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BRIEF REPORT

Reduced Cell Surface Levels of C-C Chemokine Receptor 5 and Immunosuppression in Long Coronavirus Disease 2019 Syndrome

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In an exploratory trial treating "long COVID" with the CCR5-binding antibody leronlimab, we observed significantly increased blood cell surface CCR5 in treated symptomatic responders but not in nonresponders or placebo-treated participants. These findings suggest an unexpected mechanism of abnormal immune downmodulation in some persons that is normalized by leronlimab.

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"Long COVID" is characterized by chronic and often debilitating symptoms following acute coronavirus disease 2019 (COVID-19). A significant hurdle to characterizing and diagnosing long COVID is the complexity and heterogeneity of symptoms reported among sufferers; an international cohort of 3762 participants from 56 countries identified 203 symptoms (in 10 organ systems) that persisted at least 4 weeks after a confirmed diagnosis of COVID-19 [1]. In the United States, 10%–30% of the estimated 120 million people infected with severe acute respiratory syndrome coronavirus 2 may suffer long COVID; its diagnosis and management are thus an urgent health priority.

C-C chemokine receptor 5 (CCR5) plays key roles in several diseases [2]. Multiple recent genetic association studies have reported an association between CCR5 and the severity of COVID-19 [3–5]; thus, this receptor could also be involved in the pathogenesis of long COVID. Leronlimab is a CCR5-binding humanized immunoglobulin G_4 monoclonal antibody that has been tested in extensive human trials for the treatment of human immunodeficiency virus type 1 infection [6–9] and has been suggested to improve lymphopenia, particularly CD8 T-cell levels, by resolving inappropriate inflammation in acute severe COVID-19 [10].

METHODS

Based on the hypothesis that long COVID is mediated by persisting inflammation that remains after acute COVID-19, we performed an exploratory trial in 55 individuals with long COVID. Participants (Supplementary Table 1) were randomly assigned to receive weekly subcutaneous doses of either leronlimab (700 mg) or saline placebo for 8 weeks. The demographics for these groups were relatively similar, although participants randomized to placebo were slightly older on average (51.6 years) compared with participants randomized to leronlimab (45.5 years). Changes in 24 common symptoms (Supplementary Figure 1) were compared in participants receiving either leronlimab or placebo. The primary end point was change in symptom severity through day 56 (a numerically negative change indicating improvement). All symptoms were scored as 0–4 or 0–3 (Supplementary Figure 1), and composite symptom scores were unweighted. Exploratory end points included changes in peripheral blood leukocyte CCR5 cell surface levels, immune cell phenotypes, and plasma cytokines.

RESULTS

The mean symptom score changes from baseline to the latest available time point from day 30–56 for leronlimab vs placebo were −16.0 and −12.0, respectively; adjusting for prespecified covariates, the adjusted mean difference was −1.0 (not statistically significant; Supplementary Table 2). For several symptoms, there was a numerically higher percentage of participants with reduced raw symptom scores for leronlimab compared with placebo treatment (Supplementary Figure 2), reaching borderline statistical significance without correction for multiple comparisons due to the exploratory nature of this pilot study.

Overall cell surface CCR5 levels showed significant ($P < .0001$) increases from baseline to day 56 (week 8) among leronlimab-treated participants but not placebo-treated participants (Figure 1A). When participants with symptom improvement ("responders") were considered separately from nonresponders (Figure 1B), cell surface CCR5 levels showed significant ($P < .0001$) increases from baseline to day 56 among leronlimab-treated responders but not treated nonresponders. In contrast, placebo-recipient participants showed no significant

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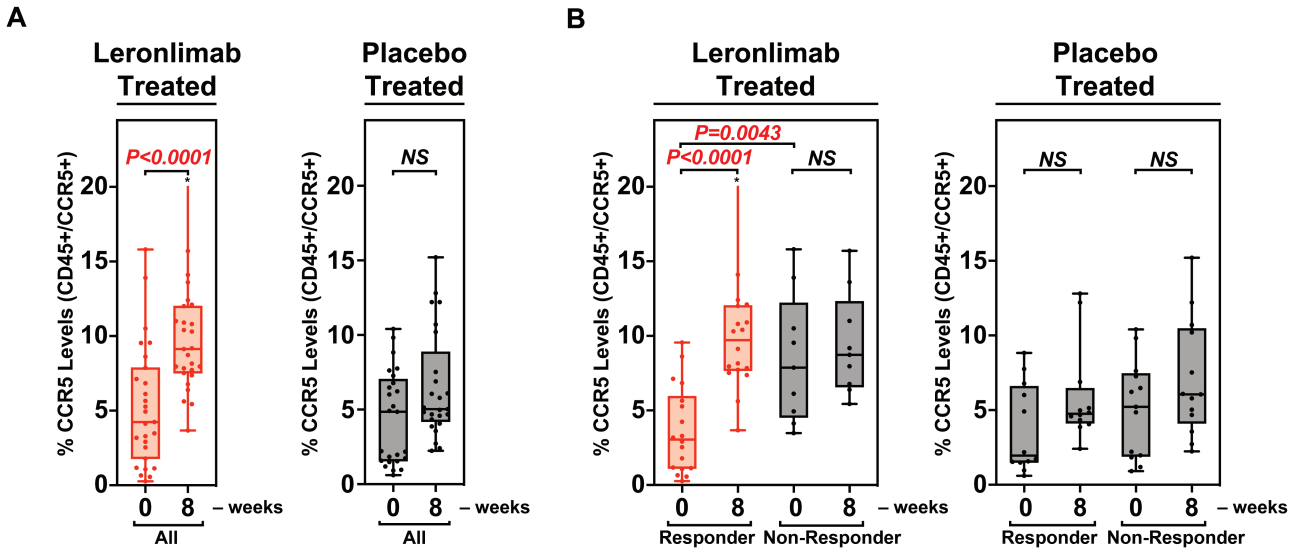

Figure 1. Percentage CCR5 levels ($CD45^+/CCR5^+$) among participants treated with leronlimab or placebo. *A,* Percentage CCR5 expressing cells in the overall leronlimab (n = 26) and placebo (n = 28) groups. *B,* Percentage CCR5 expressing cells among participants in the leronlimab and placebo groups according to participants with and without improving long coronavirus disease 2019 symptoms (14/12 and 10/18 were improved/unimproved for the leronlimab and placebo groups, respectively). The median (50th percentile; center horizontal line), interquartile range (the 25th to the 75th percentile; box), and "whiskers" (whiskers are the 0th percentile and the 100th percentile) are indicated. One data point greater than 20% (actual value of 28%) is depicted by * on the graph, but this value was included in all statistical analyses. Abbreviations: CCR5, C-C chemokine receptor 5; NS, not statistically significantly different.

difference in cell surface CCR5 levels between baseline and day 56 for both responders and nonresponders.

No differences were observed in major immune cell populations at baseline between responders and nonresponders in either the leronlimab or placebo groups (Supplementary Figure 3). However, leronlimab treatment was associated with increases in key adaptive immune cell populations (Supplementary Table 3, Supplementary Figure 4) including T cells, consistent with data from leronlimab-treated hospitalized COVID-19 patients [10], and reduced interleukin-10 and C-C chemokine ligand-2 (CCL-2) (Supplementary Figures 5 and 6).

DISCUSSION

These findings suggest an unexpected alternative mechanism for long COVID. Rather than persistent immune activation, we observed abnormal immune downmodulation, which is normalized by leronlimab. We hypothesize that this could be immune overshoot after the intense inflammation in acute COVID-19. With genetic polymorphisms reducing cell surface CCR5 levels conferring increased risk of severe COVID-19 [4], this suggests a complex role for CCR5 in balancing inflammatory and antiinflammatory effects, for example, through T regulatory cells. While leronlimab reduces the signaling of CCR5 by Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted (RANTES) [11], emerging data show that leronlimab stabilizes CCR5 expression, presumably through direct binding [12, 13], which may either change the signaling or activity of other ligands, such as Monocyte Inflammatory Protein (MIP)-1α, MIP-1β, and/or Monocyte Chemoattractant Protein (MCP-2), or increase the expression of other CCRs through heterodimerization [14]. While this is a small exploratory pilot study with potential confounders, our results support further research into the role of CCR5 in long COVID.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. S. A. K., N. Z. P., and C. R. are officers of CytoDyn Inc and report stock options and consulting fees from CytoDyn, assistance with manuscript preparation (Medical Expressions, paid by CytoDyn), and other financial or nonfinancial interests as employees of CytoDyn. J. B. S., S. G. H., and O. O. Y. are paid consultants for CytoDyn Inc. J. B. S. reports stock options available for purchase from CytoDyn and assistance with manuscript preparation (Medical Expressions, paid by CytoDyn). S. G. H. reports stock options and receipt of leronlimab for CCR5 receptor occupancy assay from CytoDyn and assistance with manuscript preparation (Medical Expression, paid by CytoDyn). O. O. Y. reports assistance in writing the manuscript (paid by CytoDyn). M. T. reports assistance with manuscript preparation (Medical Expressions, paid by CytoDyn). N. B. G. was a principal investigator for this trial, serves on

the CytoDyn Inc Scientific Advisory Board with stock options, and reports assistance with manuscript preparation (Medical Expressions, paid by CytoDyn). A. R. was a principal investigator for this trial and worked as an employee at the Center for Advanced Research & Education, Gainesville, Georgia, which is performing a clinical research trial for CytoDyn. A. R. reports assistance with manuscript preparation (Medical Expressions, paid by CytoDyn). All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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