Mini-review

Cytokine release syndrome in cancer immunotherapy with chimeric antigen receptor engineered T cells

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Article info
Article history:
Received 30 August 2013
Received in revised form 30 September 2013
Accepted 9 October 2013

Keywords:
Chimeric antigen receptor
Cytokine release syndrome
Cancer
Adverse event
Safety

Abstract
Adoptive transfer of chimeric antigen receptor (CAR)-engineered T cells is a promising new therapy for cancers. However, the safety of this approach is concerned. Cytokine release syndrome (CRS) is a common but lethal complication of CAR-T cell therapy. The development of CRS correlates with CAR structures, tumor type and burden, and patients’ genetic polymorphisms. CRS related adverse events may be reduced by designing safer CARs and CAR-T cells and following strict dose-escalation scheme. Timely and effective cytokine-directed treatment with corticosteroid and various cytokine antagonists is important to avoid CRS associated death.

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1. Introduction

Adoptive transfer of chimeric antigen receptor (CAR)-engineered T cells is a promising new therapy for cancers. Significant progresses have been made in the past decades as the optimization of the CAR design [1–3]. The results from early clinical trials have revealed a very encouraging therapeutic efficacy of the CAR-mediated immunotherapy in a variety of cancers including lymphoma, chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL) and neuroblastoma [4–9].

Although most adverse events with genetically modified T cell infusion are tolerable and acceptable in clinical trials, the safety and toxicity of CAR engineered T cell (CAR-T cell) infusion are of concern, including insertional mutagenesis, off-target effects and systemic inflammatory reaction (cytokine storm and tumor lysis syndrome) [10]. Two cases of serious adverse events following the administration of CAR-T cells were reported in 2010 [11,12]. Both the deaths seemed related to a systemic cytokine release that has been termed cytokine release syndrome (CRS). According to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAEs) Version 4.0, CRS is a disorder characterized by nausea, headache, tachycardia, hypotension, rash, and shortness of breath caused by the release of cytokines from the cells [13]. It is caused by an exaggerated systemic immune response mediated by T cells, B cells, NK cells and monocytes/macrophages which release a large amount of inflammatory mediators such as cytokines and chemokines. CRS is not a rare phenomenon in clinical setting. It occurs in graft-versus-host disease (GVHD) after transplantation, severe bacterial and viral infections, hemophagocytic lymphohistiocytosis (HLH)/macrophage activation syndrome (MAS) and monoclonal antibody (mAb) therapy [14–18]. Cytokines trigger an acute inflammatory response and induce endothelial and organ damage, which result in microvascular leakage, heart failure and even death [19–21]. Thus, it is of great importance to timely and properly manage CRS during CAR-T cell therapy. In this review, we will briefly discuss the manifestations, pathophysiology, precaution and treatment of CRS in CAR-T cell infusion.

2. Cytokine profile and clinical manifestations of CRS

The administration of biologic products resulting in CRS was firstly observed in patients with anti-CD3 mAb OKT3 therapy for organ transplantation [22]. Within 1–4 h following the antibody injection, serum levels of interferon (IFN)-γ, tumor necrosis factor (TNF)-α and interleukin (IL)-2 were markedly elevated. Severe, life-threatening CRS has been reported in six previous healthy volunteers who received intravenously infusion of TGN1412, an anti-CD28 humanized mAb that directly stimulates T cells [23]. Within 4 h after receiving the antibody, all six volunteers experienced a systemic inflammatory reaction characterized by a rapid induction of cytokines including IFN−γ, TNF−α, IL-1β, IL-2, IL-6, IL-8 and IL-10. Twelve to sixteen hours after infusion, all the patients presented
pulmonary infiltration and lung injury, renal failure and disseminated intravascular coagulation.

CRS is frequently observed in the clinical trials to treat hematological malignancies with CD19 and CD20-specific CAR-T cells as well. A variety of inflammatory cytokines and chemokines are intensively monitored before and after CAR-T cell infusion in these studies, including IFN-γ, TNF-α, IL-1β, IL-2, IL-6, IL-7, IL-8, IL-10, IL-12, sIL-2Rα, granulocyte macrophage colony stimulating factor (GM-CSF), macrophage inflammatory protein (MIP)-1 [4–8,24,25]. Based on the systemic review of six clinical trials performed in four institutions in which CRS has been reported (Table 1), we find that CRS occurs in nearly two thirds of patients treated with CAR-T cells, which usually occurs 6–20 days after CAR-T cell infusion. However, it could happen in a very short time after CAR-T cell infusion in some patients [11,12,24]. From two fatal case reports, we speculate that the time point of CRS may be related to baseline cytokine levels and the chance of CAR-T and cancer cell encountering [11,12]. When the baseline cytokine level is high at CAR-T cell infusion, or a large amount of CAR-T cells encountering with the target cells at a very short time, the CRS might be triggered earlier and enormous. All of the above cytokines are found elevated in part of the patients with CRS, while the cytokine profiles varies greatly among different individuals. IFN-γ, TNF-α and IL-6 are the most frequently monitored cytokines. IFN-γ and IL-6 are increased more than 10 folds in most patients with CRS when compared with the baseline, while TNF-α is rarely elevated in four of the six studies.

The clinical symptoms result from CRS include fever, fatigue, headache, seizure, nausea, rigors, chills, myalgia, dyspnea, acute respiratory distress syndrome (ARDS), hypotension, acute vascular leak syndrome, tachycardia, liver function impairment and renal failure (Table 1). Fever is the most frequent and may be the earliest sign of CRS, which progresses along with the development of CRS and can be completely resolved after the control of CRS [8]. Hypotension is not rare in patients with CRS, which needs immediate fluid resuscitation or vasopressor support but can be reversed in most patients after the effective cytokine directed therapy. In Grupp and Teachey et al.’s study, they found that the manifestations of CRS in their patients mimic HLH/MAST, with highly elevated serum ferritin, d-dimer, aminotransferases, lactate dehydrogenase and triglycerides, hypofibrinogenemia, and hepatosplenomegaly [8]. Furthermore, the cytokine pattern of significant elevation of IFN-γ, IL-10 and IL-6, but not TNF-α is consistent with that of HLH as well [17,26].

The severity of the CRS correlated well with the level of cytokines. Brentjens et al. found that the degree of cytokine elevation was coincident with post-infusion fevers and episodes of relative hypotension [7]. In their study, two patients with high tumor burden presented higher cytokine levels and increased fever severity and persistence accompanied by multiple organs involved, while other patients only showed mild fever or hypotension. Kochenderfer et al. evaluated the cytokine-associated toxicity by using a score system named sequential organ failure assessment (SOFA), which includes an assessment of hypotension, the platelet count, and the respiratory, liver, renal, and central nervous system functions [25]. They found that the patients with prominent elevations in serum IFN-γ and TNF-α after CAR-T cell infusion had a higher mean total SOFA score than those without elevations in IFN-γ and TNF-α.

3. Pathophysiology of CRS

The classic and basic design of a CAR includes a single chain variable fragment (scFv) targeting tumor associated antigen (TAA), an extracellular spacer/hinge region, a trans-membrane domain and an intracellular signaling domain. After the CAR-T cells encountered the tumor cells, the scFv is engaged by the TAA and the activation signal is transduced to the immunoreceptor tyrosine-based activating motif of the CD3ζ chain. The CD3ζ chain provides the requisite ‘signal 1’ resulting in T cell activation, cytokine secretion and target cell lysis [27,28]. Of the second-generation CARs, the

<table>
<thead>
<tr>
<th>Table 1</th>
<th>The cytokines and symptoms involved in CRS in the CAR-T cell clinical trials.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Targeted antigen</td>
<td>CLL</td>
</tr>
<tr>
<td>Co-stimulatory domain</td>
<td>CD19</td>
</tr>
<tr>
<td>Evaluable case number</td>
<td>8</td>
</tr>
<tr>
<td>Multiple cytokine elevation</td>
<td>5/7</td>
</tr>
<tr>
<td>Time of peak cytokines</td>
<td>&lt;2 days</td>
</tr>
<tr>
<td>Individual cytokines</td>
<td></td>
</tr>
<tr>
<td>IFN-γ</td>
<td>3/7</td>
</tr>
<tr>
<td>TNF-α</td>
<td>5/7</td>
</tr>
<tr>
<td>IL-6</td>
<td>NR</td>
</tr>
<tr>
<td>IL-10</td>
<td>NR</td>
</tr>
<tr>
<td>IL-2</td>
<td>5/7</td>
</tr>
<tr>
<td>IL-7</td>
<td>3/7</td>
</tr>
<tr>
<td>IL-8</td>
<td>NR</td>
</tr>
<tr>
<td>IL-12</td>
<td>2/7</td>
</tr>
<tr>
<td>Clinical symptoms</td>
<td>3/7</td>
</tr>
<tr>
<td>Fever</td>
<td>8/8</td>
</tr>
<tr>
<td>Rigors and chills</td>
<td>5/8</td>
</tr>
<tr>
<td>Fatigue</td>
<td>NR</td>
</tr>
<tr>
<td>Hypotension</td>
<td>2/8</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>1/8</td>
</tr>
<tr>
<td>ARDS</td>
<td>NR</td>
</tr>
<tr>
<td>Encephalopathy</td>
<td>NR</td>
</tr>
<tr>
<td>ALT/AST elevation</td>
<td>NR</td>
</tr>
<tr>
<td>Renal failure</td>
<td>1/8</td>
</tr>
<tr>
<td>Cardiac disorders</td>
<td>1/8</td>
</tr>
</tbody>
</table>

Abbreviations: MSKCC, Memorial Sloan-Kettering Cancer Center; UPenn, University of Pennsylvania; NCI, National Cancer Institute; FHCRC, Fred Hutchinson Cancer Research Center; CLL, chronic lymphocytic leukemia; ALL, acute lymphoblastic leukemia; NR, not reported. Cytokine elevation: 10 folds higher than the baseline level. Multiple cytokine elevation, three or more cytokines elevated with levels 10 folds higher than the baseline level.

* Including patients with TNF-α concentration 10 folds higher than the baseline level or higher than 50 pg/mL.
CAR activation signals, cytokine secretion and anti-tumor activity are enhanced by incorporating co-stimulatory domains like CD27, CD28, 4-1BB and ICOS [29–32]. Following the activation of the CAR-T cells, a variety of inflammatory cytokines, including IFN-γ, TNF-α, IL-1β, IL-2, IL-6, are released. IFN-γ activates the macrophages, which release cytokines including TNF-α, IL-1β, IL-6, IL-8 and IL-10 (Table 2). These cytokines augment the immune response by further enhancing the activation, proliferation and cytokine secretion of T cells except IL-10 which serves as suppressor of cellular immunity but may play limited role in this battle. The formation of a positive feedback loop between cytokines and immune cells dramatically elevate the levels of various cytokines then induce cytokine storm. This cytokine storm causes systemic inflammatory response similar to sepsis, which induces fever, headache, dizziness, nausea, rigors, chills, rash, hypotension, tachycardia and dyspnea. Acute vascular leak leads to fluid retention, generalized edema and pulmonary edema like ARDS, which are worsened by hydration commonly given to treat hypotension (Table 2). The syndrome can be associated with arrhythmia and cardiac arrest. Hepatic and renal failure can ensue.

4. Differentiation of CRS

In CAR-T cell therapy, some other complications, including tumor lysis syndrome (TLS) and severe sepsis, may mimic CAR-T cell induced CRS. Both of them may cause elevation of cytokines and organ failure, but the management are different. Thus, it is necessary to differentiate the above conditions and to give proper treatment. TLS is a disease-related emergency which has been reported in CAR-T cell therapy. In TLS, lysed tumor cells release DNA, phosphate, potassium, and cytokines. When the accumulation of phosphate, potassium, xanthine, or uric acid is more rapid than excretion, the TLS develops [33]. Although cytokines may contribute to the development of inflammation, hypotension and acute kidney injury in TLS as well, some metabolic abnormalities which are frequently found in TLS are not very common at early stage of CAR-T cell induced CRS, including hyperuricemia, hyperkalemia, hyperphosphatemia and hypocalcemia [34]. It is helpful to diagnose TLS by measuring serum potassium, phosphorus, calcium, creatinine, uric acid and urine output. Allopurinol has been prescribed for the prevention and treatment of the TLS in some CAR-T cell based clinical trials [8,11]. Severe sepsis is another common complication in cancer patients which may present CRS. The approaches like microbiologic culture, specific nucleic acid and antibody assay are well-established tools for the diagnosis of microbial infection. Furthermore, we find that the IFN-γ is rarely significantly elevated although IL-6 and IL-10 are very high in most patients with severe sepsis, which is quite different from CAR-T cell induced CRS and might be helpful for the differentiation [15,17].

5. Potential factors related to CRS

The incidence of CRS varies greatly among different clinical trials and each patient responds differently to CAR-T infusion even when the same protocol is used. The variety of cytokine profiles among different individuals and different clinical trials may be related to various CAR structures, underlying diseases and patients’ genetic polymorphisms.

5.1. CAR structure

The optimization of CAR design improves the anti-tumor efficacy of CAR-T cells. The cytokine (IFN-γ, TNF-α and IL-2) secretion of CAR-T cells after exposure to tumor cells is closely related to their killing activity. Generally, CAR-T cells with either CD28 or 4-1BB signal domain secret more cytokines than the first generation CARs after encountering the target cells [30,35]. Therefore, CRS is rarely reported in first generation CAR clinical trials but is more common in second generation CARs [29,36]. 4-1BB signaling shows reduced propensity to trigger TNF-α and IL-2 secretion

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Sources [66]</th>
<th>Principal targets and physiologic actions [66]</th>
<th>Related symptoms in CRS [67–72]</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ</td>
<td>NK cells, Th1 cells and CTLs</td>
<td>Macrophages: classical activation T cells: Th1 differentiation B cells: isotype switching to opsonizing Various cells: increases MHC expression and antigen processing to T cells</td>
<td>Fever, chills, dizziness and headache, fatigue</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Macrophages, NK cells and T cells</td>
<td>Endothelial cells: activation (inflammation) Neutrophils and macrophages: stimulates microbial activity Liver: synthesis of acute phase proteins</td>
<td>Flu-like syndrome, fever, general malaise, rigor, and watery diarrhea, vascular leak, inhibits myocardial contractility and vascular muscle tone, lung injury, synthesis of acute phase proteins</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Macrophages, DCs, fibroblasts, endothelial cells, hepatocytes</td>
<td>Endothelial cells: activation (inflammation, coagulation) Liver: synthesis of acute phase proteins</td>
<td>Flu-like syndrome, vascular leak, inhibitory actions on myocyte contractility, lung injury</td>
</tr>
<tr>
<td>IL-2</td>
<td>T cells</td>
<td>T cells: proliferation and differentiation into effector and memory T cells NK cells: proliferation and differentiation B cells: proliferation and antibody synthesis</td>
<td>Flu-like syndrome, vascular leak, inhibitory actions on myocyte contractility</td>
</tr>
<tr>
<td>IL-6</td>
<td>T cells, monocytes, macrophages, fibroblasts, and endothelial cells</td>
<td>Augment immune response B cells: proliferation of antibody-producing cells Neutrophils: stimulates production from the bone marrow Liver: synthesis of acute phase proteins</td>
<td>Fever, chills, nausea, vomiting and fatigue, vascular leak, lung injury</td>
</tr>
<tr>
<td>IL-10</td>
<td>Th2 cells and macrophages</td>
<td>Macrophages and DCs: inhibition of the expression of IL-12, costimulators and class II MHC</td>
<td>Fever, headache, back pain, dizziness</td>
</tr>
<tr>
<td>IL-12</td>
<td>Macrophages and DCs</td>
<td>T cells: Th1 differentiation, NK cells and T cells: IFN-γ synthesis, increasing cytotoxicity</td>
<td>Fever, chills, nausea/vomiting, hypotension, anorexia, myalgia, fatigue, liver toxicity</td>
</tr>
</tbody>
</table>
compared with CAR-T cells with CD28 signaling [30,37]. Thus, elevated amounts of IL-2 and TNF-α are not detected in the sera of the patients treated with 4-1BB modified CAR-T cells [8].

5.2. Tumor type and burden

In patients undergoing mAb administration, the development of CRS appears to be related to comorbidity factors. For example, CRS is more likely to occur in patients with high lymphocyte counts after rituximab injection [38]. Similar phenomenon is found in CAR-T cell therapy. In Brentjens et al.’s study, they found that the degree of cytokine elevation correlated to the bulk of residual disease at the time of adoptive T cell infusion [7]. The highest cytokine elevations, including IL-2, IFN-γ, IL-6, and IFN-inducible protein 10 (IP10), were seen in the patients with the highest tumor burden, while cytokine elevations were far more modest or undetectable in other minimal residual disease (MRD)+ and MRD– patients. The cytokine levels, the number of detectable CD19 CAR-targeted T cells and the tumor burden seemed correlated with each other in their study.

It was interesting that the CRS was not severe compared with other groups although the CAR with both CD28 and 4-1BB domains was used in Till et al.’s study [4]. The low expression of anti-CD20 CAR on the surface of T cells in this study may contribute to the low CRS. However, we speculate that the solid tumor might induce smaller amounts of cytokines than leukemias. In Kochenderfer’s study, the 4 patients who developed severe CRS, 3 were with CLL and 1 was lymphoma [25]. In another clinical trial about neuroblastoma treated with anti-GD2 CAR-T cells, no CRS and severe cytotoxicity was reported [9]. In patients with leukemias, the infused CAR-T cells are intensively activated after encountering a large amount of cancer cells in the peripheral blood, then secrete a large amount of inflammatory cytokines in a very short period of time and cause severe CRS. Of the fatal case reported by Morgan, the large number of CAR-T cells infiltrated to the lung immediately following infusion and were triggered to intensively release inflammatory cytokine before the lung meanwhile following infusion and were triggered to intensively release cytokines by the recognition of a large amount of lung epithelial cells which expressed low levels of ERBB2. Thus, the CRS rose to peak only within 4 h, which was much earlier than other clinical trials [12]. This report supports the idea that the severity of CRS might be related to the amount of antigen encountered by the CAR-T cells in a certain time.

5.3. Cytokine gene polymorphism

Polymorphisms in cytokine genes are associated with immunological responses and cytokine production, which has been clearly illustrated in sepsis. For example, IL-6-174G/C, IL-10-1082 G/A and TNF-α-308 G/A are associated with increased IL-6, IL-10 and TNF-α production during sepsis, respectively, and the latter two are even related to severer disease and septic shock [39]. Morgan et al. reported fatal case with CAR-T cell therapy due to CRS in 2010 [12]. They analyzed the patient’s DNA from the pretreatment PBMC and found that her IL-6 and IL-10 genotypes were -174G/C and -1082 G/A, which may explain why this patient developed much more severe CRS than other patients.

6. Precautions of CRS

CRS induced by CAR-T cell infusion shares many common features with that caused by mAb administration. However, unlike mAb-induced side effects could be alleviated following the excretion of the drug, CRS and its related toxicity induced by CAR-T cells could be long-lasting, as proliferating T cells will increase in numbers in vivo and eventually cause CRS. For example, the 4-1BB incorporated anti-CD19 CAR modified T cells can be expanded more than 1000 folds in CLL patients [6]. From this perspective, the precaution of CRS in CAR-T cell therapy is much more important than that in mAb treatment. The following measures might be helpful to avoid severe CRS.

6.1. Inflammatory cytokine monitoring and related gene polymorphism assessment

Inflammatory cytokine monitoring has become a standard of care in most of the clinical trials on CAR-T cell adoptive therapy. The baseline cytokine level is important for the evaluation of host immune response to CAR-T cells. On the other hand, it could be an approach to screen patients who present very high baseline cytokine level and are at high risk of severe CRS after CAR-T infusion. Furthermore, the assessment of inflammatory cytokine levels at various time points after CAR-T infusion helps to diagnose CRS early and treat the patients timely. In Memorial Sloan-Kettering Cancer Center, the first patient who received conditioning chemotherapy (cyclophosphamide) followed by anti-CD19 CAR modified T cell infusion died 2 days later [11]. Although the authors speculated that the likely cause of death was infection but not the infused CAR-T cells, it is possible that hypercytokinemia before CAR-T cell infusion may initiate and aggravate the CRS in a very short period of time and cause fatal outcome. Thus, it would be very important to clinically identify which subsets of patients are more prone to developing the complication and more cautious scheme should be established for them. As the polymorphisms of cytokine genes are related to the CRS severity, the assessment of inflammatory cytokine gene polymorphisms before CAR-T cell treatment may be helpful to recognize those patients.

6.2. Dose-escalation strategy for CAR-T cell infusion

In patients receiving donor lymphocyte infusion (DLI) after hematopoietic stem cell transplant, the adverse effects of DLI correlate with the infused T-cell numbers [40]. As we have mentioned above, the CRS caused by CAR-T cells is difficult to foresee and to be controlled. Thus, a conservative dose-escalation strategy for CAR-T cell therapy infusion is adopted by many phase I clinical trials [4,5,24,41]. It is impossible to provide reliable guidelines for proper starting doses of CAR-T cells under the current situation. The starting dose should be adjusted depending on the type of CAR and the protocol used. For example, T cells with second- or third-generation CARs should start at a lower dose than those with a first-generation CAR. Similarly, transfer of CAR-T cells into patients receiving IL-2 administration should commence at a lower dose than transfer into patients without supplementary treatment. Alternatively, one could conduct the initial dose escalation with first generation CAR-T cells. Once the first generation CAR-T cells are shown to be safe, second-, or perhaps third-generation CAR-T cells could be explored [42].

6.3. Design of short-lived CAR-T cells

It is concerned that the expansion and persistence of CAR-T cell in human body may increase the risk of severe and/or long-term adverse effects. In this sense, the short-lived CAR-T cells may be safer. Therefore, it has attracted great attention to genetically modify the T cells with mRNA CAR electroporation. Transgene expression of the in vitro transcribed RNA could be detected on the surface of the RNA-engineered T cells up to 7 days after RNA electroporation [43]. Multiple injections of these CAR-T cells mediate regression of tumor in different animal models [43,44]. Although no mRNA CAR based clinical trial has been launched, we believe that this is an important direction for CAR exploration.
Alternatively, the introduction of suicide genes into CARs may be another strategy. For example, inducible caspase 9 (icasp9) is highly effective in producing rapid (less than 120 min) apoptosis [45]. The molecule can be triggered by administration of a small molecule dimerizer that brings together two nonfunctional icasp9 molecules to form the active enzyme [46]. Thus, some investigators have incorporated icasp9 into a CAR vector targeting CD19 and demonstrated that the activation of this suicide gene rapidly induced apoptosis of CAR-modified T-cells both in vitro and in vivo [47].

6.4. Generation of less-differentiated CAR-T cells

It becomes clear that adoptive transfer of the less-differentiated CAR-T cell subsets, memory T cell (Tcm) and central memory T (Tcm) cells, is associated with long persistence, strong expansion and superior antitumor immunity [34–50]. However, the Tcm and Tscm cells present less cytokine secretion and tumor-lysis ability after encountering the specific tumor antigens in vitro study [48,51]. Thus, theoretically, the infusion of less-differentiated CAR-T cells may induce less quantity of cytokines in the early phase of CAR-T cell treatment. Such CAR-T cells could be generated from induced pluripotent stem cells or expanded in the presence of IL-7/IL-15 or IL-21 [51,52]. However, whether the subsequent expansion of the CAR-T cells will produce delayed but more indolent toxicities is unknown.

7. Treatment of CRS

CRS is an emergent and life-threatening entity. According to the data of rituximab, there were at least 9 fatalities resulting from CRS had occurred by the year of 1999, 2 years after rituximab was approved by U.S. FDA [53]. The clinical data of CAR-T cell therapy are limited as there are only few patients being treated up to now. However, 2 fatalities have been reported shortly after adoptive transfer of CAR-T cells, which were both related to CRS [11,12]. The two patients both presented respiratory distress, hypotension within several hours after CAR-T cell infusion and died several days later although intensive support had been given. The timely and active cytokine-directed therapy is critical for saving lives. Oxygen, fluid resuscitation, vasopressor and intubation supports might be required for patients with severe symptoms. Based on the available clinical trials and other related data, the following reagents could be helpful to resolve CRS.

7.1. Corticosteroids

Corticosteroids are important agents to suppress intensive inflammatory response and CRS. It has been widely used in various kinds of CRS related diseases, including severe sepsis, GVHD, HLH/MAS, monoclonal antibody administration etc. [18,54–56]. In the treatment of CAR-T cell induced CRS, it has become the first line remedy as well. The administration of methylprednisolone decreases the cytokines and relieves the related clinical signs in most patients with mild and moderate CRS [7]. Unlike mAb based cancer treatment, corticosteroids are rarely used as a premedication prior to CAR-T cell infusion due to the concern of affecting CAR-T cell efficacy.

7.2. Antagonists of cytokines

Since cytokines like TNF-α, IFN-γ and IL-6 play important roles in the CRS and their related toxicity, various cytokine antagonists have been used as potential therapeutic agents to counteract cytokine storm. In a phase II clinical study, the patients receiving the TNF-α inhibitor etanercept presented mild infusion reactions to rituximab and were not associated with severe adverse events [57]. In Teachey et al.’s study, they successfully treated an ALL case with CRS after the blinatumomab (a CD19/CD3-bispecific T-cell receptor-engaging antibody) treatment by IL-6 receptor-directed therapy with tocilizumab [58]. In another ALL case reported by Grupp et al.’s group, the patient developed severe and glucocorticoid-resistant CRS after receiving anti-CD19 CAR-T cell infusion. A single course of etanercept and tocilizumab combined anti-cytokine therapy result in rapid clinical effects: defervescence occurred within hours, and the patient was weaned from vasoactive medications and ventilator support, and the acute respiratory distress syndrome resolved [8]. The antagonists of IL-1, IL-2 and IFN-γ have not been reported to treat CAR-T cell related CRS yet. However, they have been successfully used to treat the patients with CRS caused by other etiologies and might be effective in treating CAR-T cell related CRS. The anti-IL-2 receptor antagonist antibody has been administrated with high-dose methylprednisolone to treat the CRS induced by TGN1412 [23]. The recombinant IL-1 receptor antagonist anakinra induces rapid and sustained remission of systemic juvenile idiopathic arthritis-associated MAS when combined with corticosteroids and could even successfully control intravenous steroid, immunoglobulin and cyclosporine (CsA)-resistant MAS in some cases [59,60]. Therapeutic administration of an anti-IFN-γ antibody induced recovery from HLH in mice model, as evidenced by increased survival, decreased cytokinemia and corrections of a series of clinical and histopathological manifestations [61].

7.3. Other potential chemicals

As the same patients with CRS present similar cytokine profiles and clinical manifestations with HLH/MAS [6,8], the treatment strategy of HLH might be effective in these patients. According to the guideline, for patients with HLH, corticosteroid is recommended as a first-line agent while CsA is the second line treatment for those responding poorly to corticosteroid [56,62]. CsA preferentially targets lymphocytes by inhibiting the nuclear factor of activated T-cells (NFAT) family of transcription factors that are critical for the activation of a wide array of cytokine genes and then dampens the cytokine storm effectively [63]. Many patients with HLH and MAS respond well to CsA therapy even when they are resistant to corticosteroid treatment [64]. Although there is no experience of this agent in managing CAR-T cell induced CRS, it might be a choice when CRS cannot be controlled by corticosteroid and biological reagents only.

8. Challenges and perspectives

Adaptive CAR-T cell therapy is an attractive strategy for cancer. While the antitumor effects have been greatly enhanced following the improvement of the CAR design and growth conditions [65], how best to develop a safe approach to implement this new therapeutic becomes a big concern. CRS is a double-edged sword which is closely related to the efficacy of such therapy but does harm to the host if the inflammatory response is overwhelming [10,25]. Therefore, how to balance the two aspects is an important issue in CAR-T cell therapy. The development of CRS correlates with CAR structures, underlying diseases and individual genetic background. In an attempt to improve the effectiveness of CAR-T cells by optimizing the vector structure, the scientists should also focus on the safety simultaneously, e.g., producing short-term CAR-T cells by incorporating the suicide gene or using mRNA approaches. For clinicians, the infusion of CAR-T cells should strictly follow the escalation scheme, initiating with low-dose or...
low-generation CAR-T cells. In all, CRS related mortality should be reduced by designing safer CARs, following strict dose-escalation scheme, intensively monitoring inflammatory cytokines and taking timely and effective measures including the administration of various antagonists of cytokines under the current situation. Optimal application of this new therapeutic approach on cancer patients is yet to be established.

Conflict of Interest

All the authors declare no conflict of interests.

Acknowledgement

This study was supported in part by Grants from the National Natural Science Foundation of China (Nos. 30971283, 31100638 and 81170502), the Zhejiang Provincial Natural Science Foundation of China (Nos. Y2110020 and LZ12H08001), and the PhD Programs Foundation of Ministry of Education of China (No. 2011010112038).

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