

Video Article

Induction and Monitoring of Active Delayed Type Hypersensitivity (DTH) in Rats

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Abstract

Delayed type hypersensitivity (DTH) is an inflammatory reaction mediated by CCR7- effector memory T lymphocytes that infiltrate the site of injection of an antigen against which the immune system has been primed. The inflammatory reaction is characterized by redness and swelling of the site of antigenic challenge. It is a convenient model to determine the in vivo efficacy of immunosuppressants. Cutaneous DTH can be induced either by adoptive transfer of antigen-specific T lymphocytes or by active immunization with an antigen, and subsequent intradermal challenge with the antigen to induce the inflammatory reaction in a given skin area. DTH responses can be induced to various antigens, for example ovalbumin, tuberculin, tetanus toxoid, or keyhole limpet hemocyanin (KLH).

Here we demonstrate how to induce an active DTH reaction in Lewis rats. We will first prepare a water-in-oil emulsion of KLH, our antigen of interest, in complete Freund's adjuvant and inject this emulsion subcutaneously to rats. This will prime the immune system to develop memory T cells directed to KLH. Seven days later we will challenge the rats intradermally on the back with KLH on one side and with ovalbumin, an irrelevant antigen, on the other side. The inflammatory reaction will be visible 16-72 hours later and the red and swollen area will be measured as an indication of DTH severity.

Protocol

1. Preparation of the emulsion

1. The emulsion is a 1:1 mixture of antigen (DTH can be induced to various antigens: ovalbumin, tetanus toxoid, keyhole limpet hemocyanin....) in aqueous solution added to complete Freund's adjuvant. It is very important to add the antigen to the adjuvant and not the other way round!!!
2. There is a lot of waste when making and injecting an emulsion, so always prepare 1.5 or 2 times more than what you need.
3. Dissolve antigen in saline to prepare a 2 mg/ml solution. Do not filter, as you do not want to lose the aggregates!!!
4. Put complete Freund's adjuvant H37Ra (Difco, catalog # 231131) in a tube and vortex at high speed. Add the antigen drop by drop while vortexing. When all of the antigen is added, vortex for 5 more minutes. The mixture should turn white.
5. Put the emulsion in a glass syringe and link it to another glass syringe using a 18G bridge (Fisher catalog # 14-825-17L). Send the emulsion from a syringe to the other until it becomes hard (5-10 min). It is best to use two 5 ml syringes. If more than 5 ml of emulsion are required, use several sets of 5 ml syringes.
6. The emulsion can be stored in the syringes at 4°C for 3 weeks.

2. Immunization of the rats

1. Mix the emulsion in the syringes for a few minutes and transfer to the syringe used for the injection. Inject 200 µl subcutaneously in 1-2 sites at the base of the tail using 23G needles and a Luer-Lok tip 3 ml syringe.

3. Challenge with antigen in the back

1. Seven days after immunization, shave 2 spots on the backs of the rats and inject intradermally 20 µl of a 1 mg/ml antigen solution in one spot and saline (or irrelevant antigen) in the other spot. Do NOT filter the antigen solution, as you may lose aggregates that enhance the inflammatory reaction.
2. The rats should be anesthetized to avoid movements. Inject the solution intradermally using 27G ½ needles and 1 ml syringes. Change needles between injections.
Note: the challenge can be done in the pinna of the ears instead of the back for more precise measurements. See video at <http://www.jove.com/index/Details.stp?ID=325>

4. Measurement of DTH

1. The injection site will start turning red and swelling as a sign of inflammation. Measure the diameter of the inflamed area using a ruler or, better yet, a caliper. The maximum swelling should occur between 24 and 72 hours.

Disclosures

The authors are co-founders and consultants for Airmid Inc.

Discussion

For a more precise measurement of the DTH reaction we recommend challenging the rats with the antigen in the pinna of the ear. The reaction can then be measured as a difference in ear swelling between the relevant antigen-challenged ear and the ear challenged with either saline alone or with the irrelevant antigen. A demonstration of the challenge in the ear is shown in 1.

References

1. Beeton, C., Chandy, K.G. Induction and monitoring of adoptive delayed-type hypersensitivity in rats (10/01/2007) Journal of Visualized Experiments, 8, <http://www.jove.com/index/Details.stp?ID=325>