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BRIEF REPORT

IL-7 Immunotherapy in a Nonimmunocompromised Patient With Intractable Fungal Wound Sepsis

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A nonimmunocompromised patient developed life-threatening soft tissue infection with *Trichosporon asahii*, *Fusarium*, and *Saksenaea* that progressed despite maximum antifungal therapies and aggressive debridement. Interleukin-7 immunotherapy resulted in clinical improvement, fungal clearance, reversal of lymphopenia, and improved T-cell function. Immunoadjuvant therapies to boost host immunity may be efficacious in life-threatening fungal infections.

Keywords. fungal infection; immunotherapy; interleukin-7; *Trichosporon*; wound infection.

Invasive fungal infections are a growing complication following major traumatic injuries that result in extensive soft tissue damage [1, 2]. The Department of Defense has identified combat wound infections due to invasive fungi as an emerging threat and a high priority [1, 2]. Despite aggressive surgical debridement and antimicrobial therapy that is active against the particular fungal pathogens, many infections progress, with resultant substantial morbidity and/or mortality. Progression of infection despite optimal therapy is consistent with the hypothesis that impaired host immunity may be an important pathophysiologic mechanism that renders the fungus refractory to therapy [3, 4].

Drugs that boost the host immune system are increasingly being tested in various infectious disorders in both immunosuppressed and immunocompetent patients. These immune-adjuvant therapies include interferon (IFN)-γ, checkpoint inhibitors (anti–programmed cell death 1 (anti-PD-1), granulocyte-macrophage colony-stimulating factor (GM-CSF), and interleukin (IL)-7 [5–8]. In some cases, these immune-adjuvant therapies have restored indices of immune function and lead to control of refractory infections [5–8]. Herein, we describe the use of IL-7 in a patient with life-threatening soft tissue necrotizing fungal infection that was refractory to the maximal available therapy.

PATIENT CASE

A previously healthy 21-year-old man presented to the hospital after suffering a high-velocity motorcycle accident. He suffered severe injuries including comminuted pelvic fractures, multiple extremity factures, and a catastrophic degloving injury of the buttocks and perineum with gross wound soilage. He also suffered vascular injuries that required angioembolization of his bilateral iliac arteries for hemorrhage control. On postinjury day 7, he was noted to have a rapidly progressing necrotizing soft tissue infection of his buttocks and perineum. Tissue cultures at that time demonstrated a polymicrobial infection including abundant *Acinetobacter* spp., abundant *Pseudomonas* spp. (not *Pseudomonas aeruginosa*), and moderate *Stenotrophomonas maltophila*. His wound cultures also grew *Trichosporon asahii*, *Saksenaea* spp., and *Fusarium* spp. The *Saksenaea* isolate was initially identified using conventional fungal identification methods and the sporulation inducement method for *Saksenaea* as described by Padhye and Ajello [9]. The isolate was then referred to Mayo Clinic (Rochester, MN, USA) for a species-level identification, but only a genus level could be determined. The *Saksenaea* isolate underwent susceptibility testing at the University of Texas Health San Antonio using the Clinical and Laboratory Standards Institute (CLSI) broth dilution antifungal reference method. Drug sensitivities were as follows: amphotericin B <0.03 mcg/mL, posaconazole 0.25 mcg/mL, isavuconazole 1 mcg/mL. The *Fusarium* was identified by phenotype and microscopy. *Trichosporon asahii* was identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). No sensitivities were performed on *Fusarium* or *Trichosporon asahii*.

He was aggressively treated with daily or alternating-day operative debridement, broad-spectrum parenteral and topical antibacterial and antifungal therapy. Antimicrobial therapy included ceftazidime, metronidazole, trimethoprim-sulfamethoxazole, micafungin, amphotericin B, and

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posaconazole (Figure 2), as well as VERAFLO vac instillation of antimicrobials into the wound bed. The patient's polymicrobial soft tissue infection continued to advance and was accompanied by multisystem organ failure and shock. Over a 4-week period, he required debridement of >3000 cm^2 of skin, subcutaneous tissue, and muscle involving the entire perineum, bilateral thighs, buttocks, and circumferential abdominal wall (Figure 1B). Although bacterial pathogens were rapidly eradicated from the patient's wound, cultures were persistently positive for both *Trichosporon asahii* and *Saksenaea* species despite triple antifungal therapy consisting of posaconazole, amphotericin B, and micafungin. Histopathological exam of wound biopsies showed invasive fungal elements (Figure 1E).

Despite aggressive antimicrobial and surgical management over the course of 7 weeks after the identification of the polymicrobial infection, his condition continued to worsen. The patient initially had an increased lymphocyte count of up to 2×10^3/μL, but soon developed persistent lymphopenia with neutrophilia as high as 50×10^3/μL. Therefore, immune-adjuvant therapy with recombinant human IL-7 was considered. IL-7 induces proliferation, maturation, and activation of CD4 and CD8 T cells, which are severely depleted and poorly functional in patients with life-threatening infections including those due to fungal pathogens [6, 7].

Informed consent was obtained from the patient and family, and a test dose of IL-7 (3 μg/kg ideal body weight, intramuscularly; kindly provided by Dr. Michel Morre, RevImmune) was administered on day 59 postinjury, which was well tolerated (Figure 1A). Twenty-four hours later, the dosage was increased to 10 μg/kg and continued every 3–4 days for 7 doses. The patient's clinical status showed significant improvement beginning at 4–7 days after initiation of IL-7. As illustrated by serial pictures of the wound, the most notable change following initiation of IL-7 was to slow the progression of the invasive soft tissue necrosis (Figure 1B, green arrows).

In addition to decreased infectious spread and fungal proliferation, with improved wound healing and healthier-appearing tissue margins, clinical indicators of severity of disease improved within days of IL-7 treatment initiation with improvement of the fever curve, tachycardia, and tachypnea (Supplementary Figure 1). Laboratory evidence of resolution of the fungal invasion included progressive decreases in total white blood cell count and increasing absolute lymphocyte counts (Figure 1A). The patient's absolute lymphocyte count increased >6-fold from a low of 600 lymphocytes/μL to >4000 lymphocytes/μL (upper limit of normal for absolute lymphocyte counts is 3500). Of note, the increase in the patient's absolute lymphocyte count was likely due to both the effect of IL-7 in inducing lymphocyte proliferation and to the resolution of the fungal infection.

The patient had demonstrated persistent fungal growth with rapidly extending tissue borders requiring surgical debridement over the course of 52 days before initiation of IL-7 (Supplementary Table 1). After initiation of IL-7 therapy, an increasing number of tissue cultures did not identify fungal elements, and by the fifth dose of IL-7, the patient had persistently negative cultures. Tissue histology also became negative for fungus at approximately the same time as tissue cultures (Figure 1E).

After completing IL-7 therapy, the wound beds qualitatively improved with development of healthy granulation tissue facilitating skin grafting. The patient's blood, wound, and bone cultures were negative for bacterial or fungal pathogens for 40 days after completing IL-7 therapy (see Supplementary Table 1 for complete list of cultures). The resolution in wound fungal infection facilitated definitive closure of >90% (>700 cm^2) of open wounds. Currently, the patient has a small region of exposed pelvic bone and a persistent perineal wound that remains open due to disruption of his urethra and ongoing urine drainage that is pending reconstruction. A recent biopsy of the exposed pelvic bone and the open wound margin were positive for *Trichosporon asahii*. This is being treated with an extended course of isavuconazonium. There has been no recurrence of the life-threatening necrotizing fasciitis that resolved with IL-7 therapy.

The effect of IL-7 in terms of improving the patient's T-cell function was evaluated by an ex vivo stimulation assay using anti-CD3 and anti-CD28 antibodies (BioLegend, San Diego, CA, USA) on an IFN-γ ELISPOT assay (Cellular Technologies Limited, Shaker Heights, OH, USA), which was performed as previously described [10, 11]. The total number of activated, IFN-γ-producing T cells progressively increased from baseline (before IL-7 therapy), accompanied by a 1.4-fold increase in the proportion of activated T cells (Figure 1D).

A beneficial effect of IL-7 in infectious disorders is to increase expression of lymphocyte adhesion molecules and induce lymphocyte trafficking to sites of infection [6]. Consequently, immunohistochemical staining using the lymphocyte marker anti-CD3 was performed and demonstrated a marked increase in the number of lymphocytes in the biopsies from the infected wound (Figure 1F, right-hand panel).

To measure the specific molecular effects underlying the increased T-cell responsiveness induced by IL-7, we measured intracellular levels of phospho-STAT5 (pSTAT5) in CD4 and CD8 T cells using mass cytometry. STAT5 is the primary T-cell differentiation signal downstream of the IL-7 receptor [12]. T cells were identified based on surface marker staining (CD45+/CD15-/CD66b-/CD56-/CD3+). We then measured the pSTAT5 signal intensity. IL-7 was associated with a 3-fold increase in activated STAT5 in CD4-T cells and a 2-fold

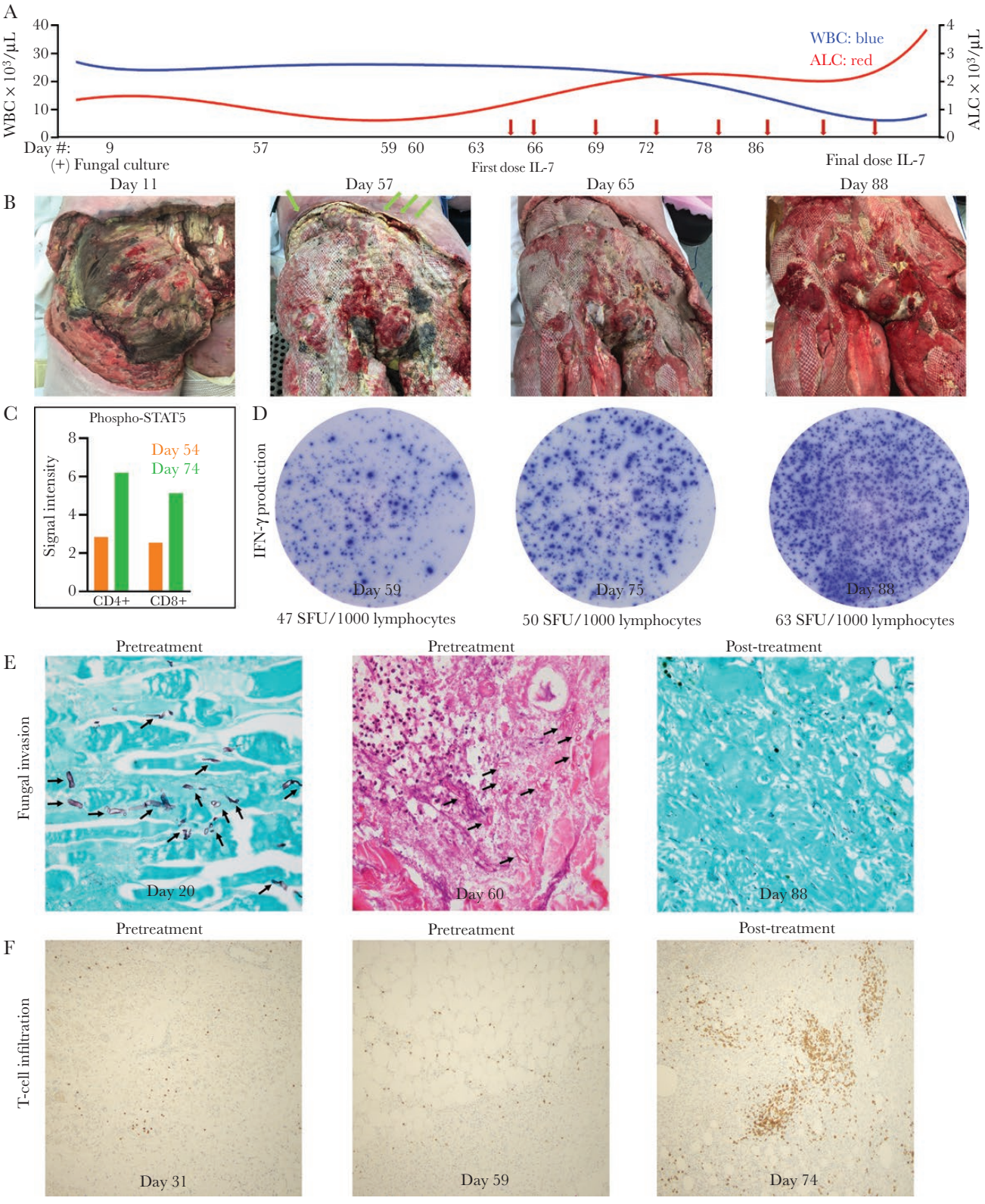

Figure 1. Serial clinical, anatomic, immunologic, and pathologic changes in an IL-7-treated patient with invasive fungal infection. A, Changes in WBC and ALC during the patient's hospital course. Days of hospitalization are indicated on the x-axis; IL-7 was initiated on day 59. Red arrows identify administration of IL-7. B, Color photographs of lower back and gluteal region demonstrating necrotic and infected areas; there was serial progression of the infection with necrotic regions, which resolved following initiation of IL-7. The green arrows in the second photo from the left show necrotic and poorly perfused margins. C, IL-7 mediates its effects to increase T-cell IFN-γ production via STAT5 signaling. CyTOF demonstrated that there was a doubling of STAT5 expression in lymphocytes obtained after initiation of IL-7 compared with pretreatment; CyTOF data are reported at the geometric mean raw signal intensity for the gated population. All 3 samples were barcoded and run in a single experiment. The data presented are

increase in CD8-T cells (Figure 1C). These data provide a putative molecular mechanism for the increase in T-cell function induced by IL-7.

Patient Consent

Informed consent was obtained from the patient and the patient's parents for therapy using IL-7 and for publication of this case report including images of the patient's wounds.

DISCUSSION

Critically ill patients with protracted sepsis typically develop profound and persistent immunosuppression [13]. Numerous pathophysiologic mechanisms drive the immune suppression including apoptosis-induced lymphocyte depletion, increased myeloid-derived suppressor cells, and T-cell exhaustion. Based upon a growing number of case reports, there is increasing recognition that therapies that boost patient immunity may be beneficial in patients with intractable infections that are nonresponsive to conventional therapies [5, 8, 13]. Particularly relevant to the present case is the use of the immune-adjuvants nivolumab (anti-PD-1) and IFN-γ in a Belgian bomb blast victim who was dying of refractory mucormycosis [5]. Immune-adjuvant therapy resulted in rapid clinical improvement, enhanced immune phenotypic markers, and fungal elimination.

Although a number of immuno-adjuvants are likely to be beneficial, IL-7 is particularly attractive because of its effects on a broad array of immune effector cells including CD4 and CD8 T cells, mucosally associated invariant T cells, and innate lymphoid cells that play key roles in pathogen elimination [8, 13]. Although not presently approved for clinical use, IL-7 is under investigation in multiple clinical trials in infectious and oncologic disorders [6–8]. IL-7 has an excellent safety profile and has been used in >450 patients with both severe infections and various cancers. A double-blind, randomized, phase 2 trial of IL-7 in patients with sepsis showed that IL-7 was well tolerated, reversed sepsis-induced lymphopenia, and enhanced T-cell activation [6]. IL-7 has also been shown to prevent lymphocyte apoptosis, improve immune function, and increase survival in a 2-hit animal model of fungal sepsis. IL-7's primary effect is on lymphocytes, but it will have indirect effects to enhance macrophage and neutrophil antimicrobial properties as well. IL-7 increases T-cell production of IFN-γ, a potent activator of macrophages. IL-7 also increases T-cell IL-17 production, which plays a critical role in fungal infections by enhancing neutrophil migration to sites of infection [6]. IL-7 should be particularly advantageous in patients with profound and persistent lymphopenia because of its potential to prevent lymphocyte apoptosis and induce lymphocyte proliferation.

Although the patient in this report had no history of recurrent fungal infections to suggest an underlying immune deficiency, persistent or recurrent mucocutaneous or invasive fungal infections developing in a "normal" host may be indicative of genetic defects in innate or adaptive immunity [14, 15]. Recently, defects in the caspase recruitment domain containing protein 9 (CARD9) have been reported to occur in patients with severe fungal infections [15].

Recently, immuno-adjuvant therapy to boost host immunity has been proposed as a potential additional powerful weapon in the armamentarium in infectious diseases [8]. The authors believe that the rather remarkable turnaround in the patient's hospital course in the current report provides further support for the concept of augmenting the integrity of the host immune system in life-threatening infections. The ability to evaluate the functional status of the patient's immune system, such as the use of the ELISpot assay in the present case (Figure 1D), will greatly advance this field by identifying patients who are immunosuppressed and enabling investigators to follow patient response to immuno-adjuvant therapies. Patients with intractable hospital-acquired infections involving multidrug-resistant bacteria or patients with invasive fungal infections are likely good candidates for immuno-adjuvant therapies because they are almost invariably immunosuppressed and have high mortality. Use of IL-7, checkpoint inhibitors, or other immune-adjuvant therapies might be considered on a compassionate basis in patients dying of these intractable fungal infections. If immunotherapy does prove to be an effective new approach in infectious diseases, it could usher in a novel path forward in the battle against increasingly deadly foes.

the geometric mean of the raw values for the gated populations (not normalized), as reported by the cytometer. D, Decreased T-cell IFN-γ production is a hallmark of T-cell exhaustion; ELISpot analysis on PBMCs were performed serially; the far-left ELISpot well had 47 IFN-γ-producing lymphocytes (depicted as SFU) per 1000 lymphocytes plated; the middle and far-right wells show a progressive increase in the both the number of lymphocytes in the PBMC fraction as well as an increase in the proportion of active IFN-γ-producing lymphocytes following CD3/CD28 stimulation. E, Immunohistochemical staining of wound margins: left panel: Gomori's methanamine silver stain highlighting invasive fungal hyphae (arrows), 400×; middle panel: hematoxylin and eosin stain showing necroinflammatory debris and refractile-appearing fungal hyphae (arrows), 400×; right panel: Gomori's methanamine silver stain highlighting shows clearance of invasive fungal microorganisms following IL-7 treatment; soft tissue necrosis is present, 400×. F, Immunohistochemical staining for $CD3^+$ T cells. Note the marked increase in the number of lymphocytes in the wound margins that occurred after initiation of IL-7, 100×. Abbreviations: ALC, absolute lymphocyte count; CyTOF, time of flight mass cytometry; IFN-γ, interferon-γ; IL-7, interleukin-7; PBMCs, peripheral blood mononuclear cells; SFU, spot-forming units; WBC, white blood cell count.

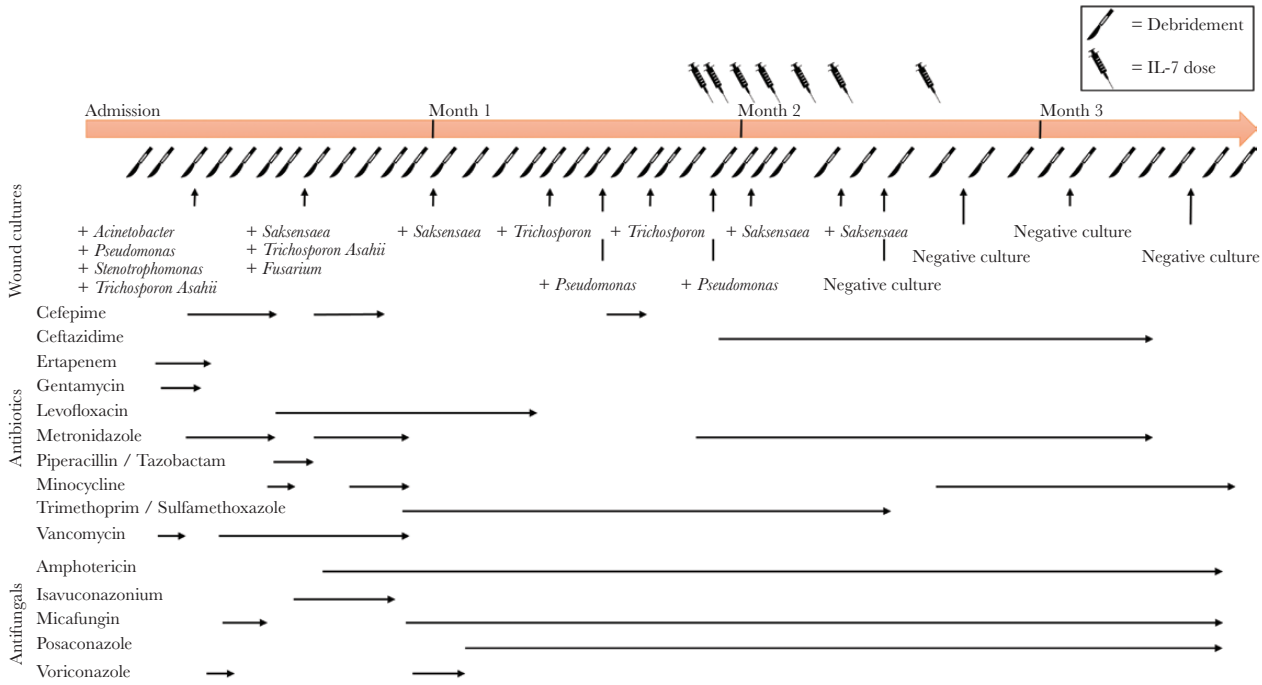

Figure 2. Time course of antimicrobial and surgical therapy. Graphical depiction of a timeline from the date of admission through the fourth month of his hospital course. This timeline demonstrates the surgical debridement occurrences, positive tissue cultures, antimicrobial therapies, and doses of recombinant human IL-7. Abbreviation: IL-7, interleukin-7.

Supplementary Data

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

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Potential conflicts of interest. R.S.H. is the principal investigator for a study of IL-7 in patients with sepsis being conducted by RevImmune. R.S.H. is also the principal investigator in the United States for a multicenter international trial of IL-7 in patients with COVID-19 being conducted by RevImmune. R.S.H. has received research funding from Transgene and has been a consultant to Transgene. No other authors have conflicts of interest relevant to the content of this manuscript.

Author contributions. I.R.T., M.B.M., M.H.H., J.P.K., J.M.L., C.M.C., A.S., J.B., S.M.M., E.M.R., R.F.P., J.P.G., K.E.R., and R.S.H. contributed to collection of clinical and laboratory data and performance of ancillary laboratory tests and contributed to clinical care of the patient. All authors contributed to the drafting and editing of the manuscript. All authors read and approved the final manuscript.

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