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Research progress on the causes and solutions of CRS during CAR-T therapy

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Abstract. CAR-T therapy is an emerging therapy used to treat cancer. Currently, CAR-T cells targeting various antigens of cancer cells have been developed. However, adverse events are still a shortcoming of this therapy. The main results of this study highlight the central role of macrophage activation and interleukin-6 (IL-6) release in the formation of CRS. Tocilizumab has been identified as an effective treatment for severe CRS, with antipyretics and pressors providing supplementary support. Future research should focus on the IL-6 mechanism to discover additional strategies for combating CRS. The symptoms of CRS range from fever to life-threatening. Lenzilumab can be used to block GM-CRS which activates macrophages, and tocilizumab is a proven mAb for the treatment of severe CRS. However, there are many treatments that have not been perfected or even developed. In the future, the mechanism of IL-6 should be studied clearly, the application range of tocilizumab should be expanded, and more medications should be developed to inhibit CRS.

Keywords: CAR-T therapy, cytokine release syndrome, macrophage activation, interleukin-6.

1. Introduction

Chimeric antigen receptor (CAR) T cell therapy is a burgeoning and appealing immunotherapy, treating cancers by modifying T cells genetically to become CAR-T cells which are able to bind with cancer surface protein and cause the apoptosis of cancer cells. CAR-T therapy can accurately and effectively target tumors, and has the possibility to cure cancer, which contributes a further development in the field of cancer treatment, and also makes it a promising and valuable therapy for research.

Major histocompatibility complex is a collection of molecules that present peptides on self and foreign cells to T cells [1], but the normal T killer cells cannot degrade cancer cells due to the absence of MHC combined with cancer surface protein. To make up for this deficiency, CAR-T cells are designed. The T cells are obtained by collecting leukocytes from patients to prevent transplant rejection. After modification, the newly formed CAR-T cells will be injected back into the patients. The CAR-T cells work when its antigen-recognition regions detect and combine with the surface antigens of cancer cells, which stimulates intracellular molecules such as CD3ζ and CD28 to produce a signal [2]. The signal is going to trigger co-stimulatory signal to activate the T cell receptor signaling pathway, so that T cells release cytotoxic proteins such as perforin to fuse with cancer cells, resulting in the death of cancer cells [3].

The idea of processing T cells was first brought up by a team at the Weizmann Institution of Science in 1989 [4], and after deep dives into the relevant fields by many scientists, it gradually became a

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practical therapy in the 21st century. CAR-T therapy has undergone five generations of improvement, spanning a period of more than 30 years [2]. The first generation began with a single intracellular signaling domain that connects to a single-chain variable fragment (scFv) at the extracellular domain, in which scFv is obtained from antibodies that is specific to antigens of certain cancer cells [2]. The structure mentioned is shown in Figure 1. From the second to the fifth generation, co-stimulatory signaling domains have been equipped to enhance the accuracy while targeting and the strength during killing process [2].

Currently, Food and Drug Administration (FDA) has given permission to 6 CAR-T therapies [5]. They are approved for the treatment of B cell and plasma cell malignancies [6]. CAR-T cell receptors that can recognize B cell antigens called CD19, CD20, CD22, or CD123 are used to combat B cell acute or chronic lymphoblastic leukemia, CD19, CD20, or CD30 are used to deal with lymphoma, and CD269 or CD138 are aimed at myeloma in clinical applications, which response roughly at a rate higher than 40% [6, 2].

However, CAR-T cell therapy has toxicity as a result of off-target effect, which means the CAR-T cell receptor may bind to antigens other than the antigens of cancer cells accidently, triggering adverse events [6]. CAR-T cells may activate other immune cells such as macrophage, resulting in release of cytokines interleukin-1 (IL-1), IL-6 and tumor necrosis factor (TNF), which generate an adverse event of inflammation named cytokine release syndrome (CRS) [6]. The severity of CRS depends on the dose of CAR-T cells in which the symptom ranges from fever to death [7]. In a lymphoma xenograft model, the high level of cytokines is a result of interaction between cancer cells and CAR-T cells [8]. In addition, the toxicity of CAR-T therapy also damages the blood-brain barrier, causing immune effector cell-associated neurotoxicity syndrome (ICANS) which is one of the adverse events.

2. The Related Causes of CRS

2.1. Macrophage activation

The release of the cytokines caused by CRS is due to the recognition and the interaction of the receptors on CAR-T cells and the surface protein of macrophages, followed by macrophage activation. The substantial increase of activated macrophage boosts the possibility of the receptors binding with the surface protein, which makes it easier for the formation of CRS. Thereby, certain pathways that are able to stimulate macrophages to release the cytokines are discussed in this paper. The interaction of CD40 and CD40 ligand (CD40L) is one of the pathways. CD40 on macrophages fit together with CD40L expressed by CAR-T cells to activate intercellular enzymes, producing messenger for the amplification of the signal, followed by the final response which is the proliferation of macrophages [8,9]. Moreover, when certain cells are damaged or enter the stage of apoptosis and necrosis, the endogenous molecules known as damage-associated molecular patterns (DAMPs) are produced [10], acting as a danger signal to engage with pattern-recognition receptors (PRRs) on macrophages so as to active macrophages [9]. What's more, CAR-T cells themselves also have the ability to produce cytokines to promote the activation of macrophages [11]. Interferon-γ is a signaling molecule, that can be secreted by CAR-T cells, that accelerates the maturation of macrophages by the induction of MHC class II proteins [12]. CAT-T cells also can produce cytokines called granulocyte–macrophage colony-stimulating factor (GM-CSF). It not only plays a role in the differentiation of macrophages from monocytes but also grows the amount of IL-6 which is one of the major causes of CRS [9,13,14].

2.2. Cytokines: IL-6

As CAR-T cell receptors bind to the surface protein of macrophages or monocytes, IL-6 and IL-1 are released to give rise to CRS after the accumulation of these two cytokines in a body [7]. IL-6 is a pro-inflammatory molecule that takes part in immune responses and inflammation [9, 15]. The process of IL-6 binding to its receptor can be classified into three patterns, displaying in figure 1.

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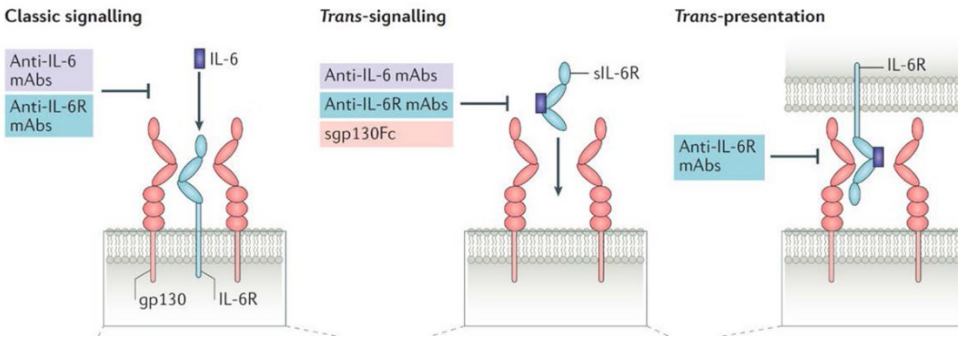

Figure 1. The three pattern of IL-6 binding to its receptor [10].

For classic signaling, IL-6 receptor on the surface membrane combine with IL-6 to form an IL-6/IL-6R complex, which stimulates a membrane-bound protein called gp130 to produce a signal and further transduction happens inside the cell [3]. In the case of trans-signaling where the cell does not have an IL-6 receptor on its membrane, IL-6 firstly binds with soluble IL-6R (SIL-6R), then the target cell with gp130 binds with IL-6/SIL-6R complex [3]. Rarely, trans-presentation occurs, which means a cell with an IL-6/IL-6R complex can bind with the gp130 on another cell with the aid of dendritic cells and receiver T cells [3]. IL-6R is mostly present on the surface membrane of immune cells and hepatocytes [9]. After IL-6 is recognized under the condition of CRS, the JAK-STAT signaling pathway is activated, in which the intercellular Janus kinase and tyrosine kinase receive the signals, resulting in the STATs being phosphorylated. It is followed by the combination with DNA inside the nucleus, thus, the instructions of the signals can be embodied by copying the target gene during the process of transcription. The process is referred in Figure 2. The function of this pathway involves the facilitation of T cells differentiation and proliferation [16]. In addition, the formation of the disease related to respiratory system and digestive system are also linked to the presence of IL-6 [15]. Nevertheless, the specific effect and mechanisms of IL-6 in the CRS remains a mystery [9].

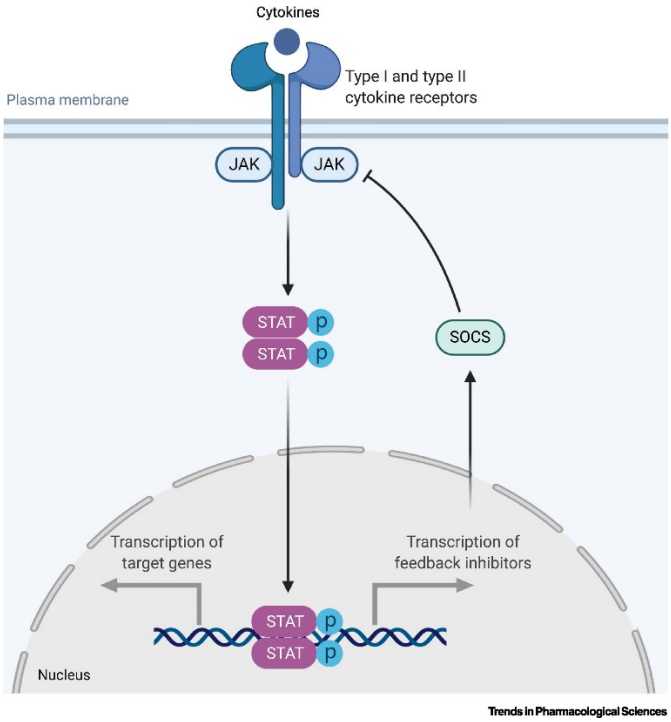

Figure 2. The JAK-STAT pathway [16].

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2.3. Other causes of CRS

According to the data from the BELINDA trial [17], ZUMA-7 trial [18] and TRANSFORM trial [19], severe CRS has been discovered in patients who are being treated for diffuse large B-cell lymphoma with tisagenlecleucel, albeit a low percentage.

3. Symptoms and problems

Grades of CRS.CRS can be categorized into 4 grades according to its severity. The lowest grade which is also the least symptomatic grade is grade 1, accompanied by a feeling of fatigue [7]. Followed by grade 2, patients get symptoms that are similar to the flu or a fever [7]. Crucially, grade 3 should be taken seriously due to its classification of severe CRS. High fever, hypoxia and hypotension are all the signs of grade 3 CRS [7]. In grade 4-the most serious level-situations that threaten lives would occur, including the precursor of heart failure and other organ failure [7].

4. Treatment and Improvement

4.1. Treatment for different grades of CRS

If a fever is developed while receiving CAR-T therapy, it is a high possibility of low grade CRS and requires immediate medical attention, which means patients should take antipyretic medicine under medical supervision [11]. Antihistamines should be used when an allergic reaction happens [11]. It is also accessible to use antibiotics that are prophylactic and empiric as treating low grade CRS. For hypotensives in grade 3, there are vasopressors for remission [7]. In cases where severe CRS is life-threatening, tocilizumab, blinatumomab and corticosteroids should be taken into account as a treatment, meanwhile the patients need to be admitted to the intensive care unit for constant monitoring [7,11].

4.2. Monitoring of cytokine

For the blockage of GM-CSF, lenzilumab, which is a monoclonal antibody (mAb), is designed to combine with GM-CSF in order to stop the recognition of its receptors [20]. In a board-approved trial which investigated the effect of diminishing GM-CSF by using lenzilumab in patients with chronic myelomonocytic leukemia, GM-CSF was successfully suppressed and the effect was sustained [21]. This result confirms that lenzilumab can be given when GM-CSF levels are high.

Tocilizumab is a mAb that is approved by FDA in treating the up regulation of IL-6 [11]. Tocilizumab targets the IL-6R to prevent the binding of IL-6. Despite the slight increase in the level of IL-6, symptoms such as hypotension will subside in a short time [22]. It can be given a second dose after 72 hours, provided the first dose did not work [22]. Therefore, it is often used as the medication of grade 3 CRS. Humanitas Cancer Center (Rozzano, Italy) authorizes a study to conduct an attempt to administer tocilizumab in grade 1 CRS. Among 23 patients, 83% had no further worsening of CRS and none of the patients in the study reached grade 3 [23]. Although the data obtained from the experiment are optimistic, it is necessary to be cautious about using preemptive tocilizumab in mild CRS owing to the possibilities of triggering neurotoxicity and ICANS [23].

5. Conclusion

In general, this article summarizes the causes of CRS, measures facing different levels of CRS and the available drug tocilizumab. Macrophage activation and IL-6 release are the key causes of CRS formation. At present, tocilizumab is already a drug for severe CRS, and antipyretics and pressors also play an auxiliary role. For the time to come, research should focus on the mechanism of IL-6 to find more ways to combat CRS. It is also essential to emphasis on overcoming other adverse reactions caused by the preemptive use of tocilizumab. In addition, more preventive drugs should be developed in order to reduce the likelihood of CRS.

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