

EDITORIAL

CAR-T CELL THERAPY CAUSES INFLAMMATION BY IL-1 WHICH ACTIVATES INFLAMMATORY CYTOKINE MAST CELLS: ANTI-INFLAMMATORY ROLE OF IL-37

Al. CARAFFA¹, C.E. GALLENGA², S.K. KRITAS³, G. RONCONI⁴,
P. DI EMIDIO⁵ and P. CONTI⁶

¹*School of Pharmacy, University of Camerino, Camerino, Italy;* ²*Department of Biomedical Sciences and Specialist Surgery, Section of Ophthalmology, University of Ferrara, Ferrara, Italy;* ³*Department of Microbiology and Infectious Diseases, School of Veterinary Medicine, Aristotle University of Thessaloniki, Macedonia, Greece;* ⁴*Clinica dei Pazienti del Territorio, Fondazione Policlinico Gemelli, Rome, Italy;* ⁵*Maxillofacial Surgery, "Mazzini" Hospital, Teramo, Italy;* ⁶*Postgraduate Medical School, University of Chieti, Chieti, Italy*

Received August 14, 2019 – Accepted October 15, 2019

Chimeric antigen receptor (CAR) T cells are genetically modified T cells that act against cancer. When CAR-T cells are administered they can trigger inflammatory cytokines and increase toxicity. Interleukin (IL)-1 is the classic cytokine that mediates inflammatory reactions including those that occur in CAR-T-cell therapy. IL-1 also induces IL-33 in mast cells (MCs), amplifying the allergic reaction. IL-37 (ILF7) is an IL-1 family member which binds IL-18 receptor alpha (IL-18R α) chain and suppresses innate and acquired immunity. IL-37 is an anti-inflammatory cytokine which inhibits pro-inflammatory cytokines including IL-1 and IL-33. Here, we hypothesize that inflammation and toxicity generated in tumor CAR-T therapy could be inhibited by IL-37, contributing to an improvement in the treatment of tumors with CAR-T therapy.

New cancer therapies open new hopes, but their toxicity is still a serious problem to be solved (1). Important adverse occurrences are allergy and inflammation, which can also cause fatal events (2). Chimeric antigen receptor T (CAR-T) has emerged as a new anti-tumor therapy, with the ability to stimulate the immune system (3). CAR-T is a genetically modified engineered receptor that produces a chimeric receptor of artificial T cells used in immunotherapy (4). This T receptor is chimeric because it has the function of binding the antigen and activating T cells, capable of affecting specific proteins.

CAR-T therapy can cause remission in patients

with tumors resistant to conventional therapies; but this therapy has turned out to be very toxic by several mechanisms, and it can also affect antigens of normal tissue associated with the tumor and may cause patient death (5). The most important toxicity of CAR-T cells is pro-inflammatory cytokine release (6). These reactions occur after treatment with CAR-T cells, provoking tumor lysis and cytokine release, followed by anaphylaxis syndrome, fever, neurological toxicity, tachycardia, and hypotension (7). In fact, in the serum of patients treated with CAR-T cells a wide variety of inflammatory cytokines are found. The inflammatory reaction produced by the

Key words: CAR-T therapy; cytokines; mast cells; chimeric antigen receptor

Corresponding Author:

Prof. Pio Conti,
Postgraduate Medical School,
University of Chieti,
Viale Unità d'Italia 73, Chieti, Italy
Tel./Fax: +39 0871 574136
e-mail: pconti@unich.it

0393-974X (2019)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties
DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

cytokines released by the CAR-T cells can activate other immune cells, such as macrophages and mast cells (MCs), which, in turn, produce other pro-inflammatory cytokines, aggravating the situation. Treatment with CAR-T can provoke a systemic inflammatory response mediated by inflammatory cytokines, such as interleukin (IL)-1 and IL-33, in frequent events (8). Therefore, it is important to apply an aggressive anti-inflammatory therapy in patients treated with CAR-T immunotherapy. In this therapy, pro-inflammatory cytokines are produced that activate the immune cells, including macrophages and MCs to produce and release other pro-inflammatory cytokines, aggravating the patient status (8). Macrophages primarily release IL-1 which in turn activates the MCs to generate IL-33, creating an allergic inflammatory phenomenon (9). In addition, IL-18, a cytokine of the IL-1 family polarizes CAR-T cells, provoking an acute inflammatory response, an effect that can be inhibited by IL-37, since it has been demonstrated that this cytokine plays its biological inhibitory role through IL-18 receptor alpha binding (IL-18R α) (10).

Mast Cells

MCs are bone marrow immune cells derived from hematopoietic stem cells and reside in all the vascularized tissues and serous cavities, where they mature (11). MCs participate in both innate and adaptive immune responses and are a potential source of cytokines/chemokines, which mediate allergic disease (12-13). They can be activated by various molecules through the Toll-like receptors, and IL-1

receptors, without degranulation and without the intervention of IgE (14-15).

In MC pathway activation, intracellular calcium (Ca⁺⁺) is regulated by PLC, and PKC results in the formation of IP3 and diacylglycerol (DAG). MAPK, ERK, JNK and p38 participate in the transcription of cytokines and subsequently the generation of proteins (11). The production of IL-1 family, is strongly involved in the inflammatory process (16).

IL-1

IL-1 is one of the most important regulators of innate immune responses and participates in numerous inflammatory processes (17). IL-1 is the classic pro-inflammatory cytokine that includes two distinct ligands: IL-1 alpha (IL-1 α) and IL-1 beta (IL-1 β), which bind the IL-R1 receptor by mediating inflammatory phenomena. IL-1 β , which is the most studied, is inducible and is released by the splitting of caspase-1 (17).

The inflammation that is created in CAR-T therapy with the activation of immune cells generates pro-inflammatory cytokines, mainly IL-1 (8). At this point IL-1 causes an inflammatory network by activating other immune cells including the MCs to produce IL-33, which actively participates in the allergic reaction.

IL-33

IL-33 (formerly IL-1F11) is a pro-inflammatory cytokine of the IL-1 family and is produced by several immune cells including macrophages and

Table I. *Organs that may be more frequently affected by toxicity after infusion of pro-inflammatory cytokine-releasing CAR-T cells.*

ORGANS	SOME EFFECTS
Brain.....	ataxia, headache, tremor, seizures
Blood.....	neutropenia, thrombocytopenia, lymphopenia
Liver.....	hyperbilirubinemia, transaminitis
Kidney.....	acute kidney injury
Lungs.....	hypoxia, tachypnea
Heart.....	hypotension, tachycardia

MCs (18). In addition, IL-33 generated by MCs contributes to allergic reactions (19). IL-33 acts through the ST-2 receptor of which there are two types, a transmembrane receptor (ST2L) and a soluble receptor (sST2) (18). IL-33 is released after cellular injury and/or necrosis by alerting. It contributes to inflammatory and allergic states and is expressed in various organs and cells, including lymphoid cells (20).

CAR-T therapy is a new potentially curative method for cancer. However, adverse reactions such as allergic phenomena, where MCs also participate, still remain obstacles to overcome. The generation of IL-33 from MCs and macrophages contributes to the inflammatory and allergic state (20). Therefore, inhibition of this cytokine with IL-37 may be a new strategy for an improvement of CAR-T therapy (21).

Interleukin-37

IL-37 has emerged as an important inhibitor of immune and inflammatory responses, and it has achieved remarkable efficacy in the treatment of inflammatory diseases (22). IL-37 binds to the IL-18R receptor and at low concentrations is capable of inhibiting innate immune reactions, reducing activation of the MyD88 receptor (23). IL-37 is mainly produced by macrophage cells after activation of the toll-like receptors (TLR) which leads to the formation of mature IL-37 through the action of caspase 1. IL-37 could abolish systemic inflammation and neurotoxicity caused by pro-inflammatory cytokines, including IL-1 and IL-33 with prolonged survival. This therapy could offer a new strategy in the treatment with CAR-T cells.

The gene expression of IL-37 shows an up-regulation of messenger RNA in tumors including melanoma. This enhancement occurs mainly in Treg cells and in granulocytes, which probably also include MCs. The up-regulation of IL-37 messenger RNA in tumors indicates a defensive state that the organism uses in neoplasia (24).

The inhibition of IL-1 and IL-33 with IL-37, a new anti-inflammatory cytokine, could be a new therapy, even if the indications of administration are still to be determined. However, treatment with IL-37 which is immune suppressant should be carried out with great

care, because it could negatively affect the immune system and may cross-react with the good receptors which are not expressed on tumor cells.

REFERENCES

1. Guedan S, Ruella M, June CH. Emerging cellular therapies for cancer. *Annu Rev Immunol* 2019; 37:145-71.
2. Kosti P, Maher J, Arnold JN. Perspectives on chimeric antigen receptor T-cell Immunotherapy for solid tumors. *Front Immunol* 2018; 9:1104.
3. Raulet DH, Vance RE, McMahon CW. Regulation of the natural killer cell receptor repertoire. *Annu Rev Immunol* 2001;19:291-330. Review.
4. Yang QY, Yang JD, Wang YS. Current strategies to improve the safety of chimeric antigen receptor (CAR) modified T cells. *Immunol Lett* 2017; 190:201-5.
5. Newick K, O'Brien S, Moon E, Albelda SM. CAR-T Cell therapy for solid tumors. *Annu Rev Med* 2017; 68:139-52.
6. Brudno JN, Kochenderfer JN. Toxicities of chimeric antigen receptor T cells: recognition and management. *Blood* 2016; 127(26):3321-30.
7. Bonifant CL, Jackson HJ, Brentjens RJ, Curran KJ. Toxicity and management in CAR-T-cell therapy. *Mol Ther Oncolytics* 2016; 3:16011.
8. Giavridis T, van der Stegen SJC, Eyquem J, Hamieh M, Piersigilli A, Sadelain M. CAR-T cell-induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade. *Nat Med* 2018; 24(6):731-38.
9. Conti P, Lauritano D, Caraffa A, et al. New insight into systemic mastocytosis mediated by cytokines IL-1 β and IL-33: Potential inhibitory effect of IL-37. *Eur J Pharmacol* 2019; 858:172473.
10. Chmielewski M, Abken H. CAR-T cells releasing IL-18 convert to T-Bet_{high} FoxO1_{low} effectors that exhibit augmented activity against advanced solid tumors. *Cell Rep* 2017; 21(11):3205-19.
11. Galli SJ, Kalesnikoff J, Grimbaldston MA, Piliponsky AM, Williams CM, Tsai M. Mast cells as "tunable" effector and immunoregulatory cells: recent advances. *Annu Rev Immunol* 2005; 23:749-86. Review.

12. Caraffa AL, Gallenga CE, Kritas SK, Ronconi G, Conti P. Impact of mast cells in systemic lupus erythematosus: can inflammation be inhibited? *J Biol Regul Homeost Agents* 2019; 33(3):669-73.
13. Taracanova A, Tsilioni I, Conti P, Norwitz ER, Leeman SE, Theoharides TC. Substance P and IL-33 administered together stimulate a marked secretion of IL-1 β from human mast cells, inhibited by methoxyluteolin. *Proc Natl Acad Sci U S A* 2018; 115(40):9381-90.
14. Gallenga CE, Pandolfi F, Caraffa AL, et al. Interleukin-1 family cytokines and mast cells: activation and inhibition. *J Biol Regul Homeost Agents* 2019; 33(1):1-6.
15. Varvara G, Tettamanti L, Gallenga CE, et al. Stimulated mast cells release inflammatory cytokines: potential suppression and therapeutical aspects. *J Biol Regul Homeost Agents* 2018; 32(6):1355-60.
16. Theoharides TC, Tsilioni I, Bawazeer M. Mast cells, neuroinflammation and pain in fibromyalgia syndrome. *Front Cell Neurosci* 2019; 13:353.
17. Cavalli G, Dinarello CA. Anakinra therapy for non-cancer inflammatory diseases. *Front Pharmacol* 2018; 9:1157. doi: 10.3389/fphar.2018.01157.
18. Dinarello CA. An IL-1 family member requires caspase-1 processing and signals through the ST2 receptor. *Immunity* 2005; 23(5):461-62.
19. Tettamanti L, Kritas SK, Gallenga CE, et al. IL-33 mediates allergy through mast cell activation: Potential inhibitory effect of certain cytokines. *J Biol Regul Homeost Agents* 2018; 32(5):1061-65.
20. Conti P, Caraffa A, Ronconi G, et al. Mast cells participate in allograft rejection: can IL-37 play an inhibitory role? *Inflamm Res* 2018; 67(9):747-55.
21. Gugliandolo A, Caraffa AL, Gallenga CE, et al. Mesenchymal stem cells and IL-37: a powerful combination. *J Biol Regul Homeost Agents* 2019; 33(4):1019-22.
22. Cavalli G, Dinarello CA. Suppression of inflammation and acquired immunity by IL-37. *Immunol Rev* 2018; 281(1):179-90.
23. Zhan Q, Zeng Q, Song R, Zhai Y, Xu D, Fullerton DA, Dinarello CA, Meng X. IL-37 suppresses MyD88-mediated inflammatory responses in human aortic valve interstitial cells. *Mol Med* 2017; 23:83-91.
24. Osborne DG, Domenico J, Luo Y, et al. Interleukin-37 is highly expressed in regulatory T cells of melanoma patients and enhanced by melanoma cell secretome. *Mol Carcinog* 2019; 58(9):1670-79.