Chapter 10

Protocols for the Induction and Evaluation of Systemic Anaphylaxis in Mice

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Abstract

Mouse models of systemic anaphylaxis are important tools for the study of mast cell function, for the elucidation of the pathomechanisms of anaphylaxis, and for identifying and characterizing potential therapies for anaphylaxis. Here, we describe two murine models of systemic anaphylaxis that have been a key part of research in these areas. In a passive model, mice are sensitized with antigen-specific IgE antibody 24 h prior to antigen challenge. In an active model, mice are instead sensitized with antigen 18–21 days prior to challenge. Hypothermia serves as the primary quantifiable indicator of anaphylaxis in these models.

Key words Anaphylaxis, Anaphylactic shock, Active systemic anaphylaxis, Passive systemic anaphylaxis, Mast cell, IgE receptor, DNP–HSA, Ovalbumin, Pertussis toxin

1 Introduction

Those who have ever experienced and survived anaphylactic shock know about the dramatic nature of this reaction. Without warning, a life-threatening, systemic allergic reaction can occur within minutes after contact with an otherwise relatively innocuous substance like peanuts or venom from a wasp sting.

There are many open questions about anaphylaxis that still need to be addressed, for example: Why do only some people experience anaphylactic shock while others do not? It is well known that many allergic patients exhibit high levels of circulating antigen-specific IgE without ever experiencing anaphylaxis, whereas other subjects with low concentrations of specific IgE in the blood can suffer anaphylactic shock [1]. It must be inferred that other factors, in addition to antigen-specific IgE, also contribute to the occurrence or the severity of anaphylaxis.

Various candidates have emerged in the clinic and at the bench. For example, clinical observations have identified certain

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drugs or vitamin D deficiency as non-IgE factors involved in occurrence and severity of anaphylaxis [2, 3]. In mouse models, we have identified a role for endothelin-1, a vasoconstrictive peptide that is up-regulated in some bacterial infections, in enhancing mast cell activation and thus likely contributing to the severity of an anaphylactic shock in mice [4]. Furthermore, there are continuing discussions about which cells and mediators are involved in anaphylaxis. While the importance of mast cells, the IgE receptor, and histamine are generally acknowledged, other cells, receptors, and mediators are also hypothesized to potentially affect an anaphylactic reaction [3, 5–7]. Additional cells and mediators include natural killer T cells, basophils, eosinophils, TRP proteins, IL-33, or PAF.

The use of mouse models of anaphylaxis is crucial to increasing our understanding of the pathomechanisms in anaphylaxis, and to identifying and characterizing potential therapeutic strategies for the treatment or the prevention of anaphylaxis. Because of the sudden and rapid reaction in the patient, the onset and course of an anaphylactic reaction can rarely be monitored, and provocation of anaphylaxis in a patient for scientific purposes is unethical.

Additionally, mouse models of systemic anaphylaxis can be utilized as model systems for in vivo analysis specifically of mast cell function. They offer unique opportunities to identify and characterize specific receptors on the mast cell surface or substances released by mast cells which might play a role in the many physiological or pathophysiological processes in which mast cells are involved.

Many different protocols for mouse models of systemic anaphylaxis have been reported in the literature. The main differences in these models are the experimental allergens (usually DNP–HSA, TNP–OVA, OVA, or BSA), the route of sensitization and challenge (i.p. or i.v.), and most importantly the method of sensitization of the mice. Passive sensitization, i.e., injection of antigen-specific IgE prior to challenge with the antigen, leads to the classical pathway of anaphylaxis involving IgE, mast cells, and histamine. Active sensitization in contrast is performed by sensitization with an allergen and adjuvant and involves IgG, macrophages, and PAF [8]. Here, we describe protocols for both passive and active systemic anaphylaxis.

It is important to note that the relevance of mouse models of systemic anaphylaxis to human anaphylaxis is not entirely clear. Therefore, as is always the case in work with mouse models, care should be taken when extrapolating experimental data to the human system.

2 Materials (*See* Note 1)

2.1 Passive Systemic Anaphylaxis

1. Mice at 6–12 weeks of age (*see* **Note 2**).
2. Monoclonal mouse anti-DNP IgE antibody (Sigma-Aldrich) (*see* **Note 3**).
3. Dinitrophenyl–human serum albumin (DNP–HSA; Sigma-Aldrich).
4. Needles and syringes for i.p. and i.v. injections (27G needles; 1 ml syringes).
5. Microprobe thermometer with a rectal probe for mice (Physitemp Instruments) (*see* **Note 4**).
6. Needles and syringes for peritoneal lavage (27G and 22G needles; 10 ml syringes).
7. May-Grünwald Stain (Sigma-Aldrich).
8. Giemsa Stain, Modified (Sigma-Aldrich).
9. McJunkin-Hayden Buffer (6.63 g of KH_2PO_4, 2.56 g of Na_2HPO_4, and double-distilled water to 1 L).
10. Cytocentrifuge or centrifuge with cytospin attachments.
11. Cytospin cuvette.
12. Cytospin paper.
13. Glass slides.
14. Phosphate-buffered saline with calcium and magnesium (PBS w/Ca & Mg) (*see* **Note 5**).

2.2 Active Systemic Anaphylaxis (ASA)

15. Mice at 6–8 weeks of age (*see* **Note 2**).
16. Ovalbumin from chicken egg white (OVA; Sigma-Aldrich).
17. Pertussis toxin from *Bordetella pertussis* (Sigma-Aldrich).
18. Aluminum potassium sulfate dodecahydrate (Sigma-Aldrich).
19. Needles and syringes for i.p. and i.v. injections (27G Needle; 1 ml syringes).
20. Microprobe thermometer with a rectal probe for mice (Physitemp Instruments) (*see* **Note 4**).

3 Methods

3.1 Passive Systemic Anaphylaxis [9]

Sensitization

1. Prepare 100 μg/ml of monoclonal mouse anti-DNP IgE antibody in 0.9 % NaCl (*see* **Note 6**).
2. Sensitize mice by intraperitoneal injection (*see* **Note 7**) with 200 μl of IgE solution (the mice will receive a total of 20 μg of IgE anti-DNP). Inject control mice with 200 μl of 0.9 % NaCl.
3. Wait 24 h before challenge.

3.2 PSA Challenge

1. Prepare 10 mg/ml of DNP–HSA in 0.9 % NaCl.
2. Measure baseline temperature using a rectal probe for mice (*see* **Note 8**).
3. Immediately challenge mice by intravenous injection with 100 μl of DNP–HSA solution (*see* **Note 9**).
4. Measure rectal temperature at 10-min intervals for the first hour, and then at 90 and 120 min following the challenge.
5. After 2 h, euthanize mice and disinfect the abdominal skin.
6. Perform a 2 cm midline abdominal incision, expose the peritoneum, and slowly inject 2 ml of 0.9 % NaCl (or medium) and 8 ml of air into the peritoneal cavity (*see* **Note 10**) using a 27G needle.
7. Gently massage the abdomen for 3 min and recover the peritoneal fluid using a 22G needle.
8. Wash the recovered cells in PBS w/Ca & Mg and resuspend at a concentration of $1–2 \times 10^6$ cells/ml PBS w/Ca & Mg.
9. Prepare cytospins following standard procedures and stain with May-Grünwald-Giemsa for analysis of mast cell degranulation [10].
10. For additional assessment of mast cell mediator release, repeat the passive systemic anaphylaxis (PSA) (**steps 1–4**) and sacrifice the mice after the first temperature measurement (10 min after induction of anaphylaxis). Collect whole blood (for example by cardiac puncture) and peritoneal lavage fluid (PLF). Leave blood sample for at least 1 h to clot, centrifuge the sample at ($1{,}000–2{,}000 \times g$) for 20 min, and remove the serum from the clot by gently pipetting off into a clean tube. To assess mast cell activation, measure mMCP-1 and/or histamine by ELISA in serum and PLF.

3.3 Active Systemic Anaphylaxis (ASA) Sensitization

1. Prepare a solution of 1 mg/ml of OVA with 1 μg/ml of Pertussis toxin and 10 mg/ml of aluminum potassium sulfate as adjuvants in saline solution. Prepare control solution identically, but without OVA.
2. Actively sensitize mice by injecting 100 μl of OVA solution intraperitoneally.
3. Wait for 18–21 days before challenge.
4. One day before challenge collect tail vein blood in 1.5 ml polypropylene tubes for measurement of OVA-specific IgG_1 and OVA-specific IgE to verify proper sensitization (*see* **Note 11**). If not used on the same day, store serum at –80 °C.

3.4 ASA Challenge

1. Measure the baseline rectal temperature (*see* **Note 8**).
2. Prepare 10 mg/ml of OVA solution in 0.9 % saline.

3. Inject 50 μl of OVA solution (500 μg of OVA) intraperitoneally or intravenously.
4. Monitor rectal temperature and signs of morbidity at regular intervals until death or until 30 min following challenge, whichever is first. Morbidity (shivering, reduced activity) should be closely monitored and documented according to the respective regulations. Mice should be sacrificed immediately if they reach or surpass the previously defined humane endpoint.

4 Notes

1. Prepare all solutions using sterile 0.9 % NaCl. Prepare and store all reagents at 2–8 °C unless indicated otherwise. Diligently follow all waste disposal regulations. Perform all animal work in accordance with the national guidelines on the care and use of animals for scientific purposes.
2. The number of mast cells differs between mouse strains and increases with age of mice. Higher mast cell numbers lead to a more pronounced temperature drop.
3. Prepare a stock solution in NaCl. Store aliquots for long-term storage at −20 °C and do not refreeze after thawing. IgE working solutions should be discarded if not used within 12 h.
4. Alternatively, subcutaneously implanted transponders (e.g., BMDS-Bio Medic Data Systems) can be used to monitor temperature.
5. The use of PBS w/Ca & Mg improves cell adhesion to slides.
6. Our preferred model of PSA uses monoclonal mouse anti-DNP IgE antibodies and DNP–HSA. However, a variety of substances have been used in other models.
7. Sensitization and challenge can be i.p. or i.v., with similar results. In the case of i.p. injections, be careful not to inject into the intestine as the sensitization will fail. This will be noticeable only after the mice have been challenged the next day and may lead to false-negative results.
8. Because the change in temperature can be quite small, it is important to carefully control for factors that might affect body temperature, such as the number of mice per cage, the time of day they are tested, and the amount of handling and stress each mouse experiences during the procedures.
9. Like the sensitization, the challenge can be performed by i.v. or i.p. injection. If the effect of a substance on the outcome of the anaphylactic reaction is to be tested, the site of the antigen injection should differ from the route of administration of the test substance. For example, if the substance in question is

injected i.p., DNP–HSA should be injected i.v. into the tail vein and vice versa.

10. Always place needles in the lateral side through the abdominal muscles.
11. This is of special importance if different genotypes are compared. Any difference observed in the biological response could be either due to differences in the challenge phase and the respective cells and mediators involved during challenge, or in the sensitization phase, for example by effects on immunoglobulin levels.

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