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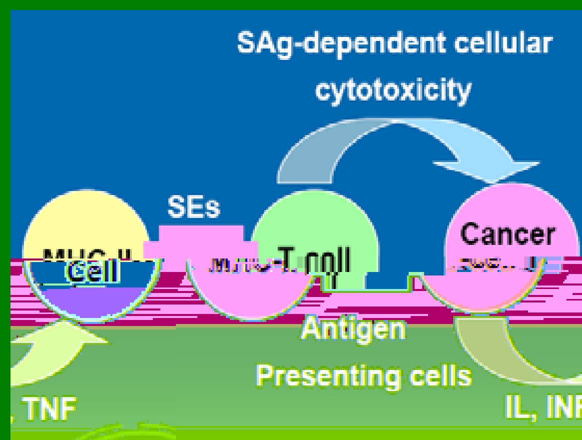

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Research advances on immunopharmacology and cancer therapy of Staphylococcal enterotoxins

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Abstract At present, we knew that staphylococcal enterotoxin (SE) has 14 serotypes (SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, SEJ, SEK, SEL,SEM, SEN and SEO), among them SEC has three subtype SEC1, SEC2, SEC3. In recent years, SEP, SEQ, SER and SEU were found. SEs are powerful superantigens that stimulate non-specific T-cell proliferation produced by *Staphylococcus aureus* and draw considerable attention as ideal drugs for cancer therapy. The study of SEs is concerned by the whole world all the time. The relative research literatures are more than 4500 papers which were searched from Medline database. The clinical results can be showed that the biotech-product is value to research and develop based on clinical studies in more than 3000 cancer patients in China. In this review, we summarized the recent advances on immunopharmacology and cancer therapy of SEs. A number of reports suggested several regions of the SEs important for superantigen activity. Deletions in the N- and C-terminal portions of SEA, SEB, and SEC result in loss of T-cell stimulatory activity and decreased resistance to proteases. Over the past few decades, several studies have been conducted on the nature of SEs, and the molecular basis of the superantigen activities of SEs has been extensively studied. The research for tumor is to improve the drugability of SEs. Many researchers carried on the recombination research to SEA, SEB, and SEC, in order to get new type SE product, they also got some progress on the aspect of keeping its anti cancer ability and decreasing the side effect of alimentary canal. The past study summarized and stated the anti-cancer mechanism of superantigen as the following 4 aspects: (1) cancer cell apoptosis mechanism, (2) Cancer cell solution mechanism, (3) Clearing mechanism, and (4) Mechanism of preventing metastasis. Through many years in clinical application, SEC injection, (highly agglutinative staphylococcin, HAS) showed its six kinds of clinical pharmacology effects: (1) activate T cells and make

described.[2] More than a decade of research on SAgs has revealed fascinating facets of its structure, properties and biological effects. At present, we knew that SE has 14 serotypes (SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, SEJ, SEK, SEL, SEM, SEN and SEO), among them SEC has three subtype SEC1, SEC2, SEC3. In recent years, SEP, SEQ, SER and SEU were found.

SEs are powerful SAgs that stimulate non-specific T-cell proliferation produced by *Staphylococcus aureus* and draw considerable attention as ideal drugs for cancer therapy. The filtrate of *S. aureus* culture has been used as new drug of Staphylococcal enterotoxin C (SEC) injection, named highly agglutinative staphylococcin (HAS) in clinic for 10 years in China and proved to be effective.[3] The study of SEs is concerned by the whole world all the time. The relative research literatures are more than 4500 papers in Medline database. The clinical results can be showed that the biotech-product is value to research and develop based on clinical studies in more than 3000 cancer patients in China. In this review, we summarized the recent advances on immunopharmacological and cancer therapeutics of SEs.

Molecular structure characteristics

The staphylococcal and streptococcal toxins with SAg-like properties are 23- to 29-kilodalton (kd) proteins. Staphylococcal enterotoxins (SEs) and a group of related proteins made by Streptococci cause food poisoning and shock in man and animals. These proteins share an ability to bind to human and mouse major histocompatibility complex proteins. The complex ligand so formed has specificity for a particular part of T-cell receptors, V , and by engaging V can stimulate many T cells. It is likely that some or all of the pathological effects of these toxins are caused by their ability to activate so many T cells quickly.

The SEs are intermediately sized proteins. The sequences of some of these products were established by analyses of the proteins. The complete primary amino acid sequences of the SEs and related proteins are shown aligned, with the exception of the sequences of the exfoliating toxins, which are shown aligned with each other, but not with the remaining toxins. The exfoliating toxin sequences are shown here for completeness, and because these toxins have properties related to those of the others (Fig 1).

Toxins shown are as follows: SEA to SEE, *S. aureus*. A to E; SPE A and C, Streptococcus pyogenes toxins A and C; TSST1, *S. aureus* toxic shock-associated toxin; ETA and ETB, *S. aureus* exfoliating toxins A and B. Data are from (9-17). Residues that are identical or that have changed to an anmino acid with similar properties among at least two of the following: SEA, SEE, and SED, are highlighted in pink. Residues that are identical or that have changed to an amino acid with similar properties among at least two of the following: SEB, SECI, and SEC3, are highlighted in blue. Residues that are identical, or that have changed to an amino acid with similar properties among at least two of SEA, SEE, and SED and at least two of SEB, SEC1, and SEC2, are highlighted in yellow.

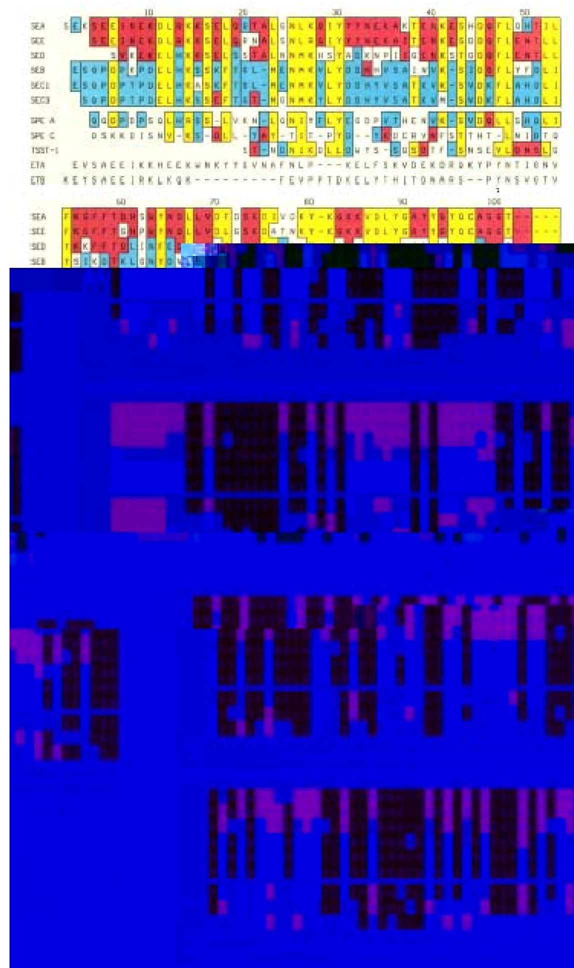


Fig 1. Comparison of the primary sequences of the SEs and their relatives. (from Science, 1990; 248:1066)
The correct figures of "The staphylococcal enterotoxins and their relatives" by Marrack P and Kappler J. Science, 1990; 248: 705

Studies of the SEs and related proteins have provided scientists with rich and unexpected vision of the complex relationships between bacteria and their hosts, and have also yielded some insight into what might have been expected to be a totally unrelated subject, namely the T cell repertoire.

SAgs include a class of certain bacterial and viral proteins exhibiting highly potent lymphocyte-transforming (mitogenic) activity towards human and or other

mammalian T lymphocytes. Unlike conventional antigens, SAgs bind to certain regions of major histocompatibility complex (MHC) class II molecules of antigen-presenting cells (APCs) outside the classical antigen-binding groove and concomitantly bind in their native form to T cells at specific motifs of the variable region of the   chain (V) of the T-cell receptor (TCR). This interaction triggers the activation (proliferation) of the targeted T lymphocytes and leads to the in vivo and in vitro release of high amounts of various cytokines and other effectors by immune cells. Each SAg interacts specifically with a characteristic set of V   motifs. The repertoire of the SAgs comprise 24 and 8 proteins, respectively. The staphylococcal SAgs include (1) the classical enterotoxins A, B, C (and antigenic variants), D, E, and the recently discovered enterotoxins G to U, (2) toxic shock syndrome toxin-1(TSST-1), (3) exfoliatins A and B. The streptococcal SAgs include the classical pyrogenic exotoxins A and C and the newly identified pyrogenic toxins, G, H, I, J, SMEZ, and SSA. The structural and genomic aspects of these toxins and their molecular relatedness are described as well as the available 3-D crystal structure of some of them and that of certain of their complexes with MHC class II molecules and the TCR, respectively.[4,5]

The classical staphylococcal and streptococcal toxins can be categorized into three distinct amino acid–sequence homology groups.[6] The staphylococcal enterotoxin serotypes SEA, SED, and SEE are closely related. Staphylococcal enterotoxin serotypes SEB, SEC1, SEC2, SEC3, and the streptococcal pyrogenic exotoxins A and C (SPE-A and C) form the second homology group. The third homology group comprises TSST-1 and SPEB, which share key amino acid residues with the other toxins but exhibit only weak sequence homology overall. However, data from X-ray crystallography of SEB2 and TSST-1 indicate that these representatives of the two groups have considerable similarities in their three-dimensional structures. The pyrogenic toxins bind to MHC class II molecules and this complex, in turn, stimulates T cells. In contrast, MHC-independent binding induces T-cell anergy. It is likely that all pyrogenic toxins share a common mode for binding MHC class II molecules, with additional stabilizing interactions that are unique to each toxin. A second, zinc-dependent molecular binding mode for SEA and SEE increases T-cell signaling and may account for the greater toxicities of these toxins. In conventional antigenspecific responses, the cluster of differentiation 4 (CD4) molecule stabilizes interactions between T-cell antigen receptors and MHC class II molecules on antigen presenting cells. The pyrogenic toxins may mimic CD4 binding and, by so doing, stimulate large numbers of T cells in a manner independent of antigen recognition. In addition, each superantigen stimulates T cells bearing characteristically distinct variable domain- (V) subsets of antigen receptors. It is thought that a massive release of cytokines (such as interferon- (IFN-), interleukin-6 (IL-6), and tumor necrosis factor– (TNF-) is responsible for the systemic effects of the toxins. In contrast, the gastrointestinal illness especially prominent after ingestion of SEs is associated with histamine and leukotriene released from mast cells.

Mutant and recombinant Staphylococcal enterotoxins

A number of reports suggest several regions of the SEs important for superantigen activity. Deletions in the N- and C-terminal portions of SEA, SEB, and SEC result in loss of T-cell stimulatory activity and decreased resistance to proteases. SEA/SEE hybrid proteins indicate that specific C-terminal residues influence V   specificity. A chimeric fusion of SEB residues 1 to 138 to protein A induces T-cell proliferation. Synthetic peptides corresponding to N-terminal and central regions of SEA and SEB inhibit SE-induced T-cell proliferation and binding to MHC class II-bearing target cells. Two synthetic peptides corresponding to central regions of SEC1 stimulate human T cells. Random mutagenesis of *seb* demonstrates that three N-terminal regions of SEB are important for induction of T-cell stimulation.[7]

In 1993, Harris et al identified residues 25, 47, and 48 of SEA as being important for SEA's emetic and T-cell stimulatory activities.[8] They reported the construction of two altered *sea* genes*: sea-1351* and *sea-1364* by oligonucleotide-directed mutagenesis. *These two* altered *sea* genes contained a number of mutations designed to incorporate unique restriction enzyme sites into *sea* in order to facilitate construction of additional *sea* mutations (Fig. 2). In 1995, Harris and Betley carried out the mutant study at these positions and at additional residues which are either completely or highly conserved among the SEs.[7] For the construction of some of the mutant SEAs, they used two variants of *sea-1364*, *sea-1379* and *sea-1380.sea-1379* was constructed by performing site-directed mutagenesis on the same altered *sea* originally used to create *sea-1364*, using the mutagenic oligonucleotide 59-gaaaaagat Ctgcgaaaaaagtc-39 (*Bgl*II:99). *sea-1380* was constructed by performing an additional round of site-directed mutagenesis on *sea-1364*, using the mutagenic oligonucleotide 59-gagagtcaTgatcaatttctg-39 (*Bsp*HI:198). The mutants of SEA were examined for emetic activity in rhesus monkeys and for the ability to induce proliferation and cytokine production in murine splenocytes. The mutant SEAs defective in T-cell activation were tested for the ability to compete with biotinylated SEA for binding to the human

MHC class II-bearing cell line Raji to see if their defects were a consequence of impaired interactions with MHC class II molecules. Mutant SEAs with substitutions at 25, 47, or 48 all had decreased T-cell stimulatory activity, with the mutants at position 47 being the most defective. Results of a competition assay for binding to the major histocompatibility complex (MHC) class II-expressing cell line Raji suggested that the decreased superantigen activities of the mutants with substitutions at positions 47 and 48 are due to poor interactions with MHC class II molecules, whereas the defects of the mutants at position 25 are a consequence of faulty interactions with T-cell receptors. To further investigate the possibility that the mutant SEAs had altered conformations, each mutant SEA was tested for susceptibility to degradation by monkey stomach lavage fluid. With respect to emetic activity in rhesus monkeys, the mutants at position 25 or 48 exhibited decreased but significant activity. Interestingly, the two mutants at position 47 had different emetic activities; SEA-F47G was nonemetic when administered intragastrically at 500 mg per animal, whereas SEA-F47S was emetic at this dosage. Since the mutants at position 47 were equally defective for superantigen activity, this further supports their suggestion of an incomplete correlation between SEA's emetic and superantigen activities.

Residues from both the N- and C-terminal portions of SEB are involved in the TCR and MHC class II binding sites.[9] The crystal structure of SEB complexed to HLA-DR1 further elucidates how SEB functions as a superantigen. No major conformational changes occur in either MHC class II or SEB upon complex formation. A model involving unconventional contacts between MHC class II and TCR explains ternary complex formation.[10]

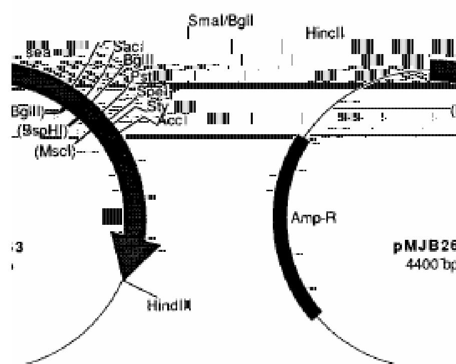


Fig 2. Map of pMJB263 (encoding *sea-1364*) indicating the unique restriction enzyme sites used to construct the mutant SEAs in this study. The restriction enzyme sites in parentheses indicate the approximate position of the restriction enzyme sites unique to *sea-1379*, *sea-1380*, and *sea-1351*. *sea-1379* lacks the *Bgl*II site contained on *sea-1364*.

The filtrate of *S. aureus* culture has been used as a novel injection for cancer therapy. The superantigen SEC has been claimed to be the only active component without certifiable evidences. For further investigations of the active components of this injection and establishment of foundations for the development of novel anti-cancer drugs, total DNA of *S. aureus* (FRI 1230) has been extracted for cloning, expressing and purifying recombinant proteins of SEM and SEN (r-SEM and r-SEN). The MTT assay of the purified r-SEM and r-SEN demonstrated that their abilities of stimulating T cells and inhibiting the proliferation of K562-ADM cells and B16 cells were equivalent to that of purified SEC2 *in vitro*. These findings suggested that SEC was not the only active component of SEC injection and the effective procedure of expression and purification may be useful for mass productions of these therapeutically important proteins.[3]

Over the past few decades, several studies have been conducted on the nature of SEs and the molecular basis of the SAg activities of SEs has been extensively studied. The research for tumor is to improve the drugability of SEs, many researchers carried on the recombination research to SEA, SEB, SEC, in order to get new type SE product, they also got some progress on the aspect of keeping its anti cancer ability and decreasing the side effect of alimentary canal. However, little is known about the mechanisms of the emetic activity of SEs. SEA, SEB, SEC2, SED, SEE, SEG, SEH, and SEI were expressed as recombinant SEs by the *Escherichia coli* expression system.

The SEA, SEB, SEC2, SED, and SEE genes were amplified by PCR from the type strains of each SE and cloned to the glutathione S-transferase fusion expression vector pGEX-6P-1. The animals were observed for emesis for 3 h after the intraperitoneal administration of SEs. The number and times of vomiting, the time to the first vomiting episode, and any behavioral changes were recorded. The 50% emetic dose (ED_{50}) was determined by the method of Reed and Muench as modified by

Matsumoto. After intraperitoneal administration, all of the SEs caused vomiting responses in the house musk shrews (Table 2).

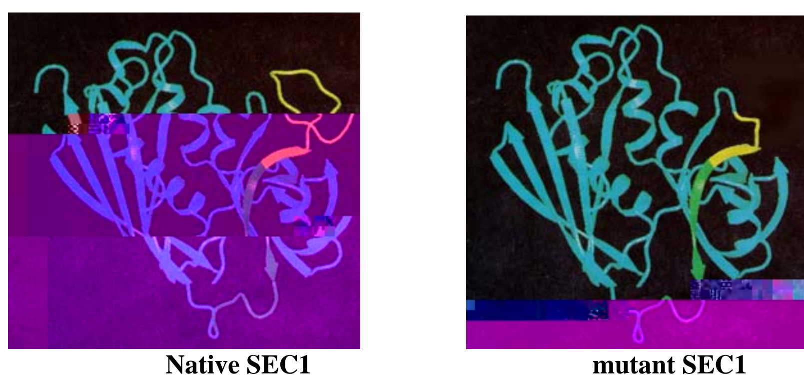
Table 1. Emetic activity of SEA and the mutant SEAs in rhesus monkeys

Sample	Resilt[a] for dose admiistered Intragastrically (μg of Toxin per animal	
	100	500
SEA	2/3	NT
SEA-L12G[b]	1/3	NT
SEA-K14G	2/3	NT
SEA-S16G	3/3	NT
SEA-N25G	0/3	1/3
SEA-N25A	0/3	2/3
SEA-K27A	2/3	NT
SEA-D45G	2/3	NT
SEA-Q46G	2/3	NT
SEA-F47G	0/3	0/6
SEA—F47S	0/3	3/3
SEA-L48G	1/6	3/3
SEA-L48A	0/3	2/3
SEA-T51A	2/3	NT

[a] Number of animals that experienced emesis/number of animals tested. NT, not tested. [b] L12G indicates that the leucine at position 12 has been replaced with a glycine residue.

All of the house musk shrews that were given doses of 1μg of SEA per animal retched and vomited. Vomiting was not induced by 0.1 μg of SEA per animal. The emetic activity of SEA in housemusk shrews was found to be dose dependent. The minimum emetic dose was 0.3μg per animal, and the ED_{50} of r-SEA for house musk shrews was estimated to be 0.4 μg per animal which was slightly higher than that of native SEA (0.21 μg/animal). This result suggested that recombinant SEs have almost the same biological activity as native SEs. With SEI, all house musk shrews vomited at a dose of 10 μg per animal. At a dose of 1 μg per animal, two out of six house musk shrews showed emetic responses. The ED_{50} of SEI for house musk shrews was 1.5 μg per animal, and the minimum emetic dose was 1 μg per animal. The vomiting induced by SEE was observed at a dose of 10 μg per animal. No house musk shrew vomited at a dose of 1 or 0.1 μg per animal. However, SEB, SED, SEC2, SEG, and SEH needed to be administered at relatively higher doses than did SEA, SEE, and SEI to elicit an emetic reaction in the house musk shrew. The minimum emetic doses of SEB and SED were 10 and 40μg per animal, respectively. The 100% emetic dose of SEB was 1,000 μg per animal; however, SED at a dose of 1,000μg per animal, did not induce vomiting in all animals tested. The minimum emetic doses of SEC2, SEG, and SEH were 1,000, 200, and 1,000 μg per animal, respectively. Moreover, SEH showed a relatively shorter latency period than did the other SEs.[11] Comparative studies using monkeys, house musk shrews, or ferrets on the emetic activity of SEs will lead to an understanding of the molecular basis of the emesis caused by SEs and study the drugablity of SEs.

Study on SEC1 mutant was cleared in the molecule difference of native and mutant (Fig 3). There is hope for to retain immunostimulation without toxicity or immunosuppression.



Native SEC1 **mutant SEC1**

Fig 3. Staphylococcal enterotoxin C1 (SEC1) mutant
Retain immunostimulation without toxicity or immunosuppression

In a paper, Xu *et al* cloned the entA gene encoding SEA from the genomic DNA of *S. aureus* (ATCC13565) by PCR, the sequence cloned was accordance with that reported in Genebank. The entA gene could be expressed effectively after inserted into plasmid pET-22b+, The r-SEA was expressed as inclusion bodies when induced by IPTG at 37 ℃ and became soluble after induced at low temperature, the soluble part is about 55% of total r-SEA products. Only one band was detected by western-blotting in expression product of BL-21 (DE3) with pET-SEA. The soluble r-SEA was purified by Ni^{++} chelating sepharose column. No other protein except r-SEA was seen in

SDS-PAGE gel stained by both Coomassie brilliant blue and silver salt, which showed that the r-SEA was purified effectively. Homology modeling of r-SEA determined the structure change was conducted, which indicated there was no apparent structure change between r-SEA and native SEA. This result was also confirmed by proliferation assay of PBMC, for the r-SEA could induced proliferation of PBMC as effectively as native SEA. The increasing anti-tumor activity of r-SEA was also detected after the spleen cell activated in vivo by r-SEA, which was accordance with others reports. This work paved the way for the further study of anti-cancer with r-SEA.[12]

Table 2. Emetic activities of SEs on house musk shrews after intraperitoneal administration

SE or Control	Dose of r-SE[a] μg/animal	No. of shrews Tested	Vomited	Latency period to first Emesis (min)[b]	No. of vomiting episodes[c]
SEA	1.0	5	5	95.4±27.1	2-7
	0.5	5	3	116±22.2	1-5
	0.3	6	1	113	1,2
	0.1	6	0		
SEI	10.0	5	5	113±23.7	1-6
	1.0	6	2	80,115	1,2
	0.1	6	0		
SEE	10.0	2	2	90,99	1,2
	1.0	2	0		
	0.1	2	0		
SEB	1000	3	3	110,90	1,2
	200	5	2	45,95	2,2
	40	6	3	77.3±34.4	1-2
	10	6	1	101	4
	1	6	0		
SED	1000	3	2	96,75	2,1
	200	2	1	48	1
	40	2	1	118	2
	10	3	9		
SEC2	1000	2	2	32,104	1,2
	200	3	0		
	40	4	0		
SEG	1000	3	1	104	2
	200	5	1	132	1
	40	5	0		
SEH	1000	2	2	`4,17	1,2
	200	2	0		
	40	3	0		
PBS		3	0		

r-SE, recombinant SE. [b] Values for three or more vomiting shrews are means and standard deviations, whereas individual values are given for one or two vomiting shrews. [c] Values for three or more vomiting shrews are given as ranges, whereas individual values are given for one or two vomiting shrews.

To study TCR V binding sites of SED. Six SED mutants were constructed by site-directed mutagenesis. The activity of promoting T-cell proliferation by the mutants was detected by ^{3}H-TdR incorporation. For the mutants with decreased mitogenic activity, flow cytometry was used detect their MHC-II binding activity and TCR V specificity. Residue N23 played an important role in the interaction of SED with human TCR V 5. Residue H26 was probably a SED binding site to human TCR V as except for TCR V 5, TCR V 8 and TCR V 12.1.Their study suggested that the residue N23 is a key TCR V binding site of SED.[13]

Immunopharmacological studies of Staphylococcal enterotoxins

SAgs tremendously activate T lymphocytes by recognizing the particular region on TCR V

has been well studied. In a study, researchers devoted the attention to evaluate SEC regarding its tumoricidal activity versus immunosuppression. The study proved with flow cytometry that SEC treatment on C57 mice resulted in a boost of the differentiation of T lymphocytes into CD4+, CD8+ subpopulations. In vitro, SEC causes increased IFN- release from human PBMC. Furthermore, in coculture SEC-treated human PBMC led to more death of cancer cell lines from a variety of origins. Systemic SEC treatment in mouse and rabbit models significantly decreases tumor growth. In tumor-bearing rabbits, tumor necrosis and strong infiltration of lymphocytes into tumor tissue were observed; the rabbits also benefit with less metastasic cancer cells in the lung. In the meantime, the induced cell immune responses, both T cell differentiation and PBMC IFN- release, declined as SEC concentration rose. Tumor growth data obtained from animal models are in accordance with the changes in immunity, in which tumor growth ceased to respond to high dosage SEC as it did to lower dosage. These observations on SEC investigation, particularly in aspect of dosage-related immunosuppression, are of significance to SEC therapeutic potential to cancer. Molecular mechanism underlying these findings warrants further intensive investigation.[14]

To engineer SAgs for cancer immunotherapy, SEA was genetically fused to the Fab region of the human colon carcinoma-reactive monoclonal antibody (mAb) C215. Fusion protein C215Fab-SEA can trigger cytotoxic T cells against C215 antigen positive tumor cells and induce tumor-suppressive cytokines. However, the antitumor effect of C215Fab-SEA is often not satisfactory because of T cell deletion after activation and failure to induce potent CTL activity after repeated administration. Lymphotactin (Lptn) is a potent chemoattractant for T cells and NK cells. To improve the therapeutic efficacy of fusion protein C215Fab-SEA researchers investigated the antitumor responses elicited by combination of C215Fab-SEA and adenovirus-mediated intratumoral Lptn gene transfer in the preestablished C215 antigen expressing B16 melanoma murine model. More significant inhibition of tumor growth and prolonged survival time were observed in tumor-bearing mice that received combined therapy of C215Fab-SEA and Ad-Lptn than those of mice treated with C215Fab-SEA or Ad-Lptn alone. The highest CTL activity of tumor-bearing mice was induced after combined therapy. Intratumoral coadministration of C215Fab-SEA and Ad-Lptn augmented splenic NK activity of tumor-bearing mice most markedly. The result demonstrated that the in vivo antitumor effect of C215Fab-SEA immunotherapy is potentiated significantly by combination with intratumoral Lptn gene transfer through more efficient induction of specific and nonspecific antitumor immune responses.[15]

A candidate B-cell SAg that has received considerable attention these days is staphylococcal protein-A (PA). It has been shown to possess multiple immunological responses. The anti-tumor property of PA is well documented in the literature in various transplantable tumors of rats and mice. Mondal *et al* found that the T-cell superantigen SEA and B-cell SAg PA induce immunomodulatory and anti-tumor activity which is strongly protentiated by PA + SEA co-administration. Combination treatment with PA and SEA prolongs the immune response in vivo, limits the development of immunological unresponsiveness and promotes maximum anti-tumor effects to tumor carrying animals, as compared with PA or SEA alone. The immune response after combined therapy is characterized by substantially augmented IFN- , TNF- , Nitric oxide and strong CTL activity. The result demonstrated that combined PA + SEA therapy induces long-term survival of the animals, carrying the Ehrlich ascites tumor.[16]

To investigate the induction of antitumor immune response by vaccination with interleukin-18 (IL-18) gene-modified, C215Fab-SEA-coated tumor cells. Immunization of the mice with the manipulated vaccine elicited protective immunity against the following tumor challenge of parental B16-C215 and wild-type B16 cells. Significant expansion of CD4+, CD8+ T cells was observed in the draining lymph node of the immunized mice when compared with that in unvaccinated mice. Higher CTL activity was induced in vaccinated mice than that in unvaccinated mice. Vaccination with IL-18 gene-modified, C215Fab-SEA-coated tumor cells elicited potent antitumor response through induction of tumor-specific immune response.[17]

Since transfection of established tumors with immunostimulatory genes, such as SAg, can elicit antitumor immunity, direct transfection of tumors with genes of SEA could probably set up a new way of immunological pathway. In a study, human hepatocellular carcinoma (HCC) cell lines stably transduced with SEA and B7.1/SEA fused genes, HHCCSEA and HHCCBS, were obtained by using the method of retroviral mediated gene transduction. The results showed that human HCC cells could express SEA gene

the reaction. Transduced HCC cells were also analyzed for HLA expression, and it was found that a majority of the cells expressed HLA-I molecules but no HLA-DR molecules. After blocking the HLA-I molecules by HLA-I mAb, the cytotoxicity of T lymphocytes dropped remarkably. The results suggested that SEA were mainly presented by HLA-I molecules, and that B7.1 and SEA could have synergistic action at the early stage of the reaction, but the relationship between them in the consequent process needs to be clarified.[18]

It is an important issues to investigate an efficient methods to induce antitumor effector T cells from peripheral blood lymphocytes of tumor patients for the development of a novel tumor immunotherapy. Nishimura *et al* established a large scale culture system of human CD4+ helper/killer T cells which have both helper and killer functions. Targeting of CD4+ helper/killer T cells to tumor using anti-CD3 x anti-c-erbB-2 mAb caused the lysis of tumor and triggering of IL-2 production. It was also demonstrated that culture of human CD4+ T cells with SEA or IL-12 caused a selective induction of Th1 type of CD4+ helper/killer T cells. IL-12 also revealed a novel effect on CD8+ CTL functions. Culture of CD8+ T cells with IL-12 resulted in the augmentation of IFN- production and cytotoxicity. Moreover, culture of tumor-infiltrating lymphocytes with IL-12 caused a marked enhancement of CD8+CTL against autologous tumor cells. These findings suggest that IL-12 will become a useful cytokine for the tumor immunotherapy. [19]

Kabi Pharmacia Therapeutics, Lund, Sweden, have recently demonstrated that the superantigen SEA conjugated to colon-carcinoma-reactive monoclonal antibodies (MAbs) directs T cells to lyse human colon-carcinoma cells, representing a potential novel tumor therapy. To further analyze the mechanism of antitumor effects of superantigen-activated T cells, Dohlsten *et al* compared the activity of free and MAb-conjugated SEA in a long term in vitro co-culture assay of human peripheral blood mononuclear cells (PBMC) and colon-carcinoma cell lines. Activation of resting T lymphocytes with SEA conjugated to the colon-carcinoma-reactive MAb C215 or free SEA resulted in strong inhibition of the growth of all studied colon-carcinoma cell lines. The growth of WiDr colon-carcinoma cells was unaffected by the presence of unactivated mononuclear cells, whereas addition of pM concentrations of SEA or C215-SEA conjugate completely suppressed tumor-cell growth. The suppressive effect was mediated by both CD4+ and CD8+ T cells and required the presence of MHC-Class II+ monocytes. The inhibition of tumor-cell growth was to a large extent mediated by soluble factors present in supernatants from SEA- or C215-SEA-activated mononuclear cells. Quantitation of cytokine mRNA in SEA-activated mononuclear cells by the reverse transcriptase-polymerase chain reaction (RT-PCR) revealed strong induction of mRNA encoding the cytokines IL-1 , IL-1 , IL-2, IL-6, TNF- , TNF- and IFN- . The use of cytokine-specific MAb demonstrated that IFN-gamma was of major importance for the tumor-growth-inhibitory activity in supernatants of SEA-activated lymphocytes. Addition of recombinant cytokines to WiDr colon-carcinoma cells showed that TNF- was able to act synergistically with IFN- to suppress tumor-cell growth. The local production of tumor-suppressive cytokines induced by MAb-targeted superantigens is likely to be of particular relevance for inhibition of the growth of tumor cells not expressing the targeted tumor-associated antigen.[20]

At Laboratory of Human Immunogenetics, Sloan-Kettering Institute for Cancer Research, New York, Goldbach-Mansky *et al* investigated the differential effect of the co-stimulatory receptor ligand molecules CD2/LFA-3, LFA-1/ICAM-1, and CD28/B7 on microbial superantigen mediated activation of CD4+ T cells. Highly purified CD4+ T cells, depleted of antigen presenting cells (APCs), do not proliferate in response to the superantigen, SEB. However, CD4+ T cells do respond to SEB in the presence of the LFA-3, ICAM-1, and B7 positive erythroleukemic cell line K562, murine L cells, human B7 transfected L cells or CD28 mAb. The kinetics of the different modes of activation are distinct. SEB induced proliferation is most efficient in the presence of autologous APCs with maximal proliferation at a log4 lower SEB concentration than when CD28 mAbs were used. SEB plus K562 activation peaks on day 7, while SEB plus CD28 mAb induced proliferative responses do not peak until day 9. Thus, superantigen mediated activation of CD4+ T cells requires co-stimulatory signals, among which CD28 has distinct and unique effects.[21]

Superantigens stimulate T cells bearing certain TCR -chain variable regions when bound to MHC II molecules. Wahlsten *et al* investigated whether the TSST1 could induce an antitumor immune response when anchored onto MHC II-negative tumor cells. Their approach was to facilitate association of TSST1 with cell membranes by fusing its coding region to the transmembrane region (TM) sequence of the proto-oncogene c-erb-B-2. TSST1-TM was expressed in bacteria with an N-terminal histidine tag and purified using nickel-agarose affinity chromatography. Purified TSST1-TM added to cultures of several different MHC II-negative tumor cells spontaneously associated with cell membranes, as detected by flow cytometry. Because superantigens can direct cell-mediated cytotoxicity against MHC II-positive cells, a TM fusion protein lacking the

TSST1 MHC II binding domain ($TSST_{88-194}$-TM) was also constructed. Tumor cells precoated with TSST1-TM or $TSST_{88-194}$-TM stimulated proliferation of human peripheral blood lymphocytes in vitro whereas uncoated tumor cells did not. Mice preimmunized with TSST1-TM- or $TSST_{88-194}$-TM-coated tumor cells mounted a systemic response that resulted in significant antitumor immunity as measured by regression of a parental tumor challenge. TSST1-TM and $TSST_{88-194}$-TM fusion proteins represent a useful new strategy for attaching SAgs or potentially other proteins onto tumor cell surfaces without genetic manipulation.[22]

Woetmann *et al* investigate SE-mediated interactions between nonmalignant T cells and malignant T-cell lines established from skin and blood of CTCL patients. The malignant CTCL cells express MHC class II molecules that are high-affinity receptors for SE. Although treatment with SE has no direct effect on the growth of the malignant CTCL cells, the SE-treated CTCL cells induce vigorous proliferation of the SE-responsive nonmalignant T cells. In turn, the nonmalignant T cells enhance proliferation of the malignant cells in an SE- and MHC class II-dependent manner. Furthermore, SE and, in addition, alloantigen presentation by malignant CTCL cells to irradiated nonmalignant CD4+ T-cell lines also enhance proliferation of the malignant cells. The growth-promoting effect depends on direct cell-cell contact and soluble factors such as interleukin-2. The study had demonstrated that SE triggers a bidirectional cross talk between nonmalignant T cells and malignant CTCL cells that promotes growth of the malignant cells. This represents a novel mechanism by which infections with SE-producing bacteria may contribute to pathogenesis of CTCL.[23]

In vivo, administration of intact superantigen in sufficient therapeutic amounts risks unwanted cytotoxicity against normal cells. Si *et al* used SEA fused with CD80 transmembrane region (SEAtm) driven by alpha-fetoprotein (AFP) enhancer/ promoter to reduce toxicity and to improve safety and efficiency in the application of SEA. The result demonstrated that SEAtm by adenovirus from the AFP enhancer/promoter (AdAFPSEA) could be expressed on the surface of AFP-producing cell line Hepa1-6 instead of non-AFP-producing cell lines. Hepa1-6 infected by recombinant adenovirus stimulated proliferation of splenocytes and activated CD4+ and CD8+ T cells in vitro. After AdAFPSEA was injected into the subcutaneously established hepatoma in vivo, the expression of SEA was detected in tumor tissues, which subsequently induced tumor-specific cytotoxic T cells in spleen. Moreover, hepatocellular carcinoma (HCC) xenografts were suppressed by treatment with AdAFPSEA and the survival time of treated mice was prolonged. These findings suggest that membrane-expressed SEA by adenovirus from AdAFPSEA can generate stronger local and systemic antitumor responses against HCC.[24]

To decrease toxicity of SEA to the normal MHC-II+ cells and to localize the immune response induced by SEA to the tumor site, some researchers genetically fused SEA with B7.1 transmembrane region (named as SEAtm) to make SEA express on the surface of tumor cells and tumor cells modified with SEAtm could induce efficient antitumor immunity *in vitro*. The tumor cell vaccines modified with multiple immune activators frequently elicited stronger antitumor immune responses than single-modified vaccines. Si *et al* modified the tumor cell vaccine with B7.1 and SEAtm to improve efficiency in the application of SEA. First, SEAtm gene was subcloned from recombinant plasmid pLXSNSEP by PCR and murine B7.1 gene was cloned from splenocytes derived from C57BL/6 mice by RT-PCR. Then, the eukaryotic co-expression vector of SEA and murine B7.1 gene was constructed and named as pcDNA-BIS. B16 cell lines stably expressing SEA and/or B7.1 were established by screening with G418 after transfection and inactivated for the preparation of tumor cell vaccines to treat mice bearing established B16 tumors. The results indicated that the dual-modified tumor cell vaccine B16/B7.1+SEAtm (B16-BIS) elicited significantly stronger antitumor immune responses in vivo when compared with the single-modified tumor cell vaccines B16/B7.1 (B16-B7.1) and B16/SEAtm (B16-SEAtm), and supported the feasibility and effectiveness of the dual-modified tumor cell vaccine with superantigen and co-stimulatory molecule.[25]

De Jonge *et al* have previously reported a bacterially produced anti-CD3 x antitumor bispecific single chain variable fragment of Ab fragment (BsscFv), which is capable of retargeting CTLs toward BCL1 tumor cells, exhibits antitumor activity in vitro. To further facilitate BsscFv production, the coding sequence was subcloned in a eukaryotic expression vector and introduced into Chinese hamster ovary cells for large-scale production. Furthermore, De Jonge *et al* determined the serum stability and the clearance rate from the circulation of BsscFv. Most important, they prove here the therapeutic value of BsscFv in the treatment of BCL1 lymphoma, a murine model for human non-Hodgkin's lymphoma. Tumor-bearing mice that were treated with rscFv in combination with SEB, human r-IL-2, or murine r-IL-12 showed long-term survival, whereas untreated mice all died. This is the first report of the successful *in vivo* use of BsscFv as an immunotherapeutic agent. Furthermore, long-term survival was the result of complete tumor removal and was not due to the induction of dormancy.[26]

In a article, Terman *et al* discussed the mechanisms by which SEs induce tumor killing and pleurodesis, and gave a review of the application of SE homolog and nucleic acid compositions as vaccines and for treatment of established tumors. In the same article, they also described the use of native SEs *ex vivo*-intratumorally and intravesicularly administered superantigens against established tumors.[27]

Immunodeficiency is a barrier to successful vaccination in individuals with cancer and chronic infection. Researchers performed a randomized phase 1-2 study in lymphopenic individuals after high-dose chemotherapy and autologous hematopoietic stem cell transplantation for myeloma. Combination immunotherapy consisting of a single early post-transplant infusion of *in vivo* vaccine-primed and *ex vivo* costimulated autologous T cells followed by post-transplant booster immunizations improved the severe immunodeficiency associated with high-dose chemotherapy and led to the induction of clinically relevant immunity in adults within a month after transplantation. Immune assays showed accelerated restoration of CD4 T cell numbers and function. Early T cell infusions also resulted in significantly improved T cell proliferation in response to antigens that were not contained in the vaccine, as assessed by responses to SEB and cytomegalovirus antigens. In the setting of lymphopenia, combined vaccine therapy and adoptive T-cell transfer fosters the development of enhanced memory T-cell responses.[28]

Since T-cell activation and T cell-derived cytokines play a role in the immune response associated with intravesical Bacillus Calmette-Guerin (BCG) application, Perabo *et al* initiated a study to explore the fundamental aspects of a potential new immunomodulatory therapy for superficial bladder cancer. Since Superantigen-induced cytotoxicity is mediated by apoptosis, the effects of SEB-SAg-activated PBMC (peripheral blood mononuclear cells) on bladder cancer cells were evaluated with regard to Fas/Fas-ligand-based interactions. Whether SEB can induce Fas-ligand expression on PBMC and the extent of cytokine secretion were examined by flow cytometry and specific ELISA. Whether the SEB-activated PBMC are able to induce apoptosis in transitional cell carcinoma cells (TCC) was evaluated in co-culture experiments. The result showed that SEB induced pronounced time- but not dose-dependent specific Fas-ligand expression on PBMC, lasting 1 h to 7 h after initiation of the experiment. Cleaved soluble Fas-ligand was detected in the culture supernatants 24 h after stimulation, but not earlier. Further, a strong time-dependent secretion of cytokines IL-2, IFN- and TNF- released from the SEB-stimulated PBMC was shown. Co-culture experiments demonstrated that SEB-activated PBMC significantly induced apoptosis in TCC cells. The released cytokines from SEB-treated PBMC demonstrated only a minor, not significant, apoptotic response in TCC cells. This first evaluation of the possible mode of action of a Superantigen opens the door for extended studies of this interesting approach to the treatment of bladder cancer.[29]

In a study, Perabo et al evaluated a new approach for the intravesical therapy of superficial bladder cancer. They investigated in coculture experiments if SEB-activated PBMCs are able to induce apoptosis in human transitional cell carcinoma (TCC) cells. Additionally, they tested the toxicity and efficacy of SEB dissolved in NaCl 0.9% administered intravesically once weekly for 6 weeks in a rat bladder cancer model. To validate the coculture in vitro findings, they evaluated tumor stage, grade, apoptotic cells in the urothelium and stroma of the bladder and infiltration of the bladder wall by lymphocytes, macrophages and mononuclear cells. Coculture experiments revealed that SEB-activated PBMCs are able to kill TCC cells by inducing apoptosis. The intravesical toxicity study with a maximum dose of 100 $\mu g \cdot ml^{-1}$ SEB demonstrated no side effects. In the intravesically SEB-treated animals (10 $\mu g \cdot ml^{-1}$), only 3 tumors remained *vs.* 15 persisting tumors in the control group. The remaining tumors of the therapy group showed a significant amount of apoptosis and granulocytes, mainly in the urothelium, whereas no relevant apoptosis or infiltration of the bladder with lymphocytes or macrophages was found in the control group. These preclinical findings suggest that SEB might be an interesting candidate for further clinical evaluation.[30]

Yi *et al* have prepared a dual-anchored tumor cell vaccine of mB7.1-GPI and SEA-TM. *In vitro* the biological activities of these vaccines were measured using a lymphocyte proliferation assay and cytokine release assay on splenocytes derived from C57BL/6 mice. The splenocytes were co-cultured with EL-4 or EL-4/mB7.1-GPI or EL-4/SEA-TM or EL-4/SEA-TM + mB7.1-GPI (treated with Mitomycin C). Lymphocyte proliferation was determined with MTT assay, the concentrations of cytokines (IL-2 and IFN-) were measured using a ELISA technique. Forty C57BL/6 mice were inoculated with EL-4 cells, after 3 days the mice were randomly divided into 5 groups with 8 in each and were treated with PBS, EL-4 cell vaccine, EL-4/mB7.1-GPI cell vaccine, EL-4/SEA-TM cell vaccine and EL-4/SEA-TM + mB7.1-GPI cell vaccine respectively, vaccines were injected three time with two-day interval. Animals were observed daily, tumor sizes were measured every third day. Twenty-five days after tumor challenge, 3 mice in each group were sacrificed and splenic lymphocytes were isolated to examine the activity of natural killer cells (NK)

and cytolytic T lymphocytes (CTL). The survival of the remaining 5 mice in each group was observed till the 90th day. The results showed that mB7.1-GPI or/and TM-SEA fusion protein was stably anchored onto the surface of EL-4 tumor cells. EL-4/mB7.1-GPI or EL-4/SEA-TM had a stronger ability to stimulate lymphocyte proliferation and IL-2 and IFN-gamma production than EL-4; while EL-4/SEA-TM + mB7.1-GPI showed a further increased ability than EL-4/mB7.1-GPI and EL-4/SEA-TM in stimulating lymphocyte proliferation and cytokine production *in vitro*. Volume of tumor was smaller and survival time of mice was longer in EL-4/mB7.1-GPI vaccine group, EL-4/SEA-TM vaccine group and EL-4/SEA-TM + mB7.1-GPI vaccine group, comparing with PBS group and EL-4 cell vaccine group. Tumor volume was much smaller and survival time of mice was much longer in EL-4/mB7.1-GPI + mB7.1-GPI vaccine group, comparing with EL-4/SEA-TM vaccine group and EL-4/mB7.1-GPI vaccine group ($P < 0.05$). Lymphocytes derived from the mice treated with EL-4/SEA-TM + mB7.1-GPI showed much higher NK activity and CTL activity than those derived from EL-4/mB7.1-GPI vaccine group and EL-4/SEA-TM vaccine group, meanwhile the NK activity and CTL activity of EL-4/mB7.1-GPI vaccine group and EL-4/SEA-TM vaccine group was higher than EL-4 vaccine group. The mB7.1-GPI or/and SEA-TM fusion protein was stably anchored onto the surface of EL-4 tumor cells. The tumor cell vaccines prepared from these cells exhibited antitumor effect. The mB7.1-GPI and SEA-TM dual-anchored tumor cell vaccine had much stronger antitumor effect than the single-anchored tumor cell vaccine.[31]

Since transfection of established tumors with immunostimulatory genes can elicit antitumor immunity, Li *et al* treat mouse HCC with *in vivo* transfection of superantigen SEA and/or costimulatory molecule CD80 and evaluated the safety and efficacy. Mice with HCC were treated with lipid-complexed plasmid DNA encoding SEA and/or CD80. Then the mice were evaluated for tumor regression, systemic immunologic responses, survival times and treatment-associated toxicity. Of all treated mice, the overall response rates (complete or partial remission) for SEA, CD80 and SEA/CD80 treated mice in this study were 65%, 60% and 75% separately, and were significantly higher than that of untreated mice. Most of the treat mice completed the therapy without any significant reaction. CTL activity increased with time of treatment and correlated temporally with an objective tumor response. Results of this study indicated that local intratumoral expression of SEA did not lead to detectable deletion or anergy of SEA-reactive spleen T cells. Survival times for hepatoma mice in this study treated by intratumoral injection of SEA, CD80 and SEA/CD80 were prolonged significantly ($P < 0.01$) compared with the control mice.[32]

To investigate the activity of Staphyloccocal enterotoxin A liposome (L-SEA) for inducing cytotoxicity of tumor infiltrating lymphocytes (TIL) against tumor cells, TIL were isolated from the tumor tissues of five hepatocellular carcinoma patients. L-SEA, SEA and IL-2 were tested in vitro for their activity levels in stimulating TIL proliferation. The TNF- and IFN- secretion and cytotoxicity of TIL against HepG-2 liver cancer cells were estimated by ELISA and MTT, respectively. Both L-SEh.3(1(o)1m 7(c)5)]TJ

control group was treated with normal saline. The percentage of tumor generation and tumor mass was measured. The results showed that the percentage of the tumor generation in the SEA-treated mice was lower than in the control group, but there was no significant difference. However, the tumor mass in the experimental group was significantly lighter than in the control group, with the difference being very significant. There were more CD4+ T cells and CD8+ T cells in the tumor of the mice treated with SEA than those of the control group. SEA has an obvious anti-tumor effect on mice gastric tumor. The mechanism might be that SEA induces the effect of SAg-dependent cell mediated cytotoxicity to the tumor cells.[35]

The antitumor effect of intra-tumoral injection of cepharanthin, a biscoclaurin alkaloid extracted from *Stephania cephalanta* Hayata, and SEB was evaluated using F344 male rats bearing transplantable rat osteosarcoma, S-SLM. A macroscopic lung metastatic nodule of tumor was transplanted into the subcutaneous back space, and 0.5 mg of cepharanthin and 2 pg of SEB were injected into the tumor on days 12, 13 and 14. On day 28, all animals were killed with an overdose of pentobarbital sodium, and the transplanted tumors and lungs were examined. The wet weight of the lungs of the rats treated with cepharanthin and SEB was significantly lower, and apoptosis in the lung metastatic nodules was significantly higher than that of the control or that of rats treated with only cepharanthin or SEB. In the transplanted tumors, infiltration of TRAP (tartrate-resistant acid phosphatase)-positive multinucleated giant cells was prominent in the rats treated with cepharanthin and SEB. These findings indicate that intra-tumoral injection of cepharanthin and SEB induced infiltration of TRAP-positive multinucleated giant cells within the transplanted rat osteosarcoma, and reduced lung metastasis.[36]

To make a study on the preparation and tissue distribution of SEA-liposomes and to provide scientific basis for the therapy of liver cancer by using SEA liposomes. SEA liposomes were prepared by reverse-phase evaporation; the diameter and entrapment efficiency (EC) of SEA liposomes were determined. ^{125}I labeled SEA solution and ^{125}I-SEA liposomes were administrated intravenously to mice, respectively. The radioactivity of the organs was determined by gamma-counter. The mean diameter and EC of SEA liposomes were 505 ± 34 nm and 44.1%±4.8%, respectively. SEA liposomes were found mainly distributed in the liver and spleen. SEA liposomes had a higher blood clearance, compared with SEA solution; SEA solution had high-radio-activity in plasma and kidney; there was statistical significance between the two groups. The preparation method of SEA liposomes is simple and repeatable. SEA liposomes possess liver-targeting properties and may provide a new application foreground for the treatment of liver cancer.[37]

Mouse factor VII (mfVII), ligand of tissue factor (TF) which is frequently over-expressed during neovascularization activated by tumor growth, was fused to SEA that mediates greater intensity of T-cell activation against tumor cells. The anti-tumor effects of the mfVII-SEA chimeric protein were evaluated. Fusion of SEA and mfVII cDNA was constructed using adenovirus vector and produced in 293 packaging cell lines. The 293 cells containing the adenovirus were administered subcutaneously to mice. Fluorescence studies at the injection site and the liver were performed 3 days later. Mouse prostatic tumor RM-1 cells and mouse sarcoma MCA 205 H12 cell lines were then used in mice to create lung metastasis and subcutaneous tumor to carry out efficacy evaluation, respectively. Adenovirus released from the injected 293 cells only infected the subcutaneous tissue at the injection site. The *in vivo* experiments in mice revealed that formation of lung metastasis was strongly inhibited by the mfVII-SEA (23± 8) compared to the vacant vector control group (193 ± 38) and PBS control group (211±42). The mfVII-SEA also strongly suppressed tumor growth at the subcutaneous injection site (342.6± 107.1) mm^3 compared to that of vacant vector control (2244.3 ± 350) mm^3 and SEA (1208.3 ± 210) mm^3 by the 23rd day. The chimeric protein mfVII-SEA significantly inhibits lung metastasis formation and local tumor growth.[38]

In a study, PNU-214565, a recombinant fusion protein consisting of the Fab of the monoclonal antibody C242 and SEA, was used in an escalating repeat dose Phase I clinical trial in patients with advanced gastrointestinal malignancies. A prior single-dose Phase I clinical trial had demonstrated safety at doses of 1.5 ng·kg^{-1} with toxicities of fever and hypotension that were not dose related. Twenty-seven patients (age range, 36-75 years; median, 62; 14 males and 13 females; 23 colorectal and 4 pancreatic) were treated with one cycle of four consecutive daily 3-h infusions of PNU-214565 at doses of 0.15 ng·kg^{-1}; 0.5, 1.5, 2.75 and 3.5 ng·kg^{-1}. All patients had a good performance status. As in the single-dose trial, fever and hypotension were the most common toxicities. Dose-limiting toxicity (DLT), consisting of transient hypotension responsive to dopamine, was experienced by one patient treated at the 2.75 ng·kg^{-1} dose level. One patient with pancreatic cancer metastatic to the liver experienced a partial response of hepatic metastases with stable pancreatic head abnormalities by computed tomography scan. Further dose escalation was suspended when two patients treated in a companion repeat dose Phase I study experienced DLT at

tolerated. Of 40 evaluable patients, 28 had disease control at 2 months and at 4 months, one patient showed partial response (PR) and 16 patients stable disease. Median survival, with minimum follow-up of 26 months was 19.7 months with 13 patients alive to date. Stratification by the Motzer's prognostic criteria highlights prolonged survival compared to published expectation. Patients receiving higher drug exposure had greater disease control and lived almost twice as long as expected, whereas the low-exposure patients survived as expected. Sustained IL-2 production after a repeated injection appears to be a biomarker for clinical effect, as the induced-IL-2 level on the day 2 of treatment correlated with survival. The high degree of disease control and the prolonged survival suggest that this treatment can be effective. These findings will be used in the trial design for the next generation of drug, with reduced antigenicity and toxicity.[42]

SEA-coated tumor cells have been shown to potently induce tumor-specific T cell response. To increase efficacy of tumor-derived exosomes to induce antitumor immune response, Xiu *et al* modified the exosomes by protein transfer of SEA tailed with a highly hydrophobic transmembrane domain (SEA-TM) and designated those SEA-TM-anchored exosomes as Exo/SEA-TM. They found the exosomes secreted from murine lymphoma E.G7-OVA cell line were round vesicles with the sizes of 40-100 nm limited by a bilayer membrane. Interestingly, the inner structures of the exosomes were visible under the transmission electron microscope; those "honeycomb-like" inner structure has not been described by other labs. Immunization with Exo/SEA-TM inhibited tumor growth and prolonged survival of the mice challenged with parental tumor cells more significantly than with exosomes (Exo) and even more than with the mixture of exosomes and SEA-TM. The results of mixed lymphocyte-tumor reaction (MLTR) showed that the increased IL-2, IFN-gamma secretion, and specific cytotoxic T lymphocyte (CTL) could be effectively induced from the splenic

blood. One half of the patients with positive cultures grew Staphylococcus aureus. This group included 11 with Sezary syndrome and 5 with rapidly enlarging mycosis fungoides plaques or tumors. All of the *S. aureus* carried enterotoxin genes. Surprisingly, 6 of 16 strains were the same toxic shock toxin-1 (TSST-1)-positive clone, designated electrophoretic type (ET)-41. Analysis of the T-cell receptor V beta repertoire in 14 CTCL patients found that only 4 had the expected monoclonal expansion of a specific V gene, whereas 10 had oligoclonal or polyclonal expansion of several V families. All patients with TSST-1+ *S. aureus* had overexpansion of V Z in blood and/or skin lesions. These studies show that S aureus containing superantigen enterotoxins are commonly found in patients with CTCL especially individuals with erythroderma where they could exacerbate and/or perpetuate stimulate chronic T-cell expansion and cutaneous inflammation. Attention to toxigenic *S. aureus* in CTCL patients would be expected to improve the quality of care and outcome of this patient population.[46]

To engineer SAgs to express tumor reactivity, Rosendahl *et al* genetically fused the Fab-part of the tumor-reactive MAb C215 and the bacterial Sag, SEA. Treatment of mice carrying established lung micrometastases of the C215-transfected syngeneic B16 melanoma with 3-4 daily injections of C215Fab-SEA resulted in strong antitumor effects, while only moderate effects were seen when treatment was given every 4th day (intermittent treatment). High serum levels of IL-2, TNF- , IFN- and strong induction of CTLs (cytotoxic T lymphocytes) were noted after priming with the fusion protein. T cells responded well to 3 daily injections of C215Fab-SEA and then gradually entered a hyporesponsive state, characterized by a reduced ability to produce IL-2, TNF- and IFN- and failure to mediate cytotoxicity *in vitro*. Intermittent treatment was characterized by increased levels of IL-10, concomitant with accentuated loss of IL-2, TNF- and IFN- production. A 10-fold increase in SEA-reactive TCR V 3+ CD4+ cells was observed in the spleen, while a loss of TCR V(beta)3+ CD8+ and V 11+ CD8+ cells was noted. This is in striking contrast to injections of native SEA which induced a marked deletion of TCR V 3+ CD4+ T cells, but not of CD8+ cells. Recovery of the TH1 cytokine profile occurred within 1-2 weeks, while restoration of cytotoxicity required several months and correlated with recovery of TCR V 3+ CD8+ and TCR V 11+ CD8+ T cells. These results show that the temporal relationship of SAg stimulations dictates the cytokine profile. Moreover, different mechanisms appear to regulate hyporespon-siveness in CD4+ and CD8+ T cells.[47]

Shimizu et al examined the effectiveness of SEB-coupled tumor cells, to induce antitumor activity. SEB was chemically conjugated to tumor cells using a heterobifunctional cross-linking agent acting through NH2 and SH groups. V beta 8+ T cells were activated and increased in number after the culture with SEB-bound Meth A cells. The cultured T cells exhibited an antitumor activity in the Winn assay. IL-2 receptor- + (IL-2R+) V 8+ T cells but not IL-2R+ V 6+ T cells increased in number in mice injected with SEB-bound Meth A cells. However, the percentages of V 8+ and V 6+ T cells did not change by this immunization. The antitumor effector cells were V 7-8- CD4+ T cells. In vivo immunization with SEB-bound cells induced a strong antitumor activity, i.e., tumor-free mice/total mice was 14 of 15 (93%) for Meth A and 7 of 15 (47%) for hepatoma MH134. The induced antitumor activity was both dose dependent and tumor specific. Treatment with SEB-bound cells prolonged the survival days of Meth A-bearing mice by 62%. These results suggest that SEB-bound tumor cells may be a powerful method for induction of *in vivo* antitumor activity.[48]

In vitro-activated T lymphocytes can be retargeted with anti-CD3 x anti-tumor bispecific antibodies (BsAb) to kill tumor cells *in vitro* and *in vivo*. Thibault *et al* performed a study to examine the systemic and intra-tumor effects of *in vivo* T cell activation with BsAb, SEB and -glucan in combination with BsAb as a retargeting agent. CL-62 melanoma cells were injected subcutaneously into C3H/ HeN mice. Mice were subsequently treated with BsAb alone or with SEB or -glucan plus BsAb. Fresh splenocytes, lymph-node cells and tumor-infiltrating lymphocytes (TIL) were tested for their proliferative response using incorporation of 3H-thymidine, and for their ability to lyse CL-62 cells in the presence or absence of BsAb in 4-hr 51Chromium release assays. Toxicity of treatments was assessed in a D-galactosamine model. BsAb, alone or combined with beta-glucan, had essentially no effect on systemic T-cell cytotoxicity, and essentially no effect on systemic proliferation, unless exogenous IL-2 was provided. At the tumor site, BsAb alone, BsAb plus -glucan, and SEB plus BsAb all significantly increased BsAb-mediated TIL cytotoxicity. In contrast to the TIL-limited effects of BsAb and of BsAb plus -glucan, SEB plus BsAb markedly increased both systemic and intra-tumor T-lymphocyte activation. Toxicity correlated with measures of systemic activation, with limited effects from BsAb alone and from beta-glucan plus BsAb, and with marked lethality from SEB plus BsAb. Overall, these results suggest moderate intra-tumor activation of TIL, but no measurable systemic activation after *in vivo* treatment with BsAb or -glucan plus BsAb. SEB plus BsAb results in complete T-cell activation in both systemic and

intra-tumor compartments, but at the expense of increased systemic toxicity. In conclusion, TIL can be activated in situ with different combinations of in vivo activants. *In vivo*-activated TIL can be retargeted with bispecific antibodies to lyse tumor cells, and may be an alternative to *ex vivo* T-cell activation and adoptive transfer therapy.[49]

To engineer SAgs for immunotherapy of human colon carcinoma, the superantigen, SEA was genetically fused to the Fab region of the colon carcinoma-reactive monoclonal antibody C242, and the effector mechanisms involved in the anti-tumor response to C242 Fab-SEA were characterized. Immunohistochemistry and computer-aided image analysis were used in studies of cryopreserved tumor tissue to evaluate the phenotype of infiltrating cells and their cytokine profiles in response to therapy. Human T cells and monocytes were recruited to the tumor area and penetrated the entire tumor mass within hours after injection of C242 Fab-SEA. The production of cytokines at the single-cell level was found to be dominated by TNF- , IL-2, IL-4, IL-5, IL-10, IL-12, IFN- , granulocyte-macrophage colony-stimulating factor, and transforming growth factor- , whereas IL-1-alpha, IL-1ra, IL-1 , TNF- , IL-3, IL-6, and IL-8 were undetectable. Most of the TNF- , IL-2, IL-12, and IFN- were made by the infiltrating human leukocytes, while the colon carcinoma cells were induced to produce IL-4, IL-10, and TNF- . Up-regulation of IFN- receptors and TNF Rp60 receptors was found, while the TNF Rp80 receptor was absent. The cytokine production, T cell infiltration, and CD95 Fas receptor expression concomitantly occurred to induce programmed cell death in the tumor cells. This was followed by a strong reduction of the tumor mass that was seen within 24 h after C242 Fab-SEA infusion. These findings demonstrate that antibody-SAg proteins efficiently recruit tumor-infiltrating lymphocytes actively producing a variety of cytokines likely to be essential for the therapeutic effects observed in the model. Although the humanized SCID model has obvious limitations in its predictive value for treatment of human cancer, Researchers believe that these results encourage clinical evaluation of antibody-targeted superantigens.[50]

The results of both laboratory and clinical research into the immunomodulatory activity of levamisole have shown a considerable degree of inconsistency and sometimes contradiction. The probably a reflection of the lack of understanding of the mechanism of action of levamisole and it is therefore necessary to base conclusions about its immunomodulatory efficacy in the treatment of disease on experimental assays that take into consideration the in vivo conditions. Abdalla *et al* have designed a investigation to compare the immunomodulatory activity of levamisole under clinically achievable and non-achievable conditions as judged by changes in the perioperative proliferative response of lymphocytes from 30 patients with colorectal cancer. The results obtained showed that proliferation in antigen (purified protein derivative, PPD)-stimulated, but not phytohaemagglutinin(PHA)- or SEB-stimulated, lymphocyte cultures was consistently and significantly augmented by levamisole in concentrations of 25 ng-25 μg·ml^{-1}. High concentrations of levamisole (25 μg·ml^{-1} and 100 μg·ml^{-1}) were inhibitory to PHA- and SEB-stimulated, but not PPD-stimulated, lymphocyte cultures, especially in the postoperative period. Of particular interest was the observation that, although levamisole temporarily lost its stimulatory activity in the postoperative period, it did enhance antigen-stimulated lymphocytes at the time of the nadir of the postoperative suppression of lymphocyte proliferation. Clinically achievable concentrations of levamisole are therefore effective both before and after operation in enhancing the response of lymphocytes to antigens.[51]

T lymphocytes generally fail to recognize human colon carcinomas, suggesting that the tumour is beyond reach of immunotherapy. In order to develop a T-cell-based therapy for colon cancer, Lando *et al* also investigated the antitumor activities of C242Fab-SEA fusion protein to human colon carcinomas. The C242Fab-SEA fusion protein targeted SEA-reactive T cells against MHC-class-II- negative human colon carcinoma cells in vitro at nanomolar concentrations. Treatment of disseminated human colon carcinomas growing in humanized SCID mice resulted in marked inhibition of tumour growth and the apparent cure of the animals. Therapeutic efficiency was dependent on the tumor specificity of the fusion protein and human T cells. Immunohistochemistry demonstrated massive infiltration of human T cells in C242Fab-SEA-treated tumors. The results merit further evaluation of C242Fab-SEA fusion proteins as immunotherapy in patients suffering from colon carcinoma.[52]

Significant numbers of infiltrating mononuclear cells are commonly observed in solid tumors, although their role in restricting tumor growth is not clear. Tumor-infiltrating lymphocytes (TIL) from 38 patients with colorectal cancer, in parallel with peripheral blood lymphocytes (PBL), were assayed to determine their ability to proliferate in response to ConA, IL-2, ConA+IL-2, phorbol 12-myristate 13-acetate (PMA)+ionomycin ionomycin (IOM), and SEB. These reagents were selected to give a range of weak to strong proliferative responses either via or independent of the T cell receptor. Proliferation of TIL was significantly lower than that of PBL in all cultures: ConA, IL-2, ConA+IL-2, PMA+IOM, and SEB. In addition to the low proliferative capacity of TIL, production of cytokines by

TIL may also play a role in control of tumor growth. The researchers have assayed IFN- production in the supernatants from 16 paired TIL and PBL cultures, and tumor necrosis factor alpha (TNF-) in 6 paired cultures. TNF- concentrations were significantly lower in TIL cultures than in PBL cultures stimulated with ConA, but no difference in control or IL-2 stimulated cultures. IFN- levels did not significantly differ between PBL and TIL cultures, indicating that despite the restricted proliferative capacity of TIL, these cells remain capable of secreting significant amounts of IFN- .[53]

Human CD4+ T cells activated with SEA were fractionated by Percoll discontinuous density gradient centrifugation to enrich SEA-reactive CD4+ T cells. The SEA-reactive CD4+ T cells showed significant cytotoxicity, so-called SAg-dependent cell-mediated cytotoxicity, against SEA-coated class II-positive tumor cells. During lysis of SEA-coated tumor cells, SEA-reactive CD4+ T cells produced high levels of IL-2 and IFN- but not IL-4 in an Ag-specific manner. The skewing of human CD4+ T cells to Th1-type helper/killer T cells was also demonstrated when SEA-reactive CD4+V beta 5.3+ clonal T cells were cultured with SEA, but not with PHA or OKT3 mAb. Interestingly, the generation of SEA-reactive helper/killer T cells was negatively regulated by IL-4, but up-regulated by IL-12. The SEA-reactive CD4+ helper/killer T cells were able to generate from PBMC of tumor patients and could be expanded to 10^9 levels in a 7-day culture. The SEA-reactive CD4+ helper/killer T cells were specifically targeted to c-erbB-2 positive human colon cancer cells using SEA-conjugated- anti-c-erbB-2 mAb. These results initially demonstrated that SEA-activated human CD4+ T cells are a Th1 type of Th cell that has both helper and killer functions which may be useful for adoptive tumor immunotherapy in combination with SEA-conjugated antitumor mAb.[54]

To develop a tumor-specific SAg for cancer therapy, Dohlsten *et al* investigated the antitumor activities of FabC215-SEA fusion protein to human colon carcinoma cells. SEA as part of a fusion protein showed a > 10-fold reduction in MHC class II binding compared to native SEA, and accordingly, the affinity of the FabC215-SEA fusion protein for the C215 tumor antigen was approximately 100-fold stronger than to MHC class II molecules. The FabC215-SEA fusion protein efficiently targeted T cells to lyse C215+ MHC class II- human colon carcinoma cells, which demonstrates functional substitution of the MHC class II-dependent presentation of SEA with tumor specificity. Treatment of mice carrying B16 melanoma cells expressing a transfected C215 antigen resulted in 85-99% inhibition of tumor growth and allowed long-term survival of animals. The therapeutic effect was dependent on antigen-specific targeting of the FabC215-SEA fusion protein, since native SEA and an antigen-irrelevant FabC242-SEA fusion protein did not influence tumor growth. The results suggest that Fab-SEA fusion proteins convey superantigenicity on tumor cells, which evokes T cells to suppress tumor growth.[55]

Binding of SEB to cultured cells and to tissue sections containing presumed target sites was detected by use of an immunofluorescence sandwich technique. A triple sandwich with successive incubations of SEB, rabbit anti-SEB, and fluorescein-conjugated goat anti-rabbit secondary antibody was applied to samples. Binding of SEB to rat basophilic leukemia (RBL) cells, mast cells of rat dorsal skin, and cells of leukocyte-enriched human plasma was observed. These results point out and reinforce the reported involvement of SEB in various biological effects that appear to implicate leukocytes, either as mast cells residing in tissues or as white cells circulating in the bloodstream.[56]

The effect of SEA was studied for its effect on the development of the Lewis carcinoma in mice. It was shown that administration of SEA immediately after the appearance of the primary node in mice after transplantation of tumor cells led to insignificant inhibition of the node growth and a slight decrease of tumor metastasizing into the lungs. Inoculation of mice after the appearance of the primary node with 1/microgram of SEA 5 times a week significantly increased their survival rate. The lack of the marked effect of SEA appears to be associated with the disturbance of the immune interferon system functioning in tumor-bearing mice, since the production of serum interferon induced by SEA in mice with tumor was considerably lower than in the intact ones.[57]

Sixteen monoclonal antibodies (MAbs) directed against TSST-1 were generated by immunization of mice with purified TSST-1 and subsequent fusion of spleen cells with myeloma cells. Antibody-producing clones, identified by an enzyme-linked immunosorbent assay, were maintained as ascites tumors, and MAbs were purified by protein A chromatography. High-titered clones were further characterized and tested for the ability to neutralize several biological activities of TSST-1. The MAbs, which are of several immunoglobulin subtypes, reacted specifically with purified TSST-1 and TSST-1 present in Staphylococcus aureus culture supernatants. Three MAbs neutralized TSST-1-induced mitogenesis in a dose-dependent manner. Three of eight MAbs tested were able to neutralize induction by TSST-1 of interleukin-1 production by human monocytes. One neutralizing MAb, 8-5-7, was tested for the ability to protect rabbits from a constant infusion of TSST-1. Rabbits given the MAb had an attenuated clinical

illness and were protected from the hypocalcemia, lipemia, and hepatic and renal insufficiency seen in control rabbits. Six of seven control rabbits died, compared with only one of seven rabbits treated with MAb 8-5-7. These experiments suggest that MAb 8-5-7 is directed against an antigenic determinant critical to the toxicity of TSST-1 and that the MAbs should be useful as probes in structure-function analyses of the TSST-1 molecule.[58]

Supernatants from cultures of normal feline lymphocytes stimulated with SEA showed antiviral activity, characterized as a gamma-like interferon. With the addition of inactivated feline leukemia virus, markedly less interferon was produced. The reduction in interferon production was not attributable to lowered lymphocyte viability or reduced mitogenic properties of SEA and appears to be a direct retroviral effect. This finding may reflect clinically relevant events that may contribute to the development of the feline or human states of acquired immunodeficiency.[59]

In tumor-bearing hosts both cellular and humoral tumor-growth-enhancing factors are present. They cause immunosuppression and facilitate the growth of tumors. Very early during tumor growth these factors are either elicited by the tumor cells or induced by the host immunocytes. Among these immunosuppressive agents, circulating immune complexes appear to play a predominant role. They also activate suppressor cell activity. Plasma adsorption of CIC and IgG by protein A of *Staphylococcus aureus* has been reported to cause tumor regression. Plasma adsorption with protein A-collodion charcoal, protein A-silica, or protein A-Sepharose also induced tumorilytic reactions. Even direct infusion of protein A induced tumor regressions in rat mammary tumors. Recent studies showing tumor regressions following *S. aureus* Wood 46 plasma adsorption or infusion of normal plasma adsorbed over *S. aureus* indicate that specific blocking factor removal by plasma adsorption may not be the mechanism for causing tumor destruction. Results indicate that *S. aureus* plasma adsorption leaches a number of staphylococcal agents. Thus, it appears that SEA, and other factors are responsible for the induction of reactions leading to tumor destruction. These results presented a unified mechanism explaining the results obtained with plasma adsorption using protein A of *S. aureus*, or *S. aureus* Wood, or direct protein A infusion.[60]

Tumoricidal responses and tumor regressions have been observed after plasma perfusion over *Staphylococcus aureus* Cowan I (SAC), or purified protein A immobilized on solid supports. The system was initially studied in a single human patient and then extended to dogs with spontaneous mammary carcinoma, an excellent model of human breast cancer. In the single patient and dogs with mammary tumors, perfusion of plasma over protein A bearing staphylococcus resulted in tumor necrosis and tumor regression. Tumor reduction or growth retardation with similar perfusion systems has been noted in various feline and rodent tumor models. Tumoricidal responses were also observed in canine tumors after perfusion over commercial protein A which was immobilized in a collodion charcoal matrix (PACC). These responses were amplified when a subtherapeutic and nontoxic dose of cytarabine was given after perfusion. Similar tumor reduction in murine and feline tumor models has been noted after perfusion of autologous serum over protein A immobilized on various other solid supports. The PACC perfusion system was extended to five consecutive patients with advanced breast adenocarcinoma. Four of five patients showed tumor regression after perfusion of small volumes of autologous or homologous plasma over PACC. Patients also experienced pyrexia, nausea, vomiting, and significant cardiopulmonary toxicity. Detailed hemodynamic studies of these effects showed that the major pathophysiology involved a decline in total peripheral resistance associated with an increase in cardiac output. With reduction of immobilized protein A quantity and diminution in plasma perfusion rate, the cardiopulmonary toxicity associated with treatments was diminished. Chemotherapy given as FAC to a single patient shortly after concluding perfusion therapy resulted in rapid regression of residual large tumor masses. Studies focusing on the mechanism of the tumoricidal responses have examined changes in sera after incubation or perfusion over immobilized SAC or PACC. Major findings include (1) the identification of protein A leaching from PACC and SAC after serum perfusion and appearing in the effluent as Clq binding oligomers composed predominantly of IgG and protein A but also containing IgA, IgM and C3 with a molecular weight range of 600,000 to 2,000,000; (2) the identification of C3a anaphylatoxins in serum perfused over PACC or SAC; (3) the recognition that several enterotoxins, in particular enterotoxin B are present in commercial protein A preparation.[61]

The past studies summarized and stated the anti cancer mechanism of SAg as the following 4 aspects: (1) cancer cell apoptosis mechanism: various kinds of cytokines after activated by T cells, such as interleukin, interferon, tumor necrosis factor, etc, to break the endothelial cell of tumor vessel, promote thrombus formation, decrease the blood supplement of tumor tissue, make tumor cell necrosis and apoptosis. The cytokines also stimulate T cells proliferation and differentiation, promote them to produce more cytokines. So that it forms endogenic cycle biological effect, accelerate the apoptosis of cancer cells; (2) Cancer cell solution mechanism: after CD4+ and CD8+T cells are

activated by SAg, then they induced to produce cytotoxic, this special fusion protein of cytotoxic which SAg depended has strong affinity, make cancer cells dissolve quickly; (3) Clearing mechanism: After T-cell is activated then produce interleukin, which may induce and activate LAK cells, this type cells have extensive anti tumor effect, and they would clear cancer cells when they are activated with great deal; and (4) Mechanism of preventing metastasis: SAg greatly activate NK cells, the protein and cytolysin on tumor cell membrane that released by these cells would make cancer death by inner fluid out flow, they play important effect in the process of immune monitoring to cancer cells, when the amounts increased, they may prevent cancer cells' metastasis. The anticancer mechanism

Table 3. Clinical application of HAS in tumor treatment

No.	Treatment and application	Cases	Reference
1	Injection in tumor area interventional therapy in liver cancer	22	62
2	Injection in liver tumor area coordination liver cancer	86	63,64
3	Partial gave medicine in tumor for bronchiogenic cancer treatment	10	65
4	Injection therapy in liver cancer excision patients' cavity	58	66
5	Injection in tumor for radiation fastness malignant tumor in cervical part treatment	33	67
6	HAS treat malignant chest fluidify	955	68-84
7	HAS treat relapse pneumothorax	29	85
8	HAS treat malignant ascites	261	86-90
9	Treat advanced stage ovarian cancer malignant ascites	119	91-93
10	HAS treat malignant hydropericardium	21	94
11	Refractoriness hypodermal effusion	5	95
12	Combined application with Cisplatin preparation	858	96-119
13	Application combined HAS with Bleocin	32	120
14	Combined 5 Fluorouracil to treat esophageal cancer	52	121
15	HAS combined with VP-16 to treat lung cancer complicating pleural effusion	136	122,123
16	HAS combined with hydroxycamptothecin to treat lung cancer	102	124,125
16	Combination with chemotherapy for non small cell lung cancer	61	126
17	Combined HAS to treat esophagus squamous cancer	28	127
18	Combination chemotherapy with HAS for treatment cancer ascites	100	88
19	Combination radiotherapy with HAS for nasopharyngeal cancer treatment	222	128,129
20	Combination chemotherapy with HAS for colon cancer treatment	68	130
21	HAS against bone marrow depression and gastrointestinal response caused by chemotherapeutics	108	131
22	Combined HAS to treat mid and late liver cancer	32	132
23	Combination HAS injection in tumor with radiation for lymph node metastatic cancer in cervical part treatment	60	133
	Total	**2976**	

Table 4. Curative effect comparison on combination HAS with chemo or radiotherapy

Cancer sort	Therapy method	Case	CR+PR （%）	Leucocytes count descent （%）	Life quality change（%）
All kinds of cancer	Single chemotherapy	114	36.8	57.0	41.1
	combined therapy	116	58.6	33.6	70.7
Nasopharyngeal cancer （50Gr）	Single radiotherapy	40	35.6		
	Combined therapy	108	80.6*		

Zhang and Yin [137] observed the curative effect on HAS combined with chemotherapeutics (FEM) in abdominal cavity to cure ascites of alimentary canal malignant tumor patients. They randomly divided 140 cases of alimentary canal malignant tumor patients into HAS Group (A) , FEM Chemotherapy Group (B) and FEM with HAS Group (C). Results are as below: the effectiveness of Group C is 34.5%, which is obvious higher than Group A (13.3%,), but there is no significant difference with Group B (20.2%)*5*. The curative effect of gastric cancer is the highest, later is pancreatic cancer and colon cancer, liver cancer is the worst. The side effect of Group A is the lowest (22.2%), Group B is the highest (97.5%), Group C (56.4%) is obvious lower than Group B. The conclusion considered that in the treatment for ascites of alimentary canal malignant tumor patients, HAS combined with chemotherapeutics through abdominal cavity, this method will improve chemotherapeutics' anticancer effect, and decrease the toxic and side effects of chemotherapy drug as well.

Malignant pleural effusion is caused by pleural metastasis of malignant tumor or pleural malignant tumor. Because of the special anatomic trait of pleura, it is not sensitive to chemotherapy all over the body, so it is hardly to reach the drug level in thoracic cavity, this is the difficult point in clinical treatment, however give drug in thoracic cavity can ensure high level chemo drug effect in pleura cancer cisplatin cell cycle-nonspecific drug, especially has strong killing effect for mitosis and DNA synthesis period, it has the strongest penetration power. After gave drug in thoracic cavity, high level cisplatin may directly or indirectly make more drugs enter into tumor inside by the transportation of blood capillary, and extend killing scope. In recent years, more reports showed the effectiveness is

between 45% and 67% when only used cisplatin, but when combination it with other chemotherapeutics, the curative effect would improve on some degree, the control group used cisplatin and vindesine for treating malignant pleural effusion patient, the total effectiveness is up to 81%, but the toxic and side effects increased significantly. HAS is a new antitumor biological response modifier, its mail ingredient is SEC which can enhance the ability of NK cell and LAK cell, improve lymphocyte transformation rate, especially has strong stimulus effect to T cells, T cell got stimulation then produce IL, IFN, TNF, CSF and a great deal of cytokines, activate the activity of immune system in organism, enhance the ability of killing tumor cells, kill tumor cells but not injure ordinary cells, and repair injured cells, recovery cells' immune function, decrease tumor relapse, cut down transfer incidence in distant place.[138] Yang Zuonan and others discuss the clinical value on combination of cisplatin with HAS for advanced tumor complicating malignant ascites treatment. The clinical results showed the effectiveness of HAS combined csplatin is 81.3%, which is obvious better than HAS or cisplatin only [139], it is also better than 73.3% of IL-2 cure malignant pleural effusion reported by Wu.[140] Ma De and others observed the curative effect and side effect on HAS combined with Cisplatin for malignant pleural effusion treatment.[141] They randomly divided 48 cases of malignant pleural effusion patients into two groups, the patients released pleural fluid as soon as possible, 24 cases of treatment group adopted HAS plus cisplatin, 24 cases of control group only used cisplatin. The effective rate of treatment group is 87.5%, the control group is 58.3%, the treatment group is obvious better than the control group. It showed HAS combined cisplatin for malignant pleural effusion treatment has good curative effect and little side effect.

Through many years' clinical application, HAS showed its six kinds of clinical pharmacology effects: (1) activate T cells and make them proliferation, improve body immune function; (2) only used HAS, it has anti cancer effect, this clinical effect is also the important evidence for its extensive application perspective; (3) raise leucocytes, it has strong effect for raising leucocytes, especially for the leucocytes descent caused by radio and chemotherapy; (4) eliminate malignant ascites, it has obvious effect when only perfused in intracavitary, when combined with chemotherapeutics, it will greatly improve the elimination coefficient of ascites. (5) improve the curative effect of radio and chemotherapy, it will obviously improve the curative effect of radio and chemotherapy when drug combination. (6) improve patients' immune function, lighten the gastrointestinal tract response caused by radio and chemotherapy. Therefore, its main indications are malignant tumor, leucocytes descent, and malignant ascites. It also used to reduce side effect caused by radio and chemotherapy and improve immune function. The main side effects are fever and partial flare, the fever will resolve by itself in short term.

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