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Mini-Osmotic Pump Infusion Model to Investigate the Systemic Effects of Chronic Continuous Exposure to Staphylococcal Superantigen in Mice

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Summary

Staphylococcus aureus can exist as a colonizer or can cause a spectrum of diseases. *S. aureus* elaborates several exotoxins and the superantigens are one among them. Staphylococcal superantigens (SSAg) cause robust activation of the immune system and acute exposure to significant amounts of SSAg can be potentially lethal. However, chronic exposure to SSAg is also possible. Administering SSAg using mini-osmotic pumps may mimic chronic recurrent exposure to SSAg. This is a relatively simple and safe way to administer purified SSAg or any other toxin/agent. In this chapter, we describe the mini-osmotic pump-mediated delivery of SSAg.

Introduction

Staphylococcus aureus is a successful pathogen. It can asymptotically colonize 20-30% of individuals either chronically or intermittently. Skin and the anterior nares are the common sites for colonization (1, 2). *S. aureus* can also cause a wide spectrum of symptomatic diseases, from localized minor skin infections to life-threatening systemic diseases such as pneumonia, sepsis and toxic shock syndrome (3).

Staphylococcus aureus produces several exotoxins, which facilitate its existence as a colonizer as well as contribute to its ability to induce highly pathogenic of invasive diseases (4, 5). The staphylococcal superantigens (SSAg) are one among them. SSAg are the most potent biological activators of the immune system known till date (6). SSAg cause immune activation in a unique manner. SSAg can bind directly to MHC class II molecules expressed on the cell surface of various professional and non-professional antigen presenting cells without undergoing any classical antigen processing steps. By directly binding to MHC class II molecules outside of the peptide-binding groove, SSAg can cross-link MHC class II molecules, activate such SSAg-presenting cells leading to production of several cytokines, chemokines and other mediators, a majority of which are proinflammatory in nature. MHC

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class II bound-SSAg can subsequently bind to certain T cell receptor α or β chain variable regions (TCR V α or β families) irrespective of the antigen specificities of those T cells. This process results in cross-linking of TCRs, rapid and robust activation of such T cells (both CD4⁺ as well as CD8⁺), production of various cytokines/chemokines and mediation of respective effector functions of such activated T cells (7). For example, activation of Th1 cells by SSAg may lead to production of Th1-type cytokines. Engagement and cross-linking of TCRs on a CD8⁺ T cell may lead to production of cytokines as well as release of perforins and granzyme, which may mediate cytotoxicity.

Acute exposure to significant amount of SSAg as in pneumonia or sepsis leads massive and rapid activation of the immune system by the above-mentioned processes. This is termed as systemic inflammatory response syndrome and generally culminates in multiple organ dysfunction syndrome, which can be lethal (8). However, in certain clinical situations, chronic exposure to small amounts of SSAg can occur e.g., chronic *S. aureus* carriers, chronic or recurrent staphylococcal skin and soft tissue infections or catheter-associated biofilm infection (9, 10). The immunopathology ensuing from chronic immune activation mediated by SSAg could be distinct from that resulting from acute exposures. The mini-osmotic pump-mediated systemic delivery of SSAg is an ideal and simple way to investigate this process. The ALZET[®] Osmotic Pumps are small, infusion pumps commonly used to deliver drugs or agents to unrestrained laboratory animals. We have demonstrated that continuous delivery of small amounts of SSAg using such mini-osmotic pumps causes a distinct systemic inflammatory disease in our humanized mouse model (11). In this chapter, we describe the protocol to investigate the systemic effects of chronic exposure to SSAg delivered using miniosmotic pumps.

Materials

1. ALZET[®] mini osmotic pumps of desired delivery period (1, 3, 7, 14 or 28 days for mice) (DURECT Corporation, Cupertino, CA). Details regarding the functioning of mini-osmotic pumps, choice of the pumps based on the delivery rate, dose, route of delivery are discussed in detail in the manufacturer's website, (<http://www.alzet.com/>).
2. Staphylococcal superantigen: Either purified SSAg or filtered bacterial culture supernatant containing SSAg, depending on the study question ¹.
3. 1× Phosphate buffered saline (PBS): Either prepared from 10× stock or prepared in the lab and filter sterilized.
4. Mice: Age, sex, strain, gene-targeted mice etc., chosen according to the study question ².

¹Staphylococcal Enterotoxins fall under the Federal Select Agents and Toxins Category. Special permits and institutional biosafety approval are needed to use SSAg. Highly purified, endotoxin reduced SSAg may be purchased from Toxin Technology Inc, Sarasota, FL).

²Appropriate Institutional Animal Care and Use Committee's approval is required for all experiments/procedures involving animals. Special handling procedures should be followed for various chemicals and reagents used including anesthetic agents and SSAg.

5. Anesthetic agent(s): Preferred by the investigator from the list of agents approved for rodent use as well as approved by Institutional Animal Care and Use Committee (IACUC). The inhalant anesthetic, Isoflurane is ideal for such minor procedures. It is safer, easy to induce and maintain anesthesia and has a rapid recovery time from anesthesia. However, special equipment and approved laboratory setup to quench Isoflurane are needed for safe handling/administration of Isoflurane.
6. Surgical tools: Scissors, scalpel, tissue forceps, curved artery clamps, wound closure stainless steel clips and applicators; all suitable for rodent surgery and sterilized prior to use.
7. Animal Prep tools: Small clipper, hand-held vacuum to remove clippings, topical antiseptics to prepare surgical site, sterile gloves

Methods

Loading miniosmotic pumps

1. Prepare the SSAg at desired concentration in required volume of PBS corresponding to the capacity of miniosmotic pumps and number of pumps to be loaded ³. (Always prepare the SSAg slightly in excess to adjust for pipetting loss and loss during loading the miniosmotic pumps using a syringe).
2. Load the miniosmotic pumps with appropriate volume of the SSAg solution or PBS alone as directed by the manufacturer using the provided blunt needle.
3. Carefully insert the flow moderator into the sleeve as directed by the manufacturer.
4. Set aside the loaded miniosmotic pumps in labeled sterile petridishes and store aseptically till implantation into mice ⁴.

Surgical implantation in mice

1. Induce and maintain anesthesia using appropriate protocol.
2. Remove fur on the flank of anesthetized mice using a clipper.
3. Disinfect surgical site with povidone iodine swab and then wipe with alcohol wipes.
4. For subcutaneous implantation, a small (1-2 cm) skin incision is made using surgical scissors. A sub-cutaneous pocket is created using blunt artery forceps to accommodate the pump. The pump is gently inserted into the sub-cutaneous pouch with the opening of the moderator away from the incision site. The skin wound is closed with surgical clips. To place the pumps intra-abdominally, a small incision is carefully made on the abdominal muscularis layer taking care not to injure/damage internal organs or blood vessels. The wound site is wetted with sterile saline. The

³Other drugs or agents may be mixed with SSAg based on their solubility in PBS and experimental requirement.

⁴It is important to note that the mini-osmotic pump will not start releasing SSAg immediately after implantation in mice. This lag period varies depending on the type of pump used. For immediate release of the SSAg from the pumps after implantation, follow the priming steps described in the manufacturer's website depending on the type of osmotic pump used.

pump is gently pushed inside the abdominal cavity taking care not to damage any tissue/organs. The abdominal opening is closed with sutures as per IACUC recommended guidelines and the skin wound is closed with surgical clips.

5. While mice are still under anesthesia, inject saline (for prevent dehydration), analgesics or antibacterials as approved and mandated by IACUC policies.
6. Return mice to heated blankets to maintain body temperature till animals completely recover from anesthesia.

Post-operative follow-up and experimental procedures

7. Surgical site is monitored daily to ensure the presence of intact wound clips and for any abnormal wound discharge or infection. Body weight, animal activity and any abnormal changes in activity/behavior are noted.
8. Wound clips are removed 7 days later, if miniosmotic pumps of greater than 7 days duration are implanted.
9. During the experimental duration, animals can be bled at IACUC recommended intervals to measure serum cytokines or other analyses.
10. At the time of termination of experiment, animals are euthanized as per IACUC guidelines.
11. Immediately after euthanasia, collect blood by cardiac puncture using a syringe and needle in serum separation tubes. Keep the serum tubes refrigerated until sera are separated, aliquoted and stored frozen at -80°C for measuring cytokine or other immunological/biochemical analyses.
12. Collect spleen, thymus and lymph nodes and process for flow cytometric analyses or other experiments, accordingly.
13. Collect small pieces of organs in buffered formalin for histopathological analyses or in appropriate fixative for electron micrography.
14. Collect pieces of organs in Tissue-Tek Optimum Cutting Compound (Sakura FineTek USA INC) for cryopreservation and subsequent immunochemical analyses.
15. Collect organs in RNAlater (Life Technologies) for subsequent RNA-related assays, if needed.

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