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Case Report

Induction of Cytokine Storm with Organ Dysfunction and Ineffective Response to Tocilizumab and Steroids by CAR-T Cell Therapy is Rescued by Mesenchymal Stem Cells: A Case Report

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ABSTRACT: Two of the main toxicities linked with chimeric antigen receptor T (CAR-T) cell therapy are cytokine release syndrome (CRS) and immune effector cell associated neurotoxicity syndrome (ICANS). It is believed that an inflammatory factor storm resulting in organ dysfunction is the fundamental pathophysiology, and this has a significant impact on the survival rate following CAR-T cell therapy. While steroids and other IL-6R antagonists like Tocilizumab are useful treatment approaches, not all patients benefit from them. Following CD19-targeted CAR-T cell infusion, a 3-year-old kid with relapsed refractory B-cell acute lymphoblastic leukemia (ALL) started to have CRS on Day 1. Despite receiving steroids and tocilizumab early on, his illness worsened and eventually led to multiple organ failure, particularly heart failure. After receiving mesenchymal stem cells (MSCs) as a rescue treatment on Days 17 and 20, the patient's clinical symptoms were stabilized by Day 25. Negative microscopic residual disease (MRD) was achieved with CAR-T therapy, and CAR expression was maintained in peripheral blood until Day 75. The case implies that MSC may be a useful treatment for multi-organ dysfunction that results from severe CRS and ICANS brought on by CAR-T cell therapy.

Keywords: Chimeric antigen receptor T cell; Cytokine release syndrome; Organ dysfunction; Mesenchymal stem cell

1. Introduction

Following the binding of chimeric antigen receptor T (CAR-T) cells to the target antigen to lyse tumor cells, macrophages and monocytes become activated. This leads to a significant release of inflammatory cytokines, including IL-6, IL-1β, IL-2, TNF-α, IFN-γ, GM-CSF, iNOS, and so forth, which is known as cytokine release syndrome (CRS) [1,2]. Inflammatory agents can cause vascular instability, capillary leakage, and consumption coagulopathy by

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damaging endothelial cells and disrupting the tissue barrier [3]. Organ dysfunction involving the cardiac, pulmonary, hepatic, renal, and gastrointestinal systems may result from severe CRS [4]. Immune effector cell-associated neurotoxicity syndrome (ICANS) usually develops in conjunction with neurotoxicity or after CRS symptoms resolve [5]. The U.S. Food and Drug Administration (FDA) has approved steroids and the IL-6 receptor antagonist tocilizumab for the treatment of CRS, whereas dexamethasone, a corticosteroid, is advised for the treatment of ICANS. For CRS and ICANS, no specific preventive therapy is currently advised [6,7]. Additionally, analysis of the ZUMA-1 clinical trial revealed that tocilizumab enhanced neurotoxicity when used as a preventative measure early on. Although glucocorticosteroids can cross the blood-brain barrier (BBB) and significantly reduce the inflammatory response, opinions on whether or not they could impair the effectiveness of CAR-T cell therapy are divided [8].

The well-known stromal cells known as MSCs have the ability of immunomodulatory effects, hematopoietic reconstitution and tissue-repair effects without being limited by the major histocompatibility complex (MHC). It is unknown at this time if MSCs aid in the suppression of CRS and ICANS and encourage organ healing. Here, we describe a case of multi-organ failure following CAR-T cell-induced CRS and ICANS that was treated with MSCs.

The guardian of the child provided their written informed consent to participate in this study. Written informed consent was obtained from the individuals for the publication of any potentially identifiable images or data included in this article.

2. Case Presentation

A male child was admitted to our hospital with "pallor" in February 2017. Bone marrow investigation confirmed the diagnosis of acute lymphoblastic leukemia (ALL, common B-cell type, intermediate risk). Following the first round of induction chemotherapy, the patient experienced remission and was treated with normal consolidation chemotherapy. The disease recurred in September 2018, and post-chemotherapy bone marrow (35% naive lymphocytes) demonstrated the ineffectiveness of the relapse protocol treatment. It was intended to use CD19 CAR-T cell therapy (NCT03391739). However, preparatory chemotherapy with the FC regimen (Flu 13.75 mg qd×3 + CTX 165 mg qd×3) was first administered from Day 4 to Day 2 due to the rapid growth of peripheral blood leukocytes. CD19 CAR-T cells were given at 4.2×10^6/kg after leukocytes decreased.

The body temperature increased abruptly on the first day after CD19 CAR-T cell infusion. Antibacterial therapy was administered as the CT scan suggested a pulmonary infection. On Day 4, the temperature reached 41.6 ℃ and the level of IL-6 increased to 133.6 pg/mL, indicating the onset of CRS. Tocilizumab (4 mg/kg qd) was administered to control CRS (grade 2). On Day 5, the child had high fever, dyspnea with oxygen saturation of 93%–95%, and IL-6 increased sharply to more than 5000 pg/mL. Then Tocilizumab was raised to 4 mg/kg every 12 hours and combined with methylprednisolone at 3 mg/kg every day. However, the symptoms worsened with time, culminating in a huge serous effusion and the detection of significant levels of CAR19 T cells and IL-6. Thus, Tocilizumab (8 mg/kg q8h) and methylprednisolone (3 mg/kg q12h), fresh plasma transfusion, and other symptomatic supportive care were continued. Figure 1A depicts the CRS and ICANS creation and management process. Figure 1B displays the variations in IL-6, procalcitonin (PCT), lactate dehydrogenase (LDH), C-reactive protein (CRP), and body temperature.

On Day 7, the child's dyspnea was slightly improved, but he became unresponsive, somnolent, and had generalized skin cyanosis. When the heart rate dropped to 50 beats/min, myocardial enzymology was significantly increased, NT-pBNP reached a peak of 16 525 pg/ml, and cardiac ultrasonography revealed a left ventricular ejection fraction (LVEF) of 54% (Figure 2A). On the CT scan, there was interlobular edema and pleural effusion but no evident lesions on the craniums (Figure 2B). The concentration of IL-6 in the cerebrospinal fluid (CSF) was up to 393.8 pg/mL and CSF pressure was 220 mmH2O with no detectable CAR-T cells. Methylprednisolone was replaced with dexamethasone (0.5 mg/kg q12h and 2 mg by sheath injection), as ICANS and multiple-organ failure were taken into account. On Day 10, peripheral blood leukocytes rose to 10.34×10^9/L with 98% expressing the CD19 CAR phenotype (Figure 2C). Nonetheless, the patient exhibited cardiac failure symptoms such as low blood pressure, oliguria and persistent skin cyanosis, as well as respiratory failure, as evidenced by decreased oxygen saturation and a cough with blood in the sputum. As a result, a diagnosis of grade 4 CRS with multi-organ failure and life-threatening consequences was made. In addition, the results of cardiac ultrasonography suggested a drop to 42% LVEF, and the level of Creatine Kinase was 17 times higher than the normal value. The patient requires mechanical ventilation to maintain blood oxygen saturation because of left heart failure, cytokine infiltration, irregular coagulation, and thrombocytopenia that exacerbated pulmonary hemorrhage (Figure 2D). Given the organ tissue damage from severe CRS following CAR-T treatment, a treatment dose umbilical cord MSC was administered on Day 17. The umbilical cord mesenchymal stem cells were

donated by healthy mothers, approved by the ethics committee of national engineering research center for cell products. The very next day, the patient's skin cyanosis and lung rales were markedly reduced and LVEF was slightly improved (46%). On Day 20, another dose of MSC was administered based on the response to the first infusion. As cardiac function gradually recovered, alveolar exudation and hemoptysis decreased while oxygen saturation increased. On Day 25, the patient's vital signs were stable, LVEF increased to 55%, and tracheal intubation was removed. MRD was still negative on Day 75, and 1.8% of memory CD19 CAR-T cells, defined as $CD45RO^{+}CD62L^{+}CAR^{+}$, were detectable in peripheral blood (Figure 2E).

After receiving a cord blood transfusion later, the child remained in full remission for 3.5 years after the procedure.

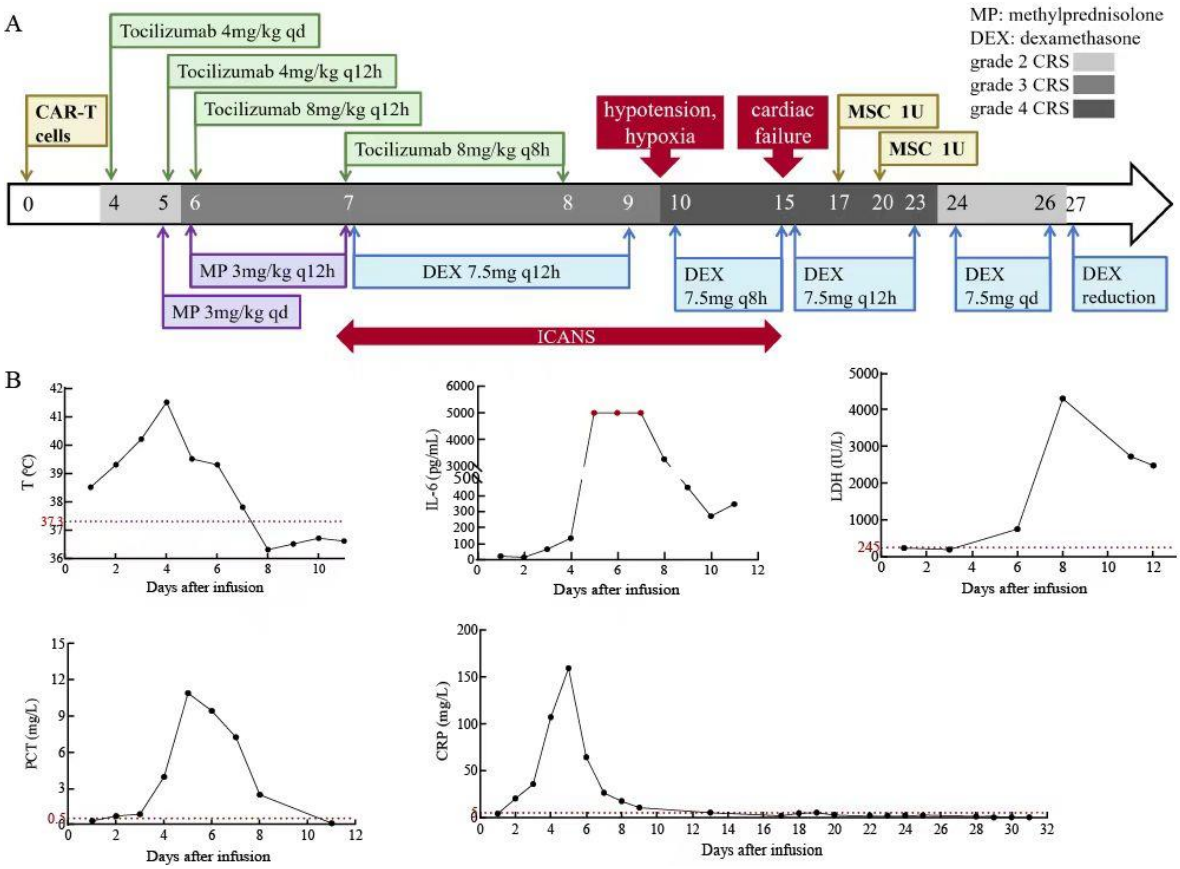

Figure 1. The development and management of CRS and ICANS after CAR T-cell therapy. A: Tocilizumab and methylprednisolone were used on the first and second day after the development of CRS and ICANS. Dexamethasone was the pivotal treatment for ICANS. Only MSCs successfully rescued multi-organ failure. B: Changes in temperature, IL-6 (pg/mL, ref: 0.37–0.46), LDH (IU/L, ref: 109–245), PCT (mg/L, ref: 0–0.5) and CRP (mg/L, ref: 0–5.00). When IL-6 is greater than the maximum range of detection 5000, denoted by 5000.

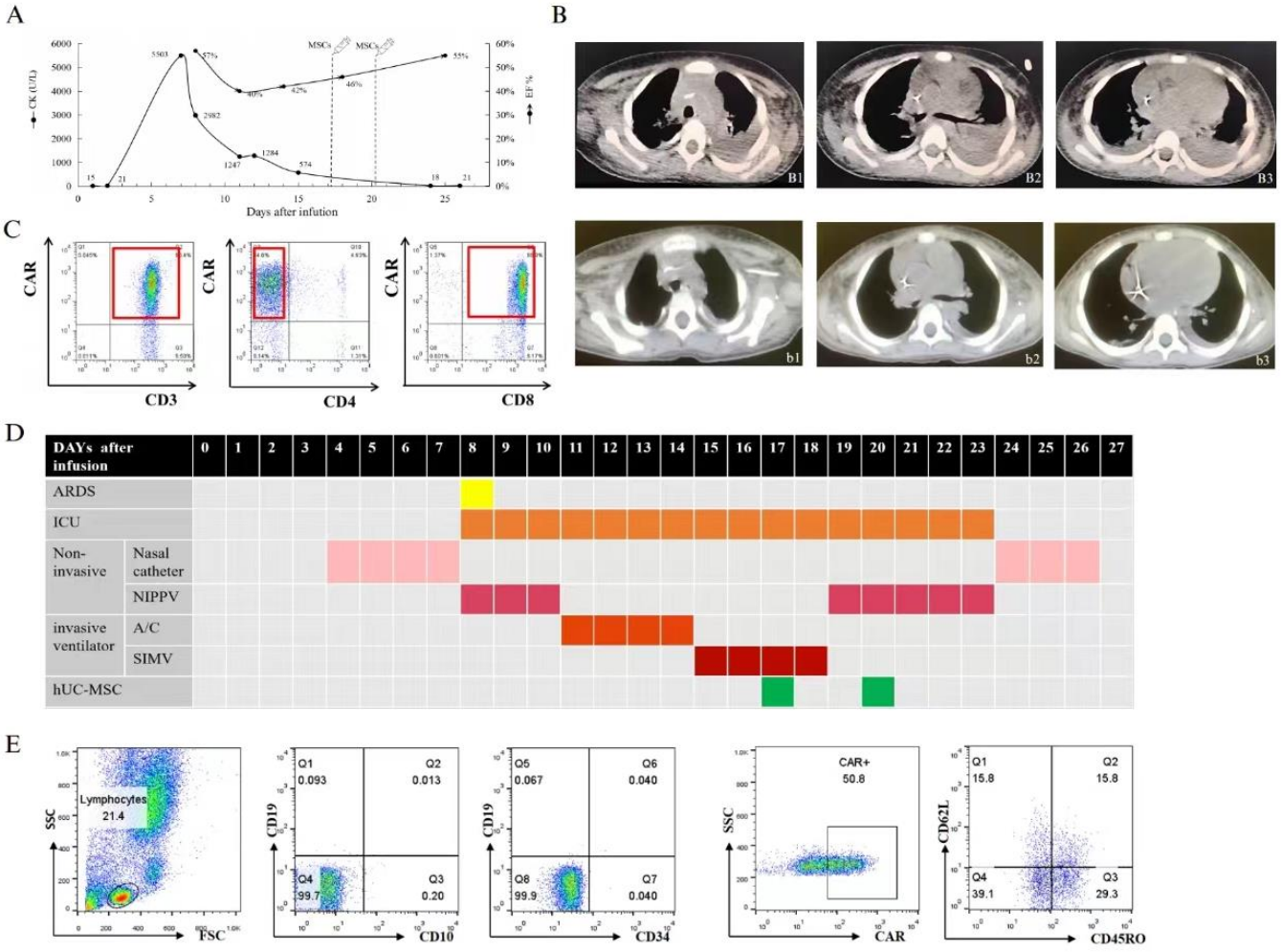

Figure 2. MSC rescued multi-organ failure from CRS and did not affect the expansion of CAR-T cells. (**A**): Changes in LVEF and creatine kinase assessing cardiac function after CAR-T cell infusion. (**B**): The typical CT images of the lung. B1–B3, CT images on Day 8 indicated that there are pleural effusion and interlobular edema. b1–b3, CT images on Day 21 indicated the relief in both left and right lung. Most of the effusion lightened or even disappeared. (**C**): Flow cytometry for peripheral blood lymphocyte phenotype on Day 10 after CAR19 T-cell transfusion. The CD19 CAR phenotype was expressed in 98% of peripheral blood lymphocytes. (**D**): The major respiratory support treatments after CAR-T cells infusion. The use of MSC shortened the patient's ventilator use time and accelerated transferred out of ICU. (**E**): Flow cytometry analysis showed 1.8% memory CD19 CAR-T cells, defined as $CD45RO^{+}CD62L^{+}CAR^{+}$, in peripheral blood lymphocytes at Day 75 after CD19 CAR-T cell transfusion.

3. Discussion

The CAR-T cell is an effective therapy for relapsed refractory hematological diseases with remission rates as high as 90%. When B-cell hematological malignancies are treated with CAR-T cells, CRS occurrences range from 50% to 90%, with severe CRSs (grades 3 and 4) carrying a substantial risk of death. Our case suffered from multiple organ dysfunction induced by severe CRS (sCRS) and there was no consensus on the treatment. Although not for CRS, MSCs were utilized to treat organ damage in conjunction with other immunotherapies [9].

Nevertheless, the risk for CRS is remarkably associated with tumor burden [4]. In our case, the child, who suffered from relapsed refractory B-ALL, was treated with CAR-T cells as a bridge to transplantation [8]. Given the child's obvious high-risk for CRS, Tocilizumab was started at an early stage and dexamethasone was thereafter administered at the maximum tolerated dose. The child continued to have life-threatening consequences from multiple organ failure in spite of these interventions. No consensus exists on effective measures to prevent CRS. Several studies found that JAK and mTOR inhibitors are effective in preventing the development of CRS in humanized NSG mice by inhibiting the active signaling pathways of associated inflammatory [10,11]. Current treatments include Tocilizumab, which specifically inhibits IL-6, and steroids, which improve symptoms but not the prognosis. On the other hand, active T cells might be the cause of sCRS. The expansion of CAR-T cells is associated with the structural composition of CAR as costimulatory domains, and the *in vivo* environment of the recipient as elevated cytokines like IL-6, IL-21, and IL-7

[12]. A sharp increase of CAR-T cells in peripheral blood was found on Day 10, so we speculated that it was due to the increasing of the level of several cytokines. Recently, it was found that off-target effects may also be involved in the development of ICANS due to the presence of CD19 expression in human brain mural cells by single-cell RNA sequencing analysis [13]. In this case, the high concentration of IL-6 was detected in the cerebrospinal fluid but no CAR-T cells, suggesting that the occurrence of ICANS may be more related to the intracranial accumulation of cytokines due to BBB damage.

Mesenchymal stem cells (MSCs) are a subpopulation of stromal stem cells with powerful immunomodulatory, hematopoietic support, and tissue repair functions [14]. Many studies confirmed that MSCs or their exosomes can regulate macrophage polarization from a pro-inflammatory M1 type to an anti-inflammatory M2 type, resulting in a decrease in pro-inflammatory factor secretion (IL-6, NO, TNF-α, IL-1β, IL-15) and an increase in anti-inflammatory factor secretion (IL-10, IL-4, IL-13, IL-21) [15]. Our previous study revealed that MSCs may ameliorate anthracycline-induced cardiotoxicity by inhibiting myocardial infiltration of inflammatory T, B, and APC cells [16]. Some randomized controlled trials found that MSCs can improve the outcome of COVID-19 patients who also experienced cytokine storm [17]. The child's cardiac and pulmonary failure was successfully treated with MSCs, resulting in a shorter ICU stay and endotracheal intubation time. In our case, CAR-T cells were detectable till Day 75, showing that MSC did not compromise CD19-CAR T-cell effectiveness, which is consistent with previous *in vivo* experimental results [18].

In conclusion, this case study demonstrates that MSCs may be a unique approach for the clinical management of CAR-T cell problems. The patient had successful therapy with MSCs for severe CRS and ICANS, and after receiving CAR-T cell infusion, the patient experienced organ failure. Whether MSCs also contribute to persistence of memory CAR-T cells deserves further studies.

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Author Contributions

C.H., X.L., N.X., C.Y., G.H., J.R., X.Z., J.L., X.Y., H.Z., and Y.Z. contributed to the manuscript investigating; C.H. and X.L. wrote original draft; Z.H. contributed mesenchycal stem cell; G.H., T.Y., and J.H. contributed to the reviewing and editing.

Ethics Statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of National Engineering Research Center for Cell Products (protocol code LL2018-12-03, December 23, 2018).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

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Declaration of Competing Interest

The authors declared no conflict of interests.

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