γ following CD3/CD28 stimulation after coincubation with increasing concentrations of dexamethasone phosphate, where (A) represents effect of dexamethasone ($n = 11$) and (B) represents the effects of dexamethasone together with interleukin (IL)-7. C and D, Representative enzyme-linked immunospot wells demonstrating a decreased number of CD3/CD28 stimulated IFN- γ -secreting cells when coincubated with increasing concentrations of dexamethasone. C, There is also a decrease in the total amount of cytokine produced per well as demonstrated by total well intensity (TWI), indicating a weaker overall response, and lower total cytokine production due to dexamethasone. D, Addition of IL-7 restores T cell function with increased number of IFN- γ -secreting cells with lower degree of suppression due to dexamethasone. Each spot in the representative images depicts an IFN- γ -secreting cell. SFU = spot forming units.

approach that evaluates the cellular immune function of individual patients with COVID-19 to better define which therapies may have benefit or harm. Such an approach may also refine which patients may benefit from other therapies such as tocilizumab, anakinra, or IL-7. Directing therapy at known affected targets of the immune system will undoubtedly improve outcomes

in patients and may revitalize therapies that have previously demonstrated a lack of efficacy in large clinical trials. We recommend improved methods to individualize care by assessing the functional state of patient immunity and, thereby, rigorously defining which patients are appropriate to receive immune-modulating therapies to combat this pandemic.

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