Engineered Staphylococcus aureus Enterotoxin C for Oral Adjuvant Use in T Cell-Based Immunotherapy

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Abstract

Staphylococcus aureus enterotoxins are potent superantigens known for inducing strong T cell activation, which can lead to cytokine storms and significant immune responses. Recent advancements in T cell-based cancer immunotherapies, including CAR-T cell therapy and immune checkpoint inhibitors, highlight the potential role of modified superantigens as adjuvants to boost immune activation. This study proposes a modified version of Enterotoxin C (ENTC3), with targeted amino acid changes to reduce toxicity while retaining its immune-activating capabilities. By altering residues at positions 74, 145, and 149, T cell stimulation is anticipated to reduce by 30%, and emetic toxicity is expected to be eliminated. Additionally, as a foodborne toxin, Enterotoxin C's stability under heat and digestion allows for potential oral administration, enhancing its applicability as an immune adjuvant with reduced invasiveness. Experimental work will involve expression, purification, and in vivo testing of the modified enterotoxin to evaluate T cell activation and safety, supporting its potential as an effective adjuvant in immunotherapy.

Introduction

T cells have become central to cancer immunotherapy, with treatments like **immune checkpoint inhibitors** and **chimeric antigen receptor (CAR) T cell therapy** showing remarkable efficacy. Despite this, challenges remain, particularly regarding efficient immune activation and overcoming immunosuppressive tumor microenvironments. Enhanced immune activation strategies are crucial to improving the efficacy of these therapies and expanding their therapeutic applications. **Superantigens**, with their ability to robustly activate T cells, offer a potential solution as immune-modulating adjuvants(1).

Staphylococcus aureus Enterotoxins and Superantigen Properties

Staphylococcus aureus enterotoxins, including Enterotoxins A, B, and C, are well-known superantigens that differ from conventional antigens in their mechanism of T cell activation. Conventional antigens selectively activate specific T cell clones; however, superantigens bind non-specifically to the **T cell receptor (TCR)** and **major histocompatibility complex (MHC) class II molecules** on antigen-presenting cells, leading to widespread T cell activation and a massive release of cytokines. This response, although beneficial in controlled immune reactions, can lead to **cytokine storms** and severe inflammation(2).

Given their strong immune-stimulating properties, superantigens have been investigated as potential adjuvants in **cancer immunotherapy**. However, their therapeutic use is hindered by toxicity risks, including T cell overactivation and significant inflammatory responses. **Enterotoxin C** (ENTC3), a superantigen comprising 266 amino acids, presents a promising candidate for modification. By reducing its toxicity while retaining superantigen properties, a modified Enterotoxin C could function as a safer adjuvant for T cell-based immunotherapy(3).

CAR-T Cell Therapy and Immune Checkpoint Inhibition

CAR-T cell therapy involves engineering a patient's T cells to express chimeric antigen receptors that target specific tumor antigens, allowing precise targeting of cancer cells. Immune checkpoint inhibitors, such as pembrolizumab (Keytruda), block the mechanisms tumors use to evade the immune system, thus enabling natural immune responses to attack cancer cells. Both therapies rely on T cell activation, making them ideal candidates for enhanced efficacy when combined with a carefully modified superantigen(4).

Enterotoxin C as an Orally Administered Adjuvant

Being a **foodborne toxin**, Staphylococcus aureus enterotoxin C can be ingested orally, presenting unique therapeutic advantages. Unlike injected biologics, Enterotoxin C's heat stability and digestive resilience make it suitable for potential oral administration. This characteristic offers a non-invasive delivery method and may allow immune modulation with reduced toxicity. By taking advantage of this stability, modified enterotoxins could serve as flexible, patient-friendly immune adjuvants(5).

Concept of Adding Enterotoxin C to CAR-T Therapy

A modified **Enterotoxin C** can act as an adjuvant in T cell-based immunotherapies such as CAR-T therapy. By altering specific residues, the engineered protein retains superantigenic strength while reducing harmful side effects. Introducing this modified enterotoxin as an adjuvant can potentially amplify T cell activation, enabling a more effective anti-tumor response. This modified enterotoxin could thus serve as a safer immune-stimulating adjuvant that enhances therapeutic outcomes in T cell-mediated cancer treatment.

Materials and Methods

Protein Selection and Modification

The Enterotoxin C protein sequence, identified in the UniProt database (P0A0L5), was selected as the foundation for modification to create a safer adjuvant for T cell-based therapies(6). Specific residues within the sequence were chosen for alteration based on prior research aimed at reducing toxicity:

- The **74th residue**, Histidine (H), was modified to **Alanine (A)**, a change anticipated to reduce T cell stimulation by approximately 30%.
- Additionally, **Histidine residues at positions 145 and 149** were also substituted with **Alanine (A)**, as these modifications are expected to eliminate emetic toxicity while preserving the immune-activating properties of the enterotoxin(7).

The modified Enterotoxin C maintains its superantigen properties while potentially offering a safer immune activation profile. The original Enterotoxin C sequence includes a signal peptide spanning residues 1-27, followed by the active superantigen domain from residues 28 to 266.

Sequence Modifications

The specific sequence modifications were applied as follows:

- **Original Enterotoxin C Protein Sequence (yellow sections indicate residues to be modified):**
  MNKSRFISCVILIFALILVLFTPNVLAESQPDPTPDELHKSSEFTGTMGNMKYLYDDHYVS
  ATKVMSVDKFLAHDLIYNISDKKLKNYDKVKTELLNEDLAKKYKDEVVDVYGSNYYVNC
  YFSSKDNVGKVTGGKTCMYGGITKHEGNHFDNGNLQNVLIRVYENKRNTISFEVQTDK

KSVTAQELDIKARNFLINKKNLYEFNSSPYETGYIKFIENNGNTFWYDMMPAPGDKFDQS
KYLMMYNDNKTVDSKSVKIEVHLTTKNG

- **Modified Enterotoxin C Protein Sequence (orange sections indicate modified residues):**
  MNKSRFISCVILIFALILVLFTPNVLAESQPDPTPDELHKSSEFTGTMGNMKYLYDDHYVS
  ATKVMSVDKFLAADLIYNISDKKLKNYDKVKTELLNEDLAKKYKDEVVDVYGSNYYVNC
  YFSSKDNVGKVTGGKTCMYGGITKAEGNAFDNGNLQNVLIRVYENKRNTISFEVQTDK
  KSVTAQELDIKARNFLINKKNLYEFNSSPYETGYIKFIENNGNTFWYDMMPAPGDKFDQS
  KYLMMYNDNKTVDSKSVKIEVHLTTKNG

Protein Expression and Purification

We will synthesize and clone the modified Enterotoxin C gene into an expression vector. Following successful cloning, the protein will be expressed in E. coli and purified using affinity chromatography to achieve sufficient quality and yield for subsequent in vivo testing.

Animal Studies and Safety Evaluation

Future animal studies will evaluate the modified enterotoxin's immune response and safety profile as an adjuvant. These studies will include:

- **T cell Activation and Cytokine Release**: Mice treated with the modified enterotoxin will be monitored for T cell activation levels and cytokine profiles to assess immune activation.
- **Safety Evaluation**: The studies will examine potential side effects, including inflammatory responses, organ toxicity, and overall immune system impact.
- **Combination Therapy with CAR-T**: To evaluate synergistic effects on tumor suppression and survival, the modified enterotoxin will be administered with CAR-T cells in tumor-bearing mouse models.

Expected Results and Further Experiments

We anticipate that the modified Enterotoxin C will enhance T cell activation without inducing a cytokine storm or severe toxicity. The expected outcomes of initial animal studies include safe immune activation and controlled cytokine release. Future work will focus on optimizing dosages

for immune stimulation, evaluating the feasibility of oral administration, and progressing to preclinical trials in larger animal models to establish safety and efficacy before initiating human clinical trials.

Discussion

The modifications in Enterotoxin C present a promising strategy for leveraging superantigens' immune-stimulating potential while managing toxicity. Its stability and potential for oral administration enhance its suitability as a flexible and non-invasive adjuvant, well-aligned with patient-centered cancer treatment approaches. Combining this modified enterotoxin with CAR-T cell therapy could yield synergistic effects, bolstering immune activation and potentially overcoming challenges associated with tumor immune evasion. Further studies in preclinical and clinical models will be essential to validate this approach and ensure both efficacy and safety.

Conclusion

Through targeted amino acid modifications, we propose a modified Enterotoxin C with reduced toxicity as a potential adjuvant for T cell-based immunotherapies such as CAR-T and immune checkpoint inhibition. The enterotoxin's stability and suitability for oral administration increase its versatility in cancer treatment protocols. Continued research will focus on confirming the effects of this modified protein in enhancing anti-tumor immunity while ensuring patient safety.

Keywords

Staphylococcus aureus enterotoxin, superantigen, CAR-T therapy, cancer immunotherapy, adjuvant, oral administration, T cell activation

References

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