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Article *in* Current Cancer Therapy Reviews · August 2020

DOI: 10.2174/1573394716666200812150402

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Current Cancer Therapy Reviews, 2020, *16*, 1-0

REVIEW ARTICLE

The Role of Bacterial Superantigens in the Immune Response: From Biology to Cancer Treatment

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Abstract: ***Aims*:** Encouraging results have been indicated preclinically and in patients using the bacterial superantigen. This review article intends to summarize the role of the superantigens that have been recently used in the treatment of cancer. In addition, the vector systems, including lentiviral vectors, adeno-associated vector systems and retroviral vectors that are increasingly being used in basic and applied research, were discussed. Most importantly, the new CRISPR technique has also been discussed in this literature review.

***Discussion*:** More successful therapies can be achieved by manipulating bacterial vector systems through incorporating genes related to the superantigens and cytokines. The products of SAg and cytokine genes contribute to the strong stimulation of the immune system against tumor cells. They bind to MHC II molecules as well as the V beta regions of TCR and lead to the production of IL2 and other cytokines, the activation of antigen-presenting cells and T lymphocytes. Additionally, superantigens can be used to eradicate tumor cells. Better results in cancer treatment can be achieved by transferring superantigen genes and subsequent strong immune stimulation along with other cancer immunotherapy agents.

***Conclusion*:** Superantigens induce the proliferation of T lymphocytes and antigen-presenting cells by binding to MHCII molecules and V beta regions in T cell receptors. Therefore, the presentation of tumor cell antigens is increased. Additionally, the production of important cytokines by T cells and APCs contributes to the stimulation of immune response against tumor cells. The manipulation of bacterial vector systems through incorporating genes related to SAgs and other immune response factors is a good strategy for the immune system stimulating and eradicating tumor cells along with other immunotherapy agents.

ARTICLE HISTORY

Received: March 12, 2020
Revised: June 27, 2020
Accepted: July 13, 2020

DOI:
10.2174/1573394716666200812150402

Keywords: Superantigen, immunotherapy, cancer, T lymphocyte, immune response.

1. INTRODUCTION

Previous studies have confirmed the effects of bacterial antigens in the modulation of the immune system. However, the naming of staphylococcal enterotoxins as superantigens has only been carried out by kappler and marrack in the year 1990 [1]. The term "superantigen" is used for antigens that activate a large percentage of T lymphocytes. Superantigens include bacterial and viral superantigens that can activate a large number of different lymphocyte clones [1, 2]. Minor lymphocyte stimulating gene products, very low concentrations of bacterial exoproteins, phyto-hemagglutinin and concanavalin A, which stimulate a large percentage of T lymphocytes, are known as superantigens [3]. One of the most important superantigens is Staphylococcus enterotoxin B [3, 4]. In addition, a number of recent studies have shown that several bacterial mitogens can be classified as superantigens based on their properties [5, 6].

The mechanism of conventional antigens to activate T lymphocytes is different from the activation of T lymphocytes by superantigens [2, 7]. T lymphocytes only recognize continuous epitopes and are not able to recognize conformational epitopes [2, 7]. Accordingly, conventional antigens must first be identified and phagocytized by the antigen-presenting cells (APCs) to activate T lymphocytes. Within the APCs, the antigen breaks down into small peptides. Consequently, peptides are loaded into Major Histocompatibility Complex (MHC) molecules [2, 7]. It is worth noting that the MHC molecule is divided into two classes 1 and 2, having a role in the delivery of specific antigens to T lymphocytes. Thus, conventional antigens contribute to the activation of T lymphocytes specifically, but the superantigen causes a large number of T lymphocytes to be nonspecifically activated [2, 7]. Bacterial products are capable of modulating the immune response [8]. The bacterial products that alter the

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1573-3947/20 $65.00+.00

immune system play an important role in the pathogenicity of microorganisms. Numerous bacterial exo-proteins have been identified that induce non-specific activation of T lymphocytes [8]. Based on previous studies, some of these bacterial exo-proteins at very low concentrations activate T lymphocytes and contribute to the proliferation of them [5, 8]. Given that these bacterial exo-proteins stimulate a large percentage of T lymphocytes, they are more important than conventional antigens and are considered as potential lymphocytic mitogens. These bacterial exo proteins also require antigen processing similar to antigens (2). One of the most important antigen receptors, that are located on the surface of T lymphocytes and consist of two alpha and beta chains, is TCR or T Cell Receptor. The T-cell receptor identifies antigens presented by APCs and consequently, is activated [5]. Bacterial exo proteins that induce nonspecific T cell activation and act as superantigens require cells containing MHC II molecules on the surface [9]. The previous studies have shown that some bacterial superantigens, such as SEA, do not bind to cells that do not express MHC II molecules on the surface, but in cells containing MHC II molecules, this type of superantigen contributes to severe stimulation of immune system [10].

The application of superantigens in the treatment of cancer is a new topic that has been less elucidated. A deeper understanding of the mechanism of superantigens in immune system modulation has an important role in the treatment of cancer, because superantigens activate lymphocytes, increase cytokine production in immune processor cells, increase growth factor receptors on the surface of T cells, and increase cytokine production by T lymphocytes and alter immune function. An accurate understanding of these mechanisms and controlling the immune response created by superantigens will certainly lead to great success in cancer treatment.

2. INTERACTION OF SUPERANTIGENS WITH MHCII

Superantigens bind directly to the antigen-presenting cells on the outside of the MHCII molecule. There are two distinct sites for binding of the superantigens [11, 12]. One of these sites is on the alpha chain of the MHCII molecule. The other site is located on the beta chain. Binding to the beta chain is about 100-fold higher than that of the alpha chain and depends on the zinc molecule. The superantigens of SEB and TSST١ bind to the alpha chain of the MHCII molecule through their hydrophobic core in the N-terminal domain. Other superantigens also use this hydrophobic core to bind to the MHCII molecule. Only some superantigens, including SPE-H, SPE-C, and SME-Z2, lack this hydrophobic core. Most superantigens, except SSA, TSST١, and SEB, have zinc ion binding sites that cooperate in the binding of the superantigens to the MHCII molecule [11].

The binding is mediated by a bridging zinc ion which tetrahedrally cooperates three ligands from SPE-C [13] and two ligands from SEH bind to one ligand of the MHCII molecule. SEH lacks the generic site for binding to a chain of MHCII but has the strongest binding to the B chain of the MHCII molecule compared to other superantigens [14]. The interaction between MHCII and SEA superantigens through the zinc-binding site, located in the C-terminal domain of SEA, is associated with a high affinity compared to the generic site located in the N-terminal of SEA [15-17]. Superantigens that have distinct sites in both of the N- terminal and C-terminal domains constitute the trimeric superantigen-MHCII-superantigen complex [18]. Other superantigens such as SEC and SPE-A lack zinc-binding sites on the C-terminal domain. However, these superantigens contain a new zinc-binding site in N-terminal. The interaction between MHCII and superantigen that is mediated by this new zinc-binding site is associated with a lower affinity compared to the zinc-binding sites, which are located in C-terminal [19, 20]. Some superantigens form homodimers. SED forms zinc-dependent homodimers [21] and the SPE-C can form zinc-independent homodimers. The binding of these homodimers to MHCII is solely and may form either trimers or tetramers [22]. Some other superantigens, including SME-Z2, SPE-G, and SPE-H, interact with MHCII in a zinc-dependent manner. Therefore, superantigens have multiple sites for interacting with MHCII. Superantigens may interact either through ZINC dependent *via* the generic sites or a combination of both. The presence of these multiple sites gives superantigens a special arrangement through which they can potentially stimulate the immune system [23] (Fig. **1**).

3. INTERACTION OF SUPERANTIGENS WITH TCR

The main target of superantigens is T CD4+ lymphocytes [24]. Almost all superantigens react with the V beta region of TCR and cause more than 10% of resting T lymphocytes to be activated [3]. Superantigens have specific residues for particular V beta elements and react with TCR through these specific residues. Therefore, sequence diversity within TCR binding sites in superantigens obtains the cornerstone for a particular V beta repertoire for superantigens [25]. Previous studies have demonstrated that the formation of the main reaction depends on the interaction between side-chain atoms in superantigens and CDR1, 2 and HV4 regions of TCR [11]. The change in only one residue of the TCR-binding site is associated with the alteration of the superantigen specificity [26]. Superantigens using TCR binding sites constitute a shallow cavity. For example, in SEB, this shallow cavity is formed by the part of β5-strand and α5-helix, α2-helix, β2-β3 loop, and β4-strand, β4-β5 loop [27]. In concern with the reaction of superantigens with TCR, some considerations are necessary. In some superantigens, such as the TSST1, the location of TCR binding sites is different from other superantigens. It is located in C-terminal domain on the long α2-helix and between the β7-β8 and α2-β9 loops as part of the α1-helix [28, 29]. Moreover, the SEH superantigen stimulates the T cells through the direct interaction between the V-alpha domain [30] (Fig. **2**).

Fig. (1). The interaction between superantigens and TCR/MHC II molecules. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

4. STAPHYLOCOCCAL ENTEROTOXIN

Staphylococcus enterotoxin is one of the bacterial proteins that strongly stimulates the immune system and has various immunological activities. Recent studies have shown that the common superantigens of *Staphylococcus aureus* can inhibit tumor cells and repair tissues and cells. The mechanisms by which this superantigen contributes to the enhancement of the immune system are the activation of T cells and natural killer cells, increasing production of cytokines involved in T cell growth and differentiation, increasing the phagocytic ability of phagocytes, increasing white blood cells in peripheral blood and the inhibition of tumor cells [31]. Recent studies have shown that Staphylococcus enterotoxin A is one of the most potent bacterial superantigens that induce the cytotoxicity of T cells and contribute to cytokine production. In a study on colon cancer, the Fab fragment of c242 monoclonal antibody, reactive against colon carcinoma, was genetically fused with SEA. C242--FAB-SEA activated SEA-responsive T cells against tumor cells at nanomolar concentrations. The treatment of disseminated colon cancer cells from humanized SCID mice with nanomolar concentrations of this fusion protein significantly inhibited tumor growth. Additionally, immunohistochemistry showed severe infiltration of T lymphocytes in tumors treated with c242-FAB-SEA [32].

Superantigens also show antitumor activity when it is used as a vaccine. Interestingly, in one study by Chernow B *et al*., the radiated melanoma cells were injected into mice and then three days later, SEA and SEB were injected sequentially. When these mice were exposed to live melanoma cells, they killed a large fraction of the tumor cells, and the survival of the treated mice was significantly increased [33]. Studies have shown that after vaccination with radiated tumor cells, immunological memory is significantly enhanced. Accordingly, vaccination against cancer using superantigens as an adjuvant-like factor is possible. Research on SEB suggests that this superantigen has potential importance in cancer treatment. The significant successes in the field of cancer treatment in animal models using SEB have been demonstrated [34]. In one study by Perabo FG *et al*., it was found when SEB is injected in appropriate doses to animal models containing various types of tumor cells, most tumor cells belong to the different cancers are eradicated and only a small number of tumor types remain. A small number of the remaining tumors were also substantially exposed with apoptosis and infiltration of granulocytes [34].

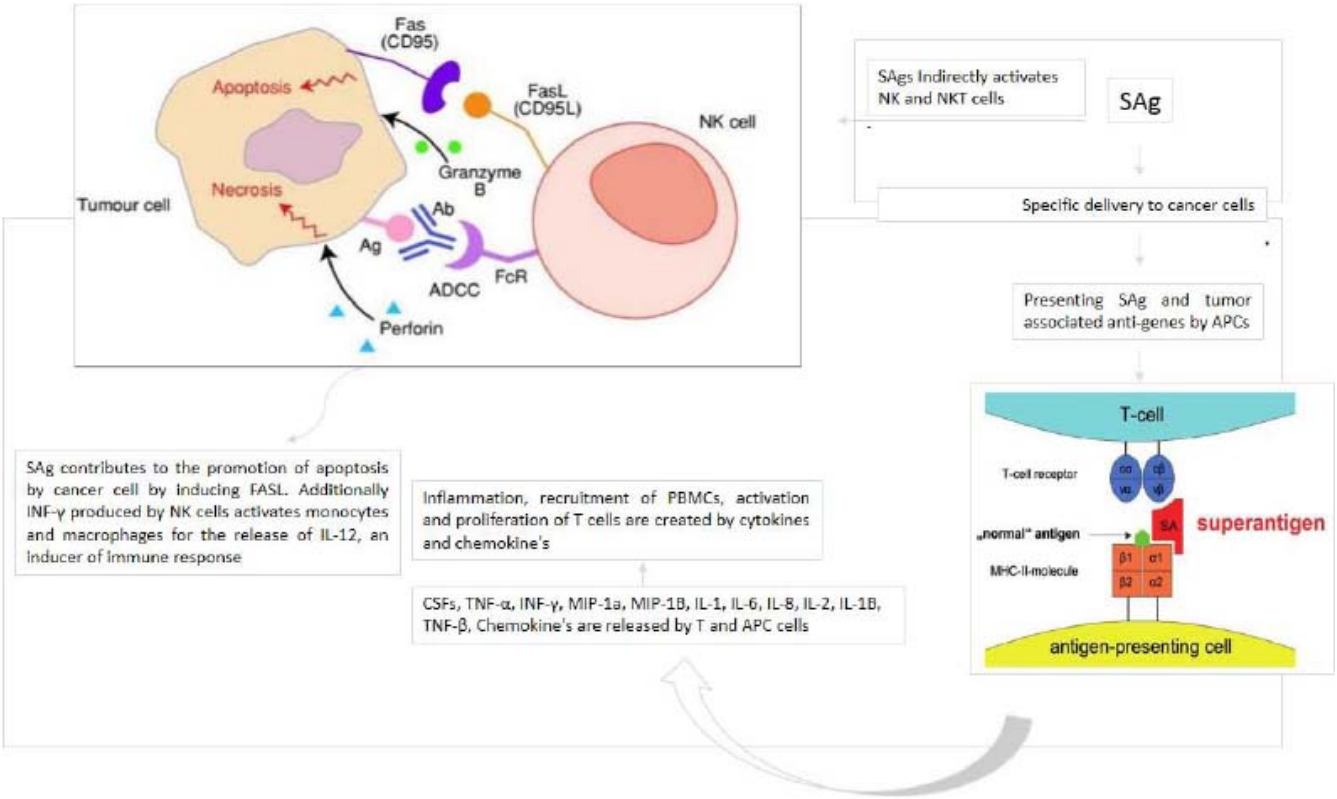

Fig. (2). The role of superantigens in the regulation of immune response. CSF: Colony Stimulating Factor, TNF: Tumor Necrosis Factor, INF: Interferon, MIP: Macrophage inflammatory protein. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Studies have shown that SEB plays a key role in reducing metastasis of breast cancer. It was also found in one study that when SEA is associated with a trans-membrane sequence of the c-erb-B2 gene derived from ovarian tumor cells, it spontaneously associates with the ovarian tumor cell membrane and results in strong proliferation of peripheral blood lymphocytes [35-38]47484950. Additionally, x in a study on the SEA superantigen drained the lymph node from the patient and exposed to the SEA superantigen for 48 hours. Subsequently, they expanded cells using interleukin-2 for 6-8 days. If these are harvested and re-injected to the patients, they will lead to a significant improvement in the disease and the residual tumors will fundamentally regress [39].

Tumor-specific monoclonal antibodies also have an important role in tumor cell elimination using superantigens. SEA fusion with tumor-specific antibodies contributes to the eradication of more than 80% of tumor cells and promotes the survival in mice models of melanoma [40-42]. Besides, the fusion of SEA with a bispecific antibody, with specificity for a tumor-specific and a T cell-specific antigen indicated complete remission in 50% of treated mice and a significant decrease in tumor growth in the remaining 50% [43].

Eva Erlandsson *et al*. showed the decreased antibody response that is commonly found in all individuals to the superantigen enhances the tumor-killing property of superantigen. Using epitope mapping, they found antibody binding sites in SEA and SEE and genetically removed the antibody-binding epitopes around the MHC-II-binding sites. Subsequently, they generated the SEA/E-120 superantigen. SEA/E-120 had a low capacity to bind to the anti-SEA/E and was more potent in the killing of tumor cells than MHCII expressing cells. In addition, due to the deletion of antibody-binding epitopes and low capacity to kill MHCII expressing cells, the toxicity of SEA/E-120 was very low [44].

5. HAS IN TUMOR THERAPY

Staphylococcus aureus metabolite forms highly agglutinative staphylococci (HAS). In fact, it is HAS, a biological response of bacteria that mediates immune system modulation. Low toxicity and strong ability to stimulate the immune system are positive advantages of these superantigens, so they can be used effectively in cancer treatment [31, 45]. According to a study by Chen *et al*., the HAS superantigen effectively increases the white blood cell count, especially neutrophils, in the tumor environment, prevents bone marrow suppression by chemotherapy drugs, and increases patient tolerance to chemotherapy [31]. Two key mechanisms that need to be considered here are that HAS alters peripheral hemogram and increases the percentage of neutrophils

Fig. (3). The mechanism involved in highly agglutinative staphylococcin (HAS) in promoting tumor cell killing. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

and white blood cells in the tumor environment [31]. Most importantly, HAS superantigen has a high ability to activate lymphocytes. Even a trace of HAS activates a large number of lymphocytes and subsequently leads to the production of different types of cytokines. It should be noted that an inaccurate dosage of HAS can lead to immune system disorders [31, 46]. HAS is an anti-tumor response modifier and its important ingredient is enterotoxin C. It strengths the activity of NK cells and increases the rate of lymphocyte transformation, augments the production of interleukins (ILs), interferons, colony stimulating factors (CSFs) and other major cytokines, thus strengthens the immune system function and the ability to kill tumor cells without damaging healthy cells. It also repairs damaged cells, restores immune system function and reduces tumor metastasis and recurrence [31, 47, 48].

For the application of superantigens in cancer treatment, four important biological processes must be considered. 1) Apoptosis: when superantigens stimulate the immune system and activate T cells, they augment cytokine production. Significant cytokines, including TNF, INFs, and ILs, destroy vascular endothelial cells of the tumor and also contribute to the migration of blood cells to cancer tissue to eradicate the tumor. Eventually, tumor necrosis and its eradication occur. Another function of cytokines produced by immune cells is to increase the differentiation of T lymphocytes. The differentiation of T cells leads to the promotion of cytokine production and acceleration of tumor eradication [31, 45, 49].

When T lymphocytes are activated by superantigens, they also induce cytotoxin production by T cells. This superantigen-dependent cytotoxin-specific fusion protein rapidly dissolves cancer cells and has a very strong affinity for tumor cells. This mechanism is called the dissolution of tumor cells [31].

Another mechanism through which superantigens can enhance the immune system, stability to eradicate tumor cells is the clearance mechanism. In the clearance mechanism, interleukins are produced by T cells to activate the lymphokine-activated killer (LAK) cells. LAK cells have potent

Fig. (4). Factors involved in promoting TSST1 mediated tumor killing. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

anti-tumor activity. After being activated, LAK cells clear tumor cells that are resistant to eradication [31].

Superantigens play an essential role in suppressing tumor cell metastasis when cancer cells are growing rapidly. They activate NK cells. Proteins and enzymes produced by NK cells destroy the cell membrane of the cancer cells and induce apoptosis in them. Thus, superantigens control immune surveillance of the tumor [31].

In a study by Qu Yunjiao *et al*., it was found HAS increases the efficacy of chemotherapy drugs in an advanced type of breast cancer. Their study also found that HAS in combination with a chemotherapy agent, vinorelbine, stimulates the immune system against tumor cells strongly. Accordingly, patients who received vinorelbine + HAS showed a significantly higher overall response rate than patients who only received vinorelbine [45]. The results of Qu Yunjiao *et al*. showed that superantigens play an essential role in controlling malignant pleural effusion (MPE). MPE is one of the relatively common complications in some different cancers. The fluid accumulates between the chest and lungs, the patient feels short of breath and the chest takes on an abnormal shape. Qu Yunjiao also demonstrated that HAS, in combination with cisplatin, increases patients' survival and decreases fluid effusion. Therefore the combination of the drug with HAS is associated with good prognosis in patients with MPE [49]. According to recent studies, local injection of HAS has a significant effect on the treatment of liver cancer. On the first day of the injection of HAS, symptoms of elevated body temperature for one to three days were seen. In some patients, the symptoms, including muscle pain and weakness, were noticed, but other discomforts were not seen. After three days, all the above symptoms disappeared and body temperature returned to normal. No renal and liver complications were noticed following the injection of HAS. ECG and other routine tests were normal. The level of AFP dropped significantly and approached to the normal. Finally, it was found that staphylococcal superantigens increase the number of anti-tumor cells. Given that they do not have significant side effects in liver cancer patients, the injection of HAS into liver cancer tissue can be associated with improving the immune system, increasing patient survival and reducing the recurrence of disease [50].

Malignant ascites are a symptom of intra-abdominal cancers in which the fluid abnormally accumulates in the peritoneal cavity. According to the recent studies, the combination of staphylococcal superantigens with 5-fluorouracil, in addition to being effective in controlling malignant ascites, enhances the immune system and induces the anti-tumor activity of the immune system. The combination of superantigen with 5-fluorouracil had significant positive effects on all

patients. A large number of patients achieved partial remission, and even some achieved complete remission. A group of other patients also stabilized. Therefore, this study suggests an effective and safe treatment in these patients through superantigens (Fig. **3**) [51].

6. TSST1 IN TUMOR THERAPY

Toxic-shock-syndrome toxin-1 (TSST-1) is a bacterial superantigen that can activate T lymphocytes and can be used in cancer immunotherapy [52]. W Wang *et al*. for investigating the immuno-modulatory effect of TSST1 selectively increased the expression of TSST1 in colorectal cancer cell lines. They also transfected several copies of HRE as an enhancer in addition to the TSST1 gene to colorectal cancer cell lines. Their study demonstrated that TSST1 contributes to the proliferation of human T cells, and the co-culture of cells containing high levels of TSST1 with PBMCs instigates a high frequency of TNF-a and IL2 secreting T cells particularly under hypoxia condition while PBMCs cultured with control cells lacked this condition. The findings of the study suggest that TSST1 plays an important role in enhancing the antitumor properties of the immune system and can be used effectively in cancer immunotherapy [52-54].

Among the superantigens, TSST1 has the lowest molecular weight [55]. The main mechanism involved in the eradication of tumor cells through the TSST1 superantigen is the non-specific binding to V beta elements in TCR molecules. Subsequently, it activates a large number of T lymphocytes. When a superantigen binds non-specifically to MHCII molecules or V beta elements of TCR, it contributes to the production of significant tumor suppressor cytokines, including TNF-α and interferon INF-γ [55, 56]. Selective delivery of superantigen to cancer cells is a key factor in the effectiveness of superantigen in eradicating tumor cells [57]. Small peptides that bind specifically to cancer cells can be used to deliver specific antigen to cancer cells. For instance, studies have shown that the HCC79 peptide (KSLSRHDHIHHH) can be used to deliver specific antigen to hepatocellular cancer cells [58].When it is specially delivered to cancer cells, its presentation binding to TCR and MHCII molecules occurs, and subsequent anti-tumor activity is increased. Finally, robust activation of T cells occurs [58].

Yong-Qiung Jiang *et al*. also made extensive efforts to treat hepatocellular carcinoma (HCC) using superantigens. They indicated that a novel peptide, named HCC79 binds to the hepatoma cell membrane with great specificity and potency. This protein does not directly affect cancer cell proliferation, but more importantly, suppresses cancer cell migration. They also found that HCC79 plays a critical role in delivering TSST1 superantigen to cancer cells for the creation of potential immunotherapy. Accordingly, they generated the fusion protein HCC79-TSST1. The fusion protein potentially bound to the cancer cell membrane and subsequently activated T lymphocytes for the anti-tumor activity. Surprisingly, TSST1-HCC79 had a much higher inhibitory effect on cancer cell progression than TSST1 alone. In addition, due to the specificity and high anti-tumor potency, HCC79 and TSST1 Sag could be developed for use in cancer immunotherapy in the future [58].

Surprisingly, superantigens, independently of MHCII, contribute to the proliferation of T lymphocytes *in vitro* as long as co-stimulatory signals were provided. Based on this information, the researchers to increase the delivery of TSST1 to the MHCII negative tumor cell membrane, fused its coding region to the TM sequence of the proto-oncogene c-erb-B-2 to construct TSST1-TM. Subsequently, MHCII negative tumor cells strongly induced T cell proliferation against tumor cells [59].

For the use of codon-optimized synthetic gene versions, recombinant superantigens for cancer treatment in recent studies have led to excellent results. It has been demonstrated that the anti-tumor activity of superantigens is due to the production of cytokine and activation of T lymphocytes because they alone cannot contribute to tumor cell killing. In a recent study, the effect of recombinant superantigens on the production of cytokines by PBMCs was evaluated. It was found that recombinant superantigens, including TSST1, SEB, SEA, and SPEA, potently induce the production of pre inflammatory cytokines, including INF Gama and IL2. Moreover, these superantigens had no cytotoxic effect on PBMCs and strongly activated T cells. The induction of the production of INF and IL2 cytokines by TSST1 was similar to SPEA. However, the induction of these cytokines by two other superantigens, including SEB and SEA, was weaker than TSST1 and SEB. Other cytokines, including IL4, IL5, and IL10, are induced by all recombinant superantigens at very low concentrations. In concern with the potency of superantigens in T cell activation, that was indicated all recombinant super antigens have a potent capability to activate T lymphocytes, but TSST1 and SEB were more potent compared to others. Superantigens alone are not capable of killing tumor cells, but by activating T cells, they induce significant apoptosis in cancer cells. Different concentrations of the TSST1 and SEA induced 80 percent apoptosis in cancer cells. Other recombinant superantigens also induced 50 to 60 percent apoptosis in cancer cells (Fig. **4**) [60].

7. STREPTOCOCCAL SUPERANTIGENS IN IMMUNOTHERAPY

Streptococcus pyogenes is one of the most important bacteria in group A *Streptococcus*, which produces various superantigens and is associated with a diverse range of clinical manifestations [61]. Eleven superantigens produced by *Streptococcus pyogenes* include SSA, SmeZ, Spe, including the serotypes of A, C, and G to M [61]. Many efforts have been made to design immune-toxins using bacterial superantigens, including *Streptococcus pyogenes* SAgs. These immune toxins, collectively called TTSs (tumor-targeted super antigen), compel T lymphocytes to recognize TAAs in a non MHC restricted manner. TTSs, recombinant proteins, that consist of a monoclonal antibody against tumor cells linked to a superantigen, activate the patient's immune system and in particular, increase CTL response [62-64].

One of the key mechanisms that play an essential role in the application of superantigens in cancer immunotherapy is the use of PBMCs [65]. The immune response to Streptococcus superantigens is quite specific at the biological and pathogenic levels because they contribute to the polyclonal activation of T-cells. According to recent studies, the incubation of PBMCs with therapeutic doses of SEB effectively induces apoptosis in cancer cells. Mechanistically, superantigens lead to the induction of FASL molecules on the surface of PBMCs. Therefore, it is likely that if PBMCs are incubated with streptococcal superantigens, PBMCs will induce apoptosis through the FASL-FAS mechanism [65, 66]. Superantigen-treated PBMCs also produce massive inflammatory cytokines, including IL2, TNF-α, and INF-γ. Subsequently, T cell proliferation, immune response activation and FAS-L-FAS mediated cytolysis are induced [61]. Interaction of streptococcal superantigens with MHCII and V beta regions of TCR molecules also produces inflammatory cytokines involved in the immune response [61]. Another important molecule that plays a key role in inducing immune responses through streptococcal superantigens is the CD28 co-stimulatory molecule. Recent studies have shown that the binding of streptococcal superantigens to CD28 molecules on T lymphocytes increases the sensitivity of T cells to the superantigen. Therefore, cytokine production, superantigen activity and the immune response are potentially increased [67-69].

SpeC, a polypeptide with 235 amino acids and a molecular weight of 24 KD, is one of the most important superantigens produced by *Streptococcus pyogenes* . This superantigen potently stimulates different clones of T cells. Unfortunately, it causes a life-threating disease called streptococcal TSS, and it makes TSS to be a barrier to use SpeC in cancer immune therapy.

According to the studies, SpeC preferentially binds and activates human Vβ2+ T cells, even though this superantigen is capable of binding to VB3, 4, 12, 15 T cell subsets [70, 71]. Some residue mutations in SpeC can dramatically reduce its toxicity, but the problem we face here is by altering some of the amino acids required for decreasing toxicity, such as ASP203 to Ala, the potency of superantigen for the activation of T cells is suppressed therefore the effect of immune therapy is neutralized [13]. However, with a compressive structural analysis of SpeC and the identification of critical amino acids involved in the biological functions of SpeC such as Tyr15 and Asp203, we gain knowledge that may also allow for SpeC to be engineered as a prototype SAg for future use in human disease particularly in cancer immunotherapy [13, 72, 73].

In a study by William B. Coley, it was found malignant tumors in patients with a concurrent bacterial infection have significantly regressed. To evaluate the accuracy of this excrement, Colley treated bone and soft tissues of sarcoma with infectious erysipelas and repetitively indicated infection associated tumors have considerably regressed [74, 75]. Patients with bacterial infections showed some important symptoms, including fever, chills, and malaise. In this excrement, Colley focused on treatment with live *Streptococcus* [74, 75]. Due to lethal infection caused by live bacteria, Colley subsequently generated Colley toxin. This toxin consists of heat-killed streptococcal and serratia marcescens organisms [75, 76].

Colley injected the toxin into more than a thousand cancer patients and believed that the immune response to the toxin would kill the tumor cells. His success in treating breast cancer, lymphoma, melanoma, and in particularly sarcoma, suggests that injecting this bacterial vaccine is the cornerstone of the development of future cancer immunotherapy. Most of Colley *et al*. successes were due to bacterial superantigens. Many studies have shown that bacterial superantigens strongly stimulate the immune system and subsequently contribute to the tumor cell killing [75, 76]. If we use *Streptococcus* superantigens instead of alive or heat-killed bacteria in this vaccine, we will still get more brilliant results. Streptococcal superantigens alone or in combination with other bacterial superantigens will repeat significant successes. Additionally, it can lead to the eradication of tumor cells if the monitoring of the patient is done carefully.

Other studies have shown that Streptococcal superantigens cause cell growth arrest. This antitumor activity may be attributed to strong immune stimulation by a systemic increase in granulocyte count and cytotoxic T cells [77]. Additionally, in a study by Elizabeth. L Brown DVM et al., it was demonstrated the injection of a vaccine containing plasmid DNA expressing *Streptococcus pyogenes* EMM55 protein has a substantial anti-tumor effect. They indicated that this vaccine contributes to the reduction of total tumor burden to 42% compared to the initial size of the tumor [78].

Other streptococcal superantigens, for example, streptococcal pyrogenic exotoxin (SPE)-C, cause the activation and expansion of T lymphocytes carrying Vβ3.2, Vβ12.5, Vβ2.1, and Vβ15.1 with a potential preference for Vβ2.1, whereas streptococcal mitogenic exotoxin (SMEZ) indicates specificity for Vβ2.1, Vβ4.1, Vβ7.3 and Vβ8.1 regions with a preference for Vβ4.1 and Vβ8.1 [79]

8. VECTOR SYSTEMS IN GENE THERAPY

Today, cancer gene therapy using a variety of vector systems has gained considerable attention. So far, several gene vectors have been developed to treat cancer and allow significant advancements in cancer treatment. The first success in treating diseases was obtained by retroviral vectors. In addition, vector biology should also be studied fundamentally because successes in cancer treatment have been overshadowed by the occurrence of vector-related cancers. Several vector systems, including lentiviral vectors, baculovirus expression vector system, and adeno-associated vector system, are increasingly being used in basic and applied research [80-84]. Besides, in cancer research, superantigen genes can be incorporated into these vectors. Consequently, the production of superantigens leads to the stimulation of immune cells against tumor cells and better results in cancer treatment.

In a study by LuciaVanrell *et al*., it was found recombinant AAV vectors have an essential role in preventing liver

cancer cell metastasis. They designed a hepato specific bidirectional and auto-regulatory tetracycline (Tet)-On system (Tet_{bidir}Alb) flanked by AAV inverted terminal repeats (ITRs). To evaluate the therapeutic effect of the recombinant AAV vector, they placed the IL12 gene into the vector. Therefore, they generated AAV-Tetbidir-Alb-expressing IL-12. Consequently, the induction of IL12 and INF-γ was performed by the vector in a murine model for hepatic colorectal metastasis. The toxicity was significantly low and AAV-Tetbidir-Alb-expressing IL-12 induced T cell memory response against liver tumor cells and prevented the establishment of tumor cell metastasis. In addition to IL12, superantigen genes can also be incorporated into the AAV-Tetbidir-Alb-expressing IL-12 system. More importantly, these superantigens can promote the stimulation of T lymphocytes against tumor cells and can lead to better results in preventing cancer cell progression [85].

Recent studies have shown that manipulation of the AAV-6 vector could potentially increase the efficiency of gene transduction to prostate, liver, and breast cancer cells. When the Arg-Gly-Asp (RGD) peptide was incorporated into the virus capsid, the specificity of this vector for gene delivery to cancer cells increases. The AAV-RGD improves gene transduction efficiency approximately three-fold in comparison with wild type AAV-6 vectors. Furthermore, it was found that mutagenesis in surface-exposed tyrosine and threonine residues involved in the intracellular trafficking of AAV vectors increased gene transduction up to 5-fold. More importantly, AAV-6-Y705-731F-T492V, in combination with RGD peptide, is the potential gene transducer vector that contributes to the promotion of gene delivery approximately 8-fold. Thus, other manipulations of this vector, such as the insertion of immune-stimulating interleukin genes and important superantigen genes, could lead to potent and specific stimulation of the immune system against tumor cells [86].

Lenti viruses are widely used in cancer therapy. In a study by Xiao Qin *et al*., it was found that increased expression of miR-199a using lentiviral vectors substantially inhibits cell proliferation through the suppression of HIF1a expression in hepatocellular carcinoma. Med19 acts as an essential factor in the proliferation of breast cancer cell lines, which suggests that the suppression of Med19 could be a promising strategy for breast cancer therapy. Recent studies indicated the increased expression of Med19 in breast cancer tissues. Med19 expression is significantly correlated with tumor progression. In a study by Li-Hua Li et al., it was demonstrated the suppression of Med19 in human breast cancer MDA-MB-231 and MCF-7 cells with lentiviruses delivering small hairpin RNA (shRNA) against Med19 contributes to the inhibition of tumor cell progression. The inhibition of Med19 expression resulted in the augmentation of cancer cells in the G0/G1 phase and significantly attenuated the proliferation of MDA-MB-231 and MCF-7 cells *in vitro*. Additionally, the suppression of MED19 expression in breast cancer using shRNA against MED19 along with integrating genes related to immune system regulation, important cytokines as well as superantigens in lentivirus vector containing shRNA against MED19, results in the suppression of tumor growth substantially [87, 88].

Another study by Flavie Sicard *et al*. demonstrated that LVs-transduced human pancreatic ductal adenocarcinoma (PDA) efficiently suppressed the expression of miR-21, both *in vitro* and *in vivo*. Subsequently, cancer cell proliferation was substantially inhibited and the apoptosis rate of PDA-derived cell lines through the mitochondrial pathway was strongly increased. *in vivo*, through the induction of apoptosis, the suppression of miR-21 inhibited the progression of a very aggressive phenotype of PDA. Furthermore, combining miR-21 targeting and chemotherapeutic agents provoked tumor regression [89]. In addition to suppressing miRNAs using lentiviral vectors, manipulating of this vector for combing of the immune-stimulating cytokines as well as superantigens can be used to make the immune system more potent against tumor cells.

Non-integrating lentiviral vectors are a potent and safe delivery system in cancer immunotherapy. Recent studies have reported that a single immunization with the integration of defective lentiviral vectors (IDLVs) congaing tumor model antigens contributes to the strong stimulation of the immune system against tumor cells. Human antigen-presenting cells have an important role in gene therapy with IDLVs. The infection of APC cells including monocyte-derived dendritic cells (DCs) and macrophages with IDLVs that contain genes involved in immune system regulation including cytokines as well as superantigens can lead to immune system stimulation against tumor cells. Surely, IDLVs can play a brilliant role in regulating the immune system and tumor cell eradication [90].

According to a recent study, systemic administration of lentiviral vectors expressing kallistatin suppresses the proliferation of metastatic cancer cells and promotes the survival of tumor-bearing patients. These results suggest that gene therapy using lentiviruses containing the kallistatin gene, which exerts anti-angiogenic and anti-metastatic activity can contribute to a promising strategy for lung cancer treatment. Additionally, this study confirmed that lentiviral vectors expressing kallistatin lead to the inhibition of migration and proliferation of endothelial cells and also reduce the invasion of tumor cells. Tumor-bearing mice models that received lentiviral vectors expressing kallistatin had low tumor nodules and longer survival than the control group. Successful therapies and better results can be achieved by manipulating these vectors and integrating genes involved in regulating the immune system. In addition, the specific stimulation of immune cells by integrating cytokine and superantigen genes can lead to the specific eradication of tumor cells [91, 92].

Other vector systems, including retroviral vectors, are indicative of cancer immune therapy. The use of retroviral vectors for gene delivery in cancer immune therapy was first introduced in 1980. The moloney MLV has a simple genome and it is commonly used as retroviral vectors in gene therapy. In the MLV genome, the polypeptides of gag and env are essential for viral replication and package. The gag, pol,

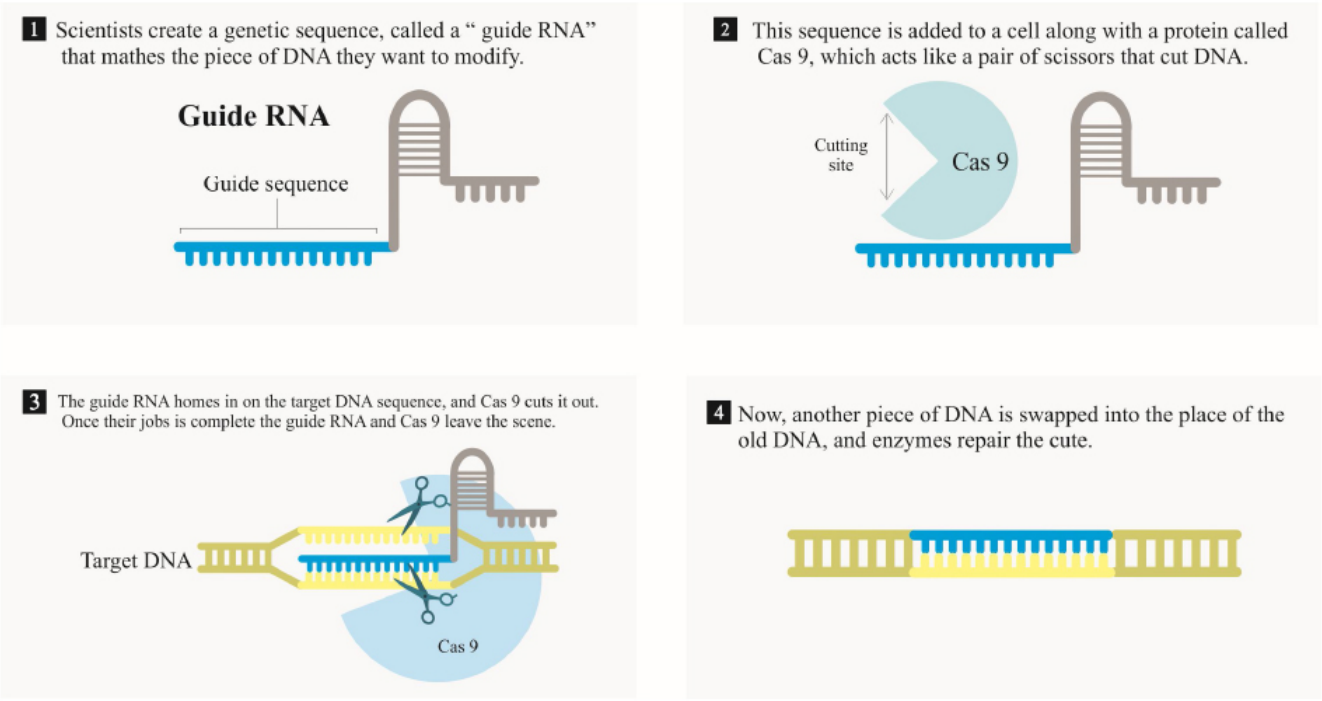

Fig. (5). Gene editing with the CRISPR/CAS9 technique. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

and env are replaced with an expression cassette containing the gene of interest that is under the control of a promoter. Like other vector systems, the indications of retroviral vectors in cancer therapy are mediated by the suppression of a specific gene by delivering siRNAs, the increasing of gene expression by integrating important immune regulating genes, *etc*. Finally, it is important to note that the manipulation of retroviral vectors and placing cytokine, superantigen and immune regulating genes can be the best strategy for eradicating tumor cells along with the suppression of genes involved in cancer cell processes including proliferation, metastasis, and angiogenesis [81].

9. CRISPR TECHNIQUE IN CANCER THERAPY

Nowadays, targeted methods of altering the genome of living cells have provided powerful tools in the treatment of genetic diseases. The CRISPR/Cas9 technique is one of the newest genome editing tools designed based on bacterial immune system gene-editing techniques. This is a very efficient, fast, and simple technique that is hoped to be able to remove, add or modify any gene in the cell. In the first step, the required sgRNA for targeting the gene of interest as well as the primers needed to determine genotypes are designed by in silico tools. In the second step, the sgRNA is cloned with an expression plasmid that contains scaffolding RNA and the Cas9 protein coding sequence. Then this plasmid that contains all of the components needed to target the gene is transferred to the target cells. At the last step, the cells that receive the plasmid are proliferated [93].

As we know, all cancers are caused by numerous mutations that contribute to abnormal proliferation and malignant phenotypes, these mutations lead to the abnormal expression of oncogenes, tumor suppressor genes, epigenetic agents and genes that induce chemotherapy resistance. The CRISPR/-Cas9 system, as a powerful and secure tool with high specificity, can modify these patterns and treatment of cancers caused by those mutations [93, 94]. The oncogenicity changes in a number of cancers cause increased proliferation of cells and malignant phenotypes. Therefore, targeting oncogenes, for instance, Tyrosine kinase receptor Erb2 by CRISPR/CAS9, can be helpful in cancer treatment [95-97]. What is important about the CRISPR technique in this study is that using this technique could modify the genome, which stimulated the immune system against tumor cells. Gene editing using this technique allows new genes to be integrated very specifically into the genome. Superantigen related genes, along with other immune-stimulating factors and, more importantly, genes that are involved in tumor suppression, can be entered into the genome using the CRISPR technique and exert their anti-tumor effects (Fig. **5**).

CONCLUSION

It is now widely demonstrated that superantigens can play a critical role in the eradication of tumor cells. Superantigens stimulate the proliferation of lymphocytes and antigen-presenting cells by binding to MHCII molecules and V beta regions in T cell receptors. Thus, the tumor antigen-presenting to the T lymphocyte and polyclonal T cell activation

is induced. Additionally, the production of important cytokines by T cells and APCs contributes to the stimulation of immune response against tumor cells. When vector systems are used to increase the expression of a particular gene or suppress a gene to treat cancer, notable successes can be achieved by incorporating genes related to superantigens or factors involved in the immune response to tumor cells. Thus superantigens, since they induce an immune response against a tumor cell if used with other cancer immunotherapy agents, can certainly play a very important role in cancer treatment.

DATA SHARING STATEMENT

We hereby state that data sharing is not applicable in our submission.

CONSENT FOR PUBLICATION

All the authors give consent for the publication of this manuscript.

FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This study was supported by Baqyatallah University of medical sciences. We gratefully thank this university. We also thank Mr. Behnam emamgolizadeh for his special assistance in preparing this article.

REFERENCES

[1] Alouf JE, Müller-Alouf H. Staphylococcal and streptococcal superantigens: molecular, biological and clinical aspects. Int J Med Microbiol 2003; 292(7-8): 429-40. http://dx.doi.org/10.1078/1438-4221-00232 PMID: 12635926

[2] DeVries AS, Lesher L, Schlievert PM, *et al.* Staphylococcal toxic shock syndrome 2000-2006: epidemiology, clinical features, and molecular characteristics. PLoS One 2011; 6(8)e22997 http://dx.doi.org/10.1371/journal.pone.0022997 PMID: 21860665

[3] White J, Herman A, Pullen AM, Kubo R, Kappler JW, Marrack P. The V β-specific superantigen staphylococcal enterotoxin B: stimulation of mature T cells and clonal deletion in neonatal mice. Cell 1989; 56(1): 27-35. http://dx.doi.org/10.1016/0092-8674(89)90980-X PMID: 2521300

[4] Janeway CA Jr, Yagi J, Conrad PJ, *et al.* T-cell responses to Mls and to bacterial proteins that mimic its behavior. Immunol Rev 1989; 107(1): 61-88. http://dx.doi.org/10.1111/j.1600-065X.1989.tb00003.x PMID: 2522086

[5] Fleischer B. A conserved mechanism of T lymphocyte stimulation by microbial exotoxins. Microb Pathog 1989; 7(2): 79-83. http://dx.doi.org/10.1016/0882-4010(89)90027-2 PMID: 2512465

[6] Callahan JE, Herman A, Kappler JW, Marrack P. Stimulation of B10.BR T cells with superantigenic staphylococcal toxins. J Immunol 1990; 144(7): 2473-9. PMID: 2138648

[7] Watson AR, Lee WT. Defective T cell receptor-mediated signal transduction in memory CD4 T lymphocytes exposed to superantigen or anti-T cell receptor antibodies. Cell Immunol 2006; 242(2): 80-90. http://dx.doi.org/10.1016/j.cellimm.2006.09.008 PMID: 17083922

[8] Misfeldt ML. Microbial "superantigens". Infect Immun 1990; 58(8): 2409-13. http://dx.doi.org/10.1128/IAI.58.8.2409-2413.1990 PMID: 2196226

[9] Fleischer B, Schrezenmeier H, Conradt P. T lymphocyte activation by staphylococcal enterotoxins: role of class II molecules and T cell surface structures. Cell Immunol 1989; 120(1): 92-101. http://dx.doi.org/10.1016/0008-8749(89)90177-9 PMID: 2522832

[10] Mollick JA, Cook RG, Rich RR. Class II MHC molecules are specific receptors for staphylococcus enterotoxin A. Science 1989; 244(4906): 817-20. http://dx.doi.org/10.1126/science.2658055 PMID: 2658055

[11] Baker MD, Acharya KR. Superantigens: structure, function, and diversity.Superantigens. American Society of Microbiology 2007; pp. 121-35.

[12] Papageorgiou AC, Acharya KR. Microbial superantigens: from structure to function. Trends Microbiol 2000; 8(8): 369-75. http://dx.doi.org/10.1016/S0966-842X(00)01793-5 PMID: 10920396

[13] Tripp TJ, McCormick JK, Webb JM, Schlievert PM. The zinc-dependent major histocompatibility complex class II binding site of streptococcal pyrogenic exotoxin C is critical for maximal superantigen function and toxic activity. Infect Immun 2003; 71(3): 1548-50. http://dx.doi.org/10.1128/IAI.71.3.1548-1550.2003 PMID: 12595474

[14] Hâkansson M, Petersson K, Nilsson H, *et al.* The crystal structure of staphylococcal enterotoxin H: implications for binding properties to MHC class II and TcR molecules. J Mol Biol 2000; 302(3): 527-37. http://dx.doi.org/10.1006/jmbi.2000.4093 PMID: 10986116

[15] Abrahmsén L, Dohlsten M, Segrén S, Björk P, Jonsson E, Kalland T. Characterization of two distinct MHC class II binding sites in the superantigen staphylococcal enterotoxin A. EMBO J 1995; 14(13): 2978-86. http://dx.doi.org/10.1002/j.1460-2075.1995.tb07300.x PMID: 7542584

[16] Fraser JD, Urban RG, Strominger JL, Robinson H. Zinc regulates the function of two superantigens. Proc Natl Acad Sci USA 1992; 89(12): 5507-11. http://dx.doi.org/10.1073/pnas.89.12.5507 PMID: 1608962

[17] Sundström M, Hallén D, Svensson A, Schad E, Dohlsten M, Abrahmsén L. The Co-crystal structure of staphylococcal enterotoxin type A with Zn2+ at 2.7 A resolution. Implications for major histocompatibility complex class II binding. J Biol Chem 1996; 271(50): 32212-6. http://dx.doi.org/10.1074/jbc.271.50.32212 PMID: 8943278

[18] Tiedemann RE, Urban RJ, Strominger JL, Fraser JD. Isolation of HLA-DR1.(staphylococcal enterotoxin A)2 trimers in solution. Proc Natl Acad Sci USA 1995; 92(26): 12156-9. http://dx.doi.org/10.1073/pnas.92.26.12156 PMID: 8618863

[19] Baker M, Gutman DM, Papageorgiou AC, Collins CM, Acharya KR. Structural features of a zinc binding site in the superantigen strepococcal pyrogenic exotoxin A (SpeA1): implications for MHC class II recognition. Protein Sci 2001; 10(6): 1268-73. http://dx.doi.org/10.1110/ps.330101 PMID: 11369867

[20] Papageorgiou AC, Collins CM, Gutman DM, *et al.* Structural basis for the recognition of superantigen streptococcal pyrogenic exotoxin A (SpeA1) by MHC class II molecules and T-cell receptors. EMBO J 1999; 18(1): 9-21. http://dx.doi.org/10.1093/emboj/18.1.9 PMID: 9878045

[21] Sundström M, Abrahmsén L, Antonsson P, Mehindate K, Mourad W, Dohlsten M. The crystal structure of staphylococcal enterotoxin type D reveals Zn2+-mediated homodimerization. EMBO J 1996; 15(24): 6832-40. http://dx.doi.org/10.1002/j.1460-2075.1996.tb01074.x PMID: 9003758

[22] Proft T, Moffatt SL, Berkahn CJ, Fraser JD. Identification and characterization of novel superantigens from Streptococcus pyogenes. J Exp Med 1999; 189(1): 89-102.

http://dx.doi.org/10.1084/jem.189.1.89 PMID: 9874566

[23] Baker MD, Acharya KR. Superantigens: structure-function relationships. Int J Med Microbiol 2004; 293(7-8): 529-37. http://dx.doi.org/10.1078/1438-4221-00298 PMID: 15149028

[24] Krakauer T. Differential inhibitory effects of interleukin-10, interleukin-4, and dexamethasone on staphylococcal enterotoxin-induced cytokine production and T cell activation. J Leukoc Biol 1995; 57(3): 450-4. http://dx.doi.org/10.1002/jlb.57.3.450 PMID: 7884317

[25] Li H, Llera A, Mariuzza RA. Structure-function studies of T-cell receptor-superantigen interactions. Immunol Rev 1998; 163(1): 177-86. http://dx.doi.org/10.1111/j.1600-065X.1998.tb01196.x PMID: 9700510

[26] Deringer JR, Ely RJ, Stauffacher CV, Bohach GA. Subtype-specific interactions of type C staphylococcal enterotoxins with the T-cell receptor. Mol Microbiol 1996; 22(3): 523-34. http://dx.doi.org/10.1046/j.1365-2958.1996.1381506.x PMID: 8939435

[27] Swaminathan S, Furey W, Pletcher J, Sax M. Crystal structure of staphylococcal enterotoxin B, a superantigen. Nature 1992; 359(6398): 801-6. http://dx.doi.org/10.1038/359801a0 PMID: 1436058

[28] Acharya KR, Passalacqua EF, Jones EY, *et al.* Structural basis of superantigen action inferred from crystal structure of toxic-shock syndrome toxin-1. Nature 1994; 367(6458): 94-7. http://dx.doi.org/10.1038/367094a0 PMID: 8107781

[29] Ohlendorf DH, *et al.* Structure of Toxic Shock Syndrome Toxin-1.Protein Toxin Structure. Springer 1996; pp. 217-29. http://dx.doi.org/10.1007/978-3-662-22352-9_11

[30] Petersson K, Pettersson H, Skartved NJ, Walse B, Forsberg G. Staphylococcal enterotoxin H induces V α-specific expansion of T cells. J Immunol 2003; 170(8): 4148-54. http://dx.doi.org/10.4049/jimmunol.170.8.4148 PMID: 12682246

[31] Tian XL, Yan Z, Chen J, Zhao WH, Guo W. Clinical application of highly agglutinative staphylococcin in cancer treatment updates of the literature. Eur Rev Med Pharmacol Sci 2016; 20(12): 2718-25. PMID: 27383328

[32] Dohlsten M, Lando PA, Björk P, *et al.* Immunotherapy of human colon cancer by antibody-targeted superantigens. Cancer Immunol Immunother 1995; 41(3): 162-8. http://dx.doi.org/10.1007/BF01521342 PMID: 7553685

[33] Kominsky SL, Torres BA, Hobeika AC, Lake FA, Johnson HM. Superantigen enhanced protection against a weak tumor-specific melanoma antigen: implications for prophylactic vaccination against cancer. Int J Cancer 2001; 94(6): 834-41. http://dx.doi.org/10.1002/ijc.1551 PMID: 11745486

[34] Perabo FG, Willert PL, Wirger A, Schmidt DH, Von Ruecker A, Mueller SC. Superantigen-activated mononuclear cells induce apoptosis in transitional cell carcinoma. Anticancer Res 2005; 25(5): 3565-73. PMID: 16101181

[35] Ma W, Yu H, Wang Q, Jin H, Solheim J, Labhasetwar V. A novel approach for cancer immunotherapy: tumor cells with anchored superantigen SEA generate effective antitumor immunity. J Clin Immunol 2004; 24(3): 294-301. http://dx.doi.org/10.1023/B:JOCI.0000025451.41948.94 PMID: 15114060

[36] Wahlsten JL, Mills CD, Ramakrishnan S. Antitumor response elicited by a superantigen-transmembrane sequence fusion protein anchored onto tumor cells. J Immunol 1998; 161(12): 6761-7. PMID: 9862706

[37] Yi P, Yu H, Ma W, Wang Q, Minev BR. Preparation of murine B7.1-glycosylphosphatidylinositol and transmembrane-anchored staphylococcal enterotoxin. A dual-anchored tumor cell vaccine and its antitumor effect. Cancer 2005; 103(7): 1519-28. http://dx.doi.org/10.1002/cncr.20943 PMID: 15739200

[38] Ma W, Yu H, Wang Q, Bao J, Yan J, Jin H. *In vitro* biological activities of transmembrane superantigen staphylococcal enterotoxin A fusion protein. Cancer Immunol Immunother 2004; 53(2): 118-24. http://dx.doi.org/10.1007/s00262-003-0437-0 PMID: 14574492

[39] Plautz GE, Barnett GH, Miller DW, *et al.* Systemic T cell adoptive immunotherapy of malignant gliomas. J Neurosurg 1998; 89(1): 42-51. http://dx.doi.org/10.3171/jns.1998.89.1.0042 PMID: 9647171

[40] Hansson J, Ohlsson L, Persson R, *et al.* Genetically engineered superantigens as tolerable antitumor agents. Proc Natl Acad Sci USA 1997; 94(6): 2489-94. http://dx.doi.org/10.1073/pnas.94.6.2489 PMID: 9122222

[41] Dohlsten M, Hedlund G, Akerblom E, Lando PA, Kalland T. Monoclonal antibody-targeted superantigens: a different class of anti-tumor agents. Proc Natl Acad Sci USA 1991; 88(20): 9287-91. http://dx.doi.org/10.1073/pnas.88.20.9287 PMID: 1924393

[42] Dohlsten M, Abrahmsén L, Björk P, *et al.* Monoclonal antibody-superantigen fusion proteins: tumor-specific agents for T-cell-based tumor therapy. Proc Natl Acad Sci USA 1994; 91(19): 8945-9. http://dx.doi.org/10.1073/pnas.91.19.8945 PMID: 8090750

[43] Takemura S, Kudo T, Asano R, *et al.* A mutated superantigen SEA D227A fusion diabody specific to MUC1 and CD3 in targeted cancer immunotherapy for bile duct carcinoma. Cancer Immunol Immunother 2002; 51(1): 33-44. http://dx.doi.org/10.1007/s00262-001-0245-3 PMID: 11845258

[44] Erlandsson E, Andersson K, Cavallin A, *et al.* Identification of the antigenic epitopes in staphylococcal enterotoxins A and E and design of a superantigen for human cancer therapy. J Mol Biol 2003; 333(5): 893-905. http://dx.doi.org/10.1016/j.jmb.2003.09.009 PMID: 14583188

[45] Yu QT, Meng ZB. Treatment of advanced breast cancer with a combination of highly agglutinative staphylococcin and vinorelbine-based chemotherapy. Eur Rev Med Pharmacol Sci 2016; 20(16): 3465-8. PMID: 27608908

[46] Cohen SG, Dzury DS, Michelini FJ. Immune responses to Staphylococcus aureus antigens. J Infect Dis 1958; 103(1): 61-6. http://dx.doi.org/10.1093/infdis/103.1.61 PMID: 13575862

[47] Zonggui JXQQX. Effect of Highly Agglutinative Staphylococcin on MDR Gene Expression and NK Cell Activity in Patients with Advanced Hepatoma. Chin J Clin Oncol 1998; •••: 7. [J].

[48] Kawaguchi-Nagata K, Okamura H, Shoji K, Kanagawa H, Semma M, Shinagawa K. Immunomodulating activities of staphylococcal enterotoxins. I. Effects on *in vivo* antibody responses and contact sensitivity reaction. Microbiol Immunol 1985; 29(3): 183-93. http://dx.doi.org/10.1111/j.1348-0421.1985.tb00818.x PMID: 3874343

[49] Yuanjiao Q, *et al.* The Therapeutic Effects of Highly Agglutinative Staphylococcin Combined with Cisplatin in the Treatment of Patients with Malignant Pleural Effusion Journal of Basic and Clinical Oncology 2010.

[50] Mu P, *et al.* Clinical research on ultrasonically guided intrahepatic injections of HAS in interventional treatment of liver carcinomas Journal of BU ON: official journal of the Balkan Union of Oncology 2016; 21(6): 1394-7.

[51] Jiemin Z, *et al.* The Therapeutic Effect of Malignant Ascites with Improved Central Venous Catheter Combined with 5-Fluorouracil and Highly Agglutinative Staphylococcin Journal of Basic and Clinical Oncology 2011; 4

[52] Wang W, Sun X, Lu L, Zheng JB, Tian Y, Wang W. Cytotoxicity of lymphocytes activated by superantigen toxic-shock-syndrome toxin-1 against colorectal cancer LoVo cells. Mol Cell Biochem 2013; 376(1-2): 1-9. http://dx.doi.org/10.1007/s11010-013-1561-6 PMID: 23340976

[53] WANG. Hypoxia microenvironment regulates immunotherapy effect of TSST-1 on CEA positive colon cancer LoVo cells. Zhongguo Zhongliu Shengwu Zhiliao Zazhi 2011; •••: 6. [J].

[54] WANG w. Packaging and identification of recombinant retrovirus dual cancer-specific expressing superantigen of TSST-1 gene Journal of Xi'an Jiaotong University (Medical Sciences) 2011; (5): 3.

[55] Marrack P, Kappler J. The staphylococcal enterotoxins and their relatives. Science 1990; 248(4956): 705-11. http://dx.doi.org/10.1126/science.2185544 PMID: 2185544

[56] Dohlsten M, Sundstedt A, Björklund M, Hedlund G, Kalland T. Superantigen-induced cytokines suppress growth of human colon-carcinoma cells. Int J Cancer 1993; 54(3): 482-8.

http://dx.doi.org/10.1002/ijc.2910540321 PMID: 8509223

[57] Dohlsten M, Hansson J, Ohlsson L, Litton M, Kalland T. Antibody-targeted superantigens are potent inducers of tumor-infiltrating T lymphocytes *in vivo*. Proc Natl Acad Sci USA 1995; 92(21): 9791-5.
http://dx.doi.org/10.1073/pnas.92.21.9791 PMID: 7568219

[58] Jiang Y-Q, Wang HR, Li HP, Hao HJ, Zheng YL, Gu J. Targeting of hepatoma cell and suppression of tumor growth by a novel 12mer peptide fused to superantigen TSST-1. Mol Med 2006; 12(4-6): 81-7.
http://dx.doi.org/10.2119/2006-00011.Jiang PMID: 16953561

[59] Si S, Sun Y, Li Z, *et al.* Gene therapy by membrane-expressed superantigen for α-fetoprotein-producing hepatocellular carcinoma. Gene Ther 2006; 13(22): 1603-10.
http://dx.doi.org/10.1038/sj.gt.3302823 PMID: 16855617

[60] Bashraheel SS, AlQahtani AD, Rashidi FB, *et al.* Studies on vascular response to full superantigens and superantigen derived peptides: Possible production of novel superantigen variants with less vasodilation effect for tolerable cancer immunotherapy. Biomed Pharmacother 2019; 115108905
http://dx.doi.org/10.1016/j.biopha.2019.108905 PMID: 31060004

[61] Commons RJ, Smeesters PR, Proft T, Fraser JD, Robins-Browne R, Curtis N. Streptococcal superantigens: categorization and clinical associations. Trends Mol Med 2014; 20(1): 48-62.
http://dx.doi.org/10.1016/j.molmed.2013.10.004 PMID: 24210845

[62] Forsberg G, Skartved NJ, Wallén-Ohman M, *et al.* Naptumomab estafenatox, an engineered antibody-superantigen fusion protein with low toxicity and reduced antigenicity. J Immunother 2010; 33(5): 492-9.
http://dx.doi.org/10.1097/CJI.0b013e3181d75820 PMID: 20463598

[63] Nathan A, Shahar M. Methods and compositions for enhancing the potency of superantigen mediated cancer immunotherapy. Google Patents 2019.

[64] Sun J, Zhao L, Teng L, *et al.* Solid tumor-targeted infiltrating cytotoxic T lymphocytes retained by a superantigen fusion protein. PLoS One 2011; 6(2)e16642
http://dx.doi.org/10.1371/journal.pone.0016642 PMID: 21311755

[65] Hedlund G, Dohlsten M, Lando PA, Kalland T. Staphylococcal enterotoxins direct and trigger CTL killing of autologous HLA-DR+ mononuclear leukocytes and freshly prepared leukemia cells. Cell Immunol 1990; 129(2): 426-34.
http://dx.doi.org/10.1016/0008-8749(90)90218-G PMID: 2383900

[66] Fuller CL, Braciale VL. Selective induction of CD8+ cytotoxic T lymphocyte effector function by staphylococcus enterotoxin B. J Immunol 1998; 161(10): 5179-86.
PMID: 9820488

[67] Proft T, Fraser JD. Streptococcal superantigens.Superantigens and Superallergens. Karger Publishers 2007; pp. 1-23.
http://dx.doi.org/10.1159/000100851

[68] Arad G, Levy R, Hillman D, Kaempfer R. Superantigen antagonist protects against lethal shock and defines a new domain for T--cell activation. Nat Med 2000; 6(4): 414-21.
http://dx.doi.org/10.1038/74672 PMID: 10742148

[69] Arad G, Levy R, Nasie I, *et al.* Binding of superantigen toxins into the CD28 homodimer interface is essential for induction of cytokine genes that mediate lethal shock. PLoS Biol 2011; 9(9)e1001149
http://dx.doi.org/10.1371/journal.pbio.1001149 PMID: 21931534

[70] Fleischer B, Necker A, Leget C, Malissen B, Romagne F. Reactivity of mouse T-cell hybridomas expressing human Vbeta gene segments with staphylococcal and streptococcal superantigens. Infect Immun 1996; 64(3): 987-94.
http://dx.doi.org/10.1128/IAI.64.3.987-994.1996 PMID: 8641811

[71] Li P-L, Tiedemann RE, Moffat SL, Fraser JD. The superantigen streptococcal pyrogenic exotoxin C (SPE-C) exhibits a novel mode of action. J Exp Med 1997; 186(3): 375-83.
http://dx.doi.org/10.1084/jem.186.3.375 PMID: 9236189

[72] Li Y, Li H, Dimasi N, *et al.* Crystal structure of a superantigen bound to the high-affinity, zinc-dependent site on MHC class II. Immunity 2001; 14(1): 93-104.
http://dx.doi.org/10.1016/S1074-7613(01)00092-9 PMID: 11163233

[73] Kasper KJ, Xi W, Rahman AK, *et al.* Molecular requirements for MHC class II α-chain engagement and allelic discrimination by the bacterial superantigen streptococcal pyrogenic exotoxin C. J Immunol 2008; 181(5): 3384-92.
http://dx.doi.org/10.4049/jimmunol.181.5.3384 PMID: 18714010

[74] Hobohm U. Fever and cancer in perspective. Cancer Immunol Immunother 2001; 50(8): 391-6.
http://dx.doi.org/10.1007/s002620100216 PMID: 11726133

[75] Linnebacher M, Maletzki C, Klier U, Klar E. Bacterial immunotherapy of gastrointestinal tumors. Langenbecks Arch Surg 2012; 397(4): 557-68.
http://dx.doi.org/10.1007/s00423-011-0892-6 PMID: 22189906

[76] Zacharski LR, Sukhatme VP. Coley's toxin revisited: immunotherapy or plasminogen activator therapy of cancer? J Thromb Haemost 2005; 3(3): 424-7.
http://dx.doi.org/10.1111/j.1538-7836.2005.01110.x PMID: 15748226

[77] Linnebacher M, Maletzki C, Emmrich J, Kreikemeyer B. Lysates of S. pyogenes serotype M49 induce pancreatic tumor growth delay by specific and unspecific antitumor immune responses. J Immunother 2008; 31(8): 704-13.
http://dx.doi.org/10.1097/CJI.0b013e3181829f62 PMID: 18779749

[78] Brown EL, *et al.* Treatment of metastatic equine melanoma with a plasmid DNA vaccine encoding streptococcus pyogenes EMM55 protein. J Equine Vet Sci 2014; 34(5): 704-8.
http://dx.doi.org/10.1016/j.jevs.2013.11.012

[79] Proft T, Fraser JD. Streptococcal superantigens: biological properties and potential role in disease.Streptococcus pyogenes: basic biology to clinical manifestations. University of Oklahoma Health Sciences Center 2016. Internet

[80] Mingozzi F, High KA. Immune responses to AAV vectors: overcoming barriers to successful gene therapy. Blood 2013; 122(1): 23-36.
http://dx.doi.org/10.1182/blood-2013-01-306647 PMID: 23596044

[81] Escors D, Breckpot K. Lentiviral vectors in gene therapy: their current status and future potential. Arch Immunol Ther Exp (Warsz) 2010; 58(2): 107-19.
http://dx.doi.org/10.1007/s00005-010-0063-4 PMID: 20143172

[82] Wold WS, Toth K. Adenovirus vectors for gene therapy, vaccination and cancer gene therapy. Curr Gene Ther 2013; 13(6): 421-33.
http://dx.doi.org/10.2174/15665232133666131125095046 PMID: 24279313

[83] Felberbaum RS. The baculovirus expression vector system: A commercial manufacturing platform for viral vaccines and gene therapy vectors. Biotechnol J 2015; 10(5): 702-14.
http://dx.doi.org/10.1002/biot.201400438 PMID: 25800821

[84] Hitchman RB, Locanto E, Possee RD, King LA. Optimizing the baculovirus expression vector system. Methods 2011; 55(1): 52-7.
http://dx.doi.org/10.1016/j.ymeth.2011.06.011 PMID: 21945427

[85] Vanrell L, Di Scala M, Blanco L, *et al.* Development of a liver-specific Tet-on inducible system for AAV vectors and its application in the treatment of liver cancer. Mol Ther 2011; 19(7): 1245-53.
http://dx.doi.org/10.1038/mt.2011.37 PMID: 21364542

[86] Sayroo R, Nolasco D, Yin Z, *et al.* Development of novel AAV serotype 6 based vectors with selective tropism for human cancer cells. Gene Ther 2016; 23(1): 18-25.
http://dx.doi.org/10.1038/gt.2015.89 PMID: 26270885

[87] Li L-H, He J, Hua D, Guo ZJ, Gao Q. Lentivirus-mediated inhibition of Med19 suppresses growth of breast cancer cells *in vitro*. Cancer Chemother Pharmacol 2011; 68(1): 207-15.
http://dx.doi.org/10.1007/s00280-010-1468-9 PMID: 20890603

[88] Jia XQ, Cheng HQ, Qian X, *et al.* Lentivirus-mediated overexpression of microRNA-199a inhibits cell proliferation of human hepatocellular carcinoma. Cell Biochem Biophys 2012; 62(1): 237-44.
http://dx.doi.org/10.1007/s12013-011-9263-8 PMID: 21847633

[89] Sicard F, Gayral M, Lulka H, Buscail L, Cordelier P. Targeting miR-21 for the therapy of pancreatic cancer. Mol Ther 2013; 21(5): 986-94.
http://dx.doi.org/10.1038/mt.2013.35 PMID: 23481326

[90] Negri DR, Bona R, Michelini Z, *et al.* Transduction of human antigen-presenting cells with integrase-defective lentiviral vector enables functional expansion of primed antigen-specific CD8(+) T cells. Hum Gene Ther 2010; 21(8): 1029-35. http://dx.doi.org/10.1089/hum.2009.200 PMID: 20210625

[91] Shiau A-L, Teo ML, Chen SY, *et al.* Inhibition of experimental lung metastasis by systemic lentiviral delivery of kallistatin. BMC Cancer 2010; 10(1): 245. http://dx.doi.org/10.1186/1471-2407-10-245 PMID: 20509975

[92] Ranjbar S, *et al.* Selective suppression of tumor cells by a tumor-specific bicistronic lentiviral vector. Turk J Biol 2016; 40(6): 1289-94. http://dx.doi.org/10.3906/biy-1512-53

[93] Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F. Genome engineering using the CRISPR-Cas9 system. Nat Protoc 2013; 8(11): 2281-308. http://dx.doi.org/10.1038/nprot.2013.143 PMID: 24157548

[94] Shalem O, Sanjana NE, Hartenian E, *et al.* Genome-scale CRISPR-Cas9 knockout screening in human cells. Science 2014; 343(6166): 84-7. http://dx.doi.org/10.1126/science.1247005 PMID: 24336571

[95] Noori-Daloii MR, Ebadi N. Pharmacogenomics and cancer stem cells. Medical Science Journal of Islamic Azad Univesity-Tehran Medical Branch 2015; 25(1): 1-15.

[96] Young R, *et al.* Crispr□Cas9 genetic screens uncover Ab cell receptor□Myd88 superpathway in diffuse large B cell lymphoma. Hematol Oncol 2017; 35: 25-5. http://dx.doi.org/10.1002/hon.2437_4

[97] Xue W, Chen S, Yin H, *et al.* CRISPR-mediated direct mutation of cancer genes in the mouse liver. Nature 2014; 514(7522): 380-4. http://dx.doi.org/10.1038/nature13589 PMID: 25119044