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Superantigens, superantigen-like proteins and superantigen derivatives for cancer treatment

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Abstract. – OBJECTIVE: Bacterial superantigens (SAgs) are proteins produced by few types of bacteria that have been linked to several human diseases. Due to their potent *in vitro* and *in vivo* tumoricidal effects, they are extensively investigated for oncological applications either alone or in combination with classical anticancer drugs. However, the intrinsic toxicity of natural SAgs stimulated the development of more effective and less toxic SAg-based immunotherapy. This review summarizes our current knowledge on SAg-based immunotherapy including SAg-like proteins and SAg derivatives, as well as their potential alone or with other therapeutic modalities including chemotherapy and radiotherapy.

Key Words:
Superantigen, Superantigen derivative, Superantigen-like, Cancer, Combination therapy

Introduction

Bacterial Superantigens (SAgs), also commonly known as erythrogenic toxins or streptococcal pyrogenic exotoxins[1], are the most potent types of T cell mitogens. SAgs are produced by only a few bacterial pathogens, including *Staphylococcus aureus*[2]. The majority are produced by the Gram-positive organisms *Staphylococcus aureus* and *Streptococcus pyogenes*[3,4]. Bacterial SAgs at a very low concentration (typically < 0.1 pg/ml) are sufficient to stimulate the T lymphocytes[5]. *In vitro* femtomolar concentrations of SAgs are able to stimulate profound proliferation and cytokine production in up to 20% of all peripheral T cells[6]. It was initially thought that the powerful immune response generated by SAgs was due to its binding to Major Histocompatibility Complex class II molecules on antigen-presenting cells and T cell receptors on T cells. Later, it was found that the staphylococcal enterotoxin B (SEB) SAg, also binds the co-stimulatory molecule CD28 suggesting a more complex mechanism of immune response[6]. Indeed, the current view is that SAgs bind to multiple coreceptors forming a costimulatory axis between coreceptors critical for T-cell activation[7]. CD28 is a homodimer expressed constitutively on T cells that interacts with its B7 coligands expressed on antigen-presenting cells, transducing the signal essential for T cell activation. The staphylococcal superantigen-like protein 1 (SSL1) specifically binds to human extracellular signal-regulated kinase 2 (hERK2), an important stress-activated kinase in mitogen-activated protein kinase signaling pathways[8]. It is now clearer that SAgs induce the release of cytokines and chemokines through multiple pathways as it was recently observed in *in vitro* experiments[9].

SAgs can cause severe poisoning and several serious human diseases. For instance, *Staphylococcus aureus* enterotoxins have potent superantigenic activity associated with frequent food poisoning outbreaks[10], toxic shock syndrome[11,12], pneumonia[13], Kawasaki disease[14-17], nasopharyngeal infections[18], atopic dermatitis (AD), and chronic rhinosinusitis (CRS) and sepsis-related infections.

SAgs can induce the production of cytokines leading to the hypothesis that viral proteins with a possible superantigen activity may be responsible for the systemic shock, acute respiratory syndrome, multiorgan failure and consequently death observed in patients with COVID-19[19]. A recent finding from *in silico* studies suggests that small insertions unique to SARS-CoV-2 (SARS-CoV-2 Spike) can display SAg activity and support this hypothesis[20]. It is relatively well established that at least one of the mechanisms by which SAgs contribute to systemic multiorgan failure is the production of a cytokine storm by enhancing the B7-2/CD28 costimulatory receptor interaction[21,22]. The formation of the B7-2/CD28 costimulatory axis is critical for full T-cell activation[21].

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Picomolar amounts of staphylococcal enterotoxin A (SEA) SAg rapidly induced cytotoxic activity against K562 and Raji cells, as well as some natural-killer (NK)-resistant tumour cell lines[23] which could be of value in therapeutic applications. Therefore, despite the serious toxic and adverse effects, SAgs are actively investigated as therapeutic tools for cancer treatment. The aim of this article is to provide a concise review of the potential application of SAgs, SAg-like proteins and SAg derivatives for cancer treatment.

SAgs for Cancer Treatment

For their ability to potentially activate T lymphocytes, SAgs have been used clinically as an immunomodifier in the treatment of tumors. The SAg Staphylococcal enterotoxin A (SEA) when co-cultured with human peripheral blood mononuclear cells (PBMCs) inhibited the proliferation and induced the death of human lung carcinoma A549 cells[24]. The Staphylococcal enterotoxin B (SEB) is an efficient activator of the antitumor immune response that leads to the eradication of tumor growth and inhibition of metastasis[25]. SEB-Superantigen-activated PBMC (peripheral blood mononuclear cells) significantly induced apoptosis in transitional cell carcinoma cells (TCC)[26]. The Staphylococcal enterotoxin C2 (SEC2) is another classical SAg with potent antitumor activity by activating T lymphocytes at very low dosage but of limited use due to toxic effects[27]. Natural superantigens have been used for cancer immunotherapy but at the cost of severe side effects due to their ability to induce high systemic levels of a large panel of inflammatory cytokines that may lead to a toxic shock syndrome and therefore attempts to translate the *in vitro* antitumor effect into clinical trials have been limited. To increase the effect of the SAgs, the concept of tumor-targeted SAgs (TTS) was established with the aim of recruiting a large number of T cells (Figure 1). In addition, because of the differential species susceptibilities, predicting the toxicity of SAgs in humans is difficult. For instance, mice are not valid human disease models because they are significantly less sensitive to toxic shock induced by bacterial SAgs when compared with primates and rabbits[28]. These limitations triggered the evaluation of other SAg alternatives such as SAg-like proteins and derivatives.

SAg-Like Proteins

The common structure and function of the SAgs are also shared by another group of staphylococcal virulence factors called the superantigen-like proteins (SAg-like proteins)[4]. Staphylococcal enterotoxin-like toxins (SEls) are proteins with similar amino acid sequences to those of classical SAgs but they exhibit low or no emetic

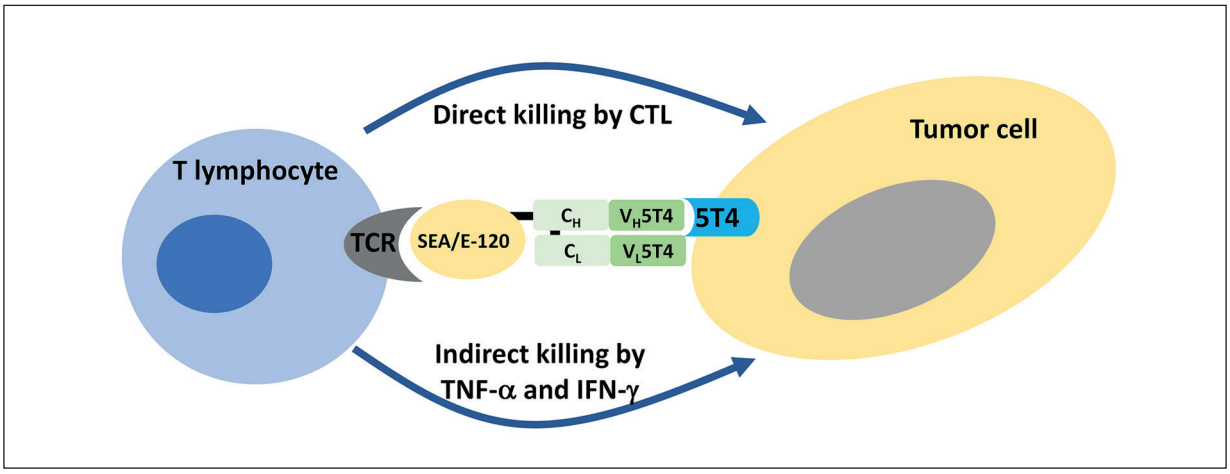

Figure 1. Potential immunotherapy using Tumor-Targeted Superantigen (SAg) (Modified from[61]). A SAg or SAg-based ligand can be linked to a tumor-specific antibody or ligand. The tumor-specific antibody/ligand binds to the tumor antigen whereas the superantigen/ligand crosslink between the major histocompatibility complex class II molecule (MHC-II) and the T cell receptor (TCR) induces T-cell hyperactivation of a T lymphocyte (shown in this figure) and monocytes/macrophages and results in the release of huge amounts of cytokines and chemokines, such as tumor necrosis factor α (TNF-α), interleukin 1 (IL-1), IL-2, interferon γ (IFN-γ). and many others. This leads to T-cell dependent tumor killing likely by apoptosis. APC = Antigen Presenting cell.

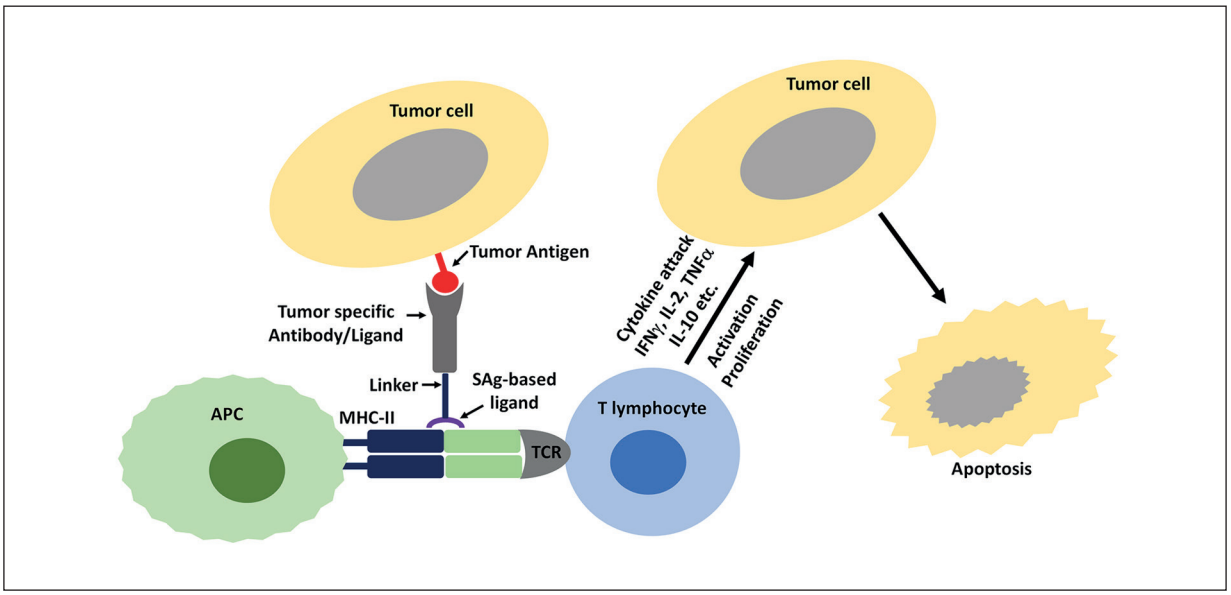

Figure 2. Potential mechanism of action of ABR-217620 (Naptumomab estafenatox; 5T4Fab-SEA/E-120). Modified from[55]. T lymphocyte activation occurs through its T-cell receptor (TCR) upon binding of the fusion protein ABR-217620 to the 5T4 tumor-associated antigen. The T cell kills tumoral cells directly by its cytotoxic T lymphocyte (CTL) activity and indirectly by producing cytokines (tumor necrosis factor [TNFa] – and interferon [IFNg).

activity and have anticancer effects by activating lymphocytes particularly $CD4^+$ and $CD8^+$ T cells[29]. Treatment with a low concentration of Staphylococcal enterotoxin-like Q (SElQ) (30 µg/mouse) could inhibit the growth of tumors by approximately 30% without significant toxicity[29]. Staphylococcal SAg-like protein 6 (SSL6) inhibited CD47 and promoted Sorafenib-induced apoptosis of hepatocellular carcinoma cells Huh-7 and MHCC97H[30] that are widely used as liver cancer models. Other staphylococcal SAg-like protein such as staphylococcal SAg-like protein 7 (SSL7) have been shown to inhibit the formation of the complement membrane attack complex (MAC) and can be useful for treating complement-mediated hemolysis[31]. Recombinant *staphylococcus aureus* SAg-like protein 7 -that inhibits the complement- decreased the rate of tumor growth in a transplantable murine colon cancer model[32]. Humanized single-domain antibody (sdAb) mimicking the C-terminal domain of SSL7 may also be efficient in inhibiting complement-mediated hemolysis of erythrocytes from patients with paroxysmal nocturnal hemoglobinuria[33]. Staphylococcal SAg-like 10 was found to bind the chemokine receptor CXCR4 expressed on human T acute lymphoblastic leukemia, lymphoma, and cervical carcinoma cell lines inhibiting CXCL12-induced cell migration[34].

SAg Derivatives

SAg derivatives have been created to reduce systemic toxicity while maintaining profound antitumor effects. SAg side-effects may be caused by nonspecific binding to class II positive cells. To overcome this, several authors created mutated staphylococcal enterotoxin A (SEA). In particular, Hansson et al[35] created a tumor-reactive SAg by engineering a fusion protein composed of a tumor-reactive mAb (C215Fab) and the bacterial SAg staphylococcal enterotoxin A (SEA) (SEA), a well-known SAg. They introduced a point mutation (D227A) at the major MHC class II binding site. The Fab–SEA D227A fusion protein showed profound antitumor effects with a markedly reduced toxicity as compared with the wild-type Fab–SEA fusion protein. Kodama et al[36], generated mutated SEA by changing Asp at position 227 of native SEA to Ala (mSEA-D227A), which has reduced affinity to MHC class II molecules, but retains the potential for T cell activation. mSEA-D227A was 500 times more tolerated compared to native SEA and had enhanced antitumor activity when conjugated to anti-MUC1 mAb.

SEA, when genetically conjugated to epidermal growth factor inhibited the proliferation of human nasopharyngeal carcinoma cell line CNE2 by promoting the proliferation of human peripheral blood mononuclear cells and

enhancing the secretion of several cytokines -such as interferon-γ, tumor necrosis factor-α, and interleukin-2 -that have broad antitumor activities[37]. Highly agglutinative staphylococcin (HAS) SAg, a derivative from *Staphylococcus aureus*, can inhibit and kill a variety of tumors cells as reviewed by Tian et al[38]. His-tagged SEC2 (SEC2-His) a derivative of the Staphylococcal enterotoxin C2 (SEC2) inhibited the growth of human colon adenocarcinoma (Caco-2) cells *in vivo*[27]. TGFαL3-SEB is another derivative created by fusing the third loop of transforming growth factor α (TGFαL3) to staphylococcal enterotoxin type B (SEB) that significantly increased the tumor volume in mice bearing breast cancer without systemic toxicity[39,40]. Bio-products made up of proteins produced by *Staphylococcus aureus* potently inhibit tumour growth in a murine model of mesothelioma[41]. The $SpeC_{D203A}$-based TTS fusion protein, an engineered human scFv that specifically targets human 5T4 (scFv5T4), was able to control the growth and spread of large tumors in an *in vivo* humanized mouse model of colon cancer[42]. The Mutant Staphylococcal enterotoxin C2 with lower toxic activity, named 2M-118 (H118A/T20L/G22E) engineered by site-directed mutagenesis effectively inhibited the growth of S180 sarcoma with acceptable toxicity in the BALB/c mice[43]. The iRGD peptide fused superantigen mutant exhibited enhanced anti-solid tumor characteristics and induced improved lymphocyte infiltration in mouse B16F10 melanoma cells[44]. SAg derivatives for cancer immunotherapy can be also created by splitting into fragments, individually inactive, until both fragments came into close proximity and reassembled into a biologically active form capable of activating T cell response[45].

SAg-Based Treatments in Clinical Trials

Few SAg-based therapies have been tested in clinical trials for cancer. The Fab-SEA fusion protein (PNU-214565) made by fusing the superantigen staphylococcal enterotoxin A (SEA) with the Fab fragment of the monoclonal antibody C242 recognizing human colorectal (CRC) and pancreatic carcinomas (PC) was tested in patients with the aim to determine the maximum tolerated dose and it was found to be safe[46,47]. Similar results were reported in a cohort of patients with advanced gastrointestinal malignancies[48]. A different SEA-based fusion protein, PNU-214936, was evaluated in patients with advanced non-small-cell lung cancer to establish the maximum tolerated dose sing a Bayesian model of Escalation with Overdose Control (EWOC)[49]. Intratumoural injection of superantigen staphylococcal enterotoxin C (SEC) in patients with hepatocellular carcinoma (HCC) after percutaneous microwave ablation (PMWA) was found to be safe and to achieve longer overall survival as well as disease free survival[50]. ABR-214936, a fusion of a Fab recognizing the antigen 5T4, and Staphylococcal enterotoxin was tested in renal cell carcinoma patients. Patients who received higher drug exposure had greater disease control and lived almost twice as long as expected[51]. ABR-217620 (Naptumomab estafenatox; 5T4Fab-SEA/E-120) is an engineered antibody-superantigen fusion protein[52] that induces T-cell mediated killing of tumor cells at concentrations around 10 pM with low toxicity and reduced antigenicity[53]. Naptumomab estafenatox was created by fusing a superantigen (SAg) to the Fab moiety of a tumor-reactive monoclonal antibody. Naptumomab estafenatox targets a 72-kDa oncofetal trophoblast protein (the 5T4 tumor antigen) expressed on many carcinomas, including renal cell carcinoma and has shown anticancer activity in these cells[54]. Fab targeting of ABR-217620 to tumor cells where the SAg portion of the fusion protein elicits a potent tumoricidal cytotoxic T response and production of cytokines (TNF-alpha and IFN-gamma) are the main proposed mechanism of action[55]. At present there are 4 registered clinical trials (www.clinicaltrials.gov: NCT00420888, NCT03983954, NCT00056537, NCT00132379) evaluating the anticancer effects of Naptumomab estafenatox in human cancers. Despite the drug was well tolerated[55] in phase I studies, a phase II/III study to determine the efficacy of naptumomab estafenatox (Nap) + IFNα *vs.* IFN in metastatic renal cell carcinoma (RCC) did not meet its primary endpoint[56].

Despite the scarcity of published clinical trials on SAg-based treatment in cancer patients, this therapeutic modality seems to be safe, well tolerated and more importantly they showed promising antitumor effects as reported in Phase I and II clinical trials.

Combination Therapy

Tumors are complex and genetically and phenotypically heterogeneous and some cancers may not be treatable with just one strategy. A combination of traditional anticancer drugs with SAg-based therapies can help to overcome the drug resistance, lessen the symptoms and improve

overall survival. Thus, effectiveness of SAgs, SAg-like proteins and SAg derivatives can be increased by combination therapy with traditional FDA-approved anticancer drugs that have been proven to give superior results when used in combination[57]. Additionally, this combination therapy can be added to tumors that are routinely treated with radiotherapy such as gliomas[58] and esophageal cancer[59]. Table I shows examples of combinations therapy using SAg-based therapies with traditional chemotherapy +/- radiotherapy.

Conclusions

Although SAgs have been associated with several diseases and serious adverse effects, they are extensively investigated for oncological applications either alone or in combination with classical anticancer drugs. The potent antitumor activity of natural SAgs is limited by their high intrinsic toxicity. However, a plethora of SAg-like proteins and derivatives with lower toxicity are already available and new ones are under development.

Table I. Combination therapy using SAg-based therapy.

SAg	Adjuvant	Cell type/tumor model	Effect	Ref.
SAg	Doxorubicin	Urothelial urinary bladder cancer	Iincreased CD4+ T cell activation	62
Tumor-targeted superantigen	Tasquinimod	Melanoma cells	Increased Tumor-specific CD8(+)	63
Microbiota-derived Staphylococcal superantigen-like protein 6 (SSL6)	Sorafenib (SFN)	Hepatocellular Carcinoma	Enhances SFN sensitivity	30
Staphylococcal enterotoxin-B	Chimeric antigen receptor (CAR) T cell	Mice bearing established E0771-Her2 tumors	Tumor-growth inhibition	64
Superantigen staphylococcal enterotoxin B	Ipilimumab and Nivolumab	Mouse MC38 and CT26 colorectal tumor models	Increased anti-Tumor activity	65
Staphylococcal enterotoxin C (SEC)	Surgery + Chemotherapy + Radiotherapy	Glioma patients	Increased "effective rate"	58
ABR-217620	Docetaxel	Patients with non-small-cell lung cancer (NSCLC), pancreatic cancer (PC), and renal cell cancer (RCC)	Evidence of immunological and antitumor activity.	55
Staphylococcal enterotoxin A (SEA) in fusion with an anti-tumor Fab-fragment	Docetaxel	B16-C215 tumors growing in the lung of C57Bl/6 mice	Prolonged long term survival	66
C215Fab-SEA fusion protein	Linomide	Syngenic B16 melanoma cells transfected with GA733-2 (a human colon cancer cell surface antigen)	> 99% reduction of liver metastasis in C57/Bl6 mice.	67
Staphylococcal enterotoxin A (SEA) in fusion with an anti-tumor Fab-fragment	Interferon alpha	B16-C215 tumors growing in the lung of C57Bl/6 mice	Synergistic anti-tumor effects, prolonged survival	68
Staphylococcal enterotoxin-A (SEA),	Staphylococcal protein-A (PA)	Animals carrying the Ehrlich ascites tumor.	Long-term survival as compared with PA or SEA alone	69
Fab-SEA	Fab-IL-2	B16 melanoma	Cure in 90% of tumo -bearing animals	70

Another approach to decrease the toxicity of SAg-based immunotherapy is to create SAg variants with less vasodilation effect but more tolerable for cancer immunotherapy as has been reported by Bashraheel et al[60]. In summary, the potent antitumor activity of naturally occurring SAgs can be exploited by identifying and developing less toxic SAg-like proteins and SAg derivatives and especially by using them in combination therapy. At present the effectiveness of this approach has been tested in few clinical trials, mostly limited to phase I and phase II studies with encouraging results in terms of safety and antitumor activity that warrants further evaluation in larger phase III studies.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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