Protocol

Repetitive Immunization at Multiple Sites (RIMMS) of Mice, Rats, and Hamsters

Edward A. Greenfield

The repetitive immunization at multiple sites (RIMMS) protocol capitalizes on the animal's innate immune system, which is genetically preprogrammed to recognize many antigens. By repetitively immunizing the animal, B cells that recognize the antigen are kept continuously expanding until the lymph nodes are harvested for hybridoma generation. This is a good method for making a more diverse repertoire of antibodies or antibodies directed against conformational epitopes.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

Reagents

Anesthetic
Animals to be immunized
Antigen of interest, prepared in phosphate-buffered saline
Complete Freund's adjuvant

Equipment

Needles
Surgical instruments
Syringes

METHOD

1. Dilute 10–100 μg of antigen in an appropriate volume of phosphate-buffered saline. Mix it 1:1 with Complete Freund's adjuvant (see Protocol: **Preparing and Using Adjuvants** [Greenfield 2018]).
2. Inject the animals subcutaneously at six sites: near the left and right inguinal lymph nodes, the left and right brachial lymph nodes, and the left and right axillary lymph nodes. Keep injection volumes to a minimum, usually 50 μL .

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The antigen must be delivered as close to the lymph node as possible for these immunizations to be successful.

3. Administer subcutaneous boosts after 2–3 d in the same six sites as in Step 2.
4. Repeat Step 3 seven more times (i.e., for a total of eight boosts).
5. Just before the eighth boost, collect a small amount of blood (see Protocol: **Sampling and Preparation of Mouse and Rat Serum** [Greenfield 2017a]). Check the antibody titer.
 - i. If the antibody titer is insufficient, rest the animals for 2–3 wk, and then reboost them using a standard immunization protocol (Protocol: **Standard Immunization of Mice, Rats, and Hamsters** [Greenfield 2020]).
 - ii. If the titer is good, the animal can be used to generate hybridomas beginning 3–4 d after the eighth boost (Proceed to Step 6).
6. Three days following the final immunization, anesthetize the animals selected for fusion (Protocol: **Administering Anesthesia to Mice, Rats, and Hamsters** [Greenfield 2019]).
7. Kill by carbon dioxide asphyxiation followed by cervical dislocation. Remove the lymph nodes for hybridoma generation (see Protocol: **Harvesting Tissue from Immunized Mice, Rats, and Hamsters** [Greenfield 2017b]).

DISCUSSION

The RIMMS protocol relies on immunization at sites draining into lymph nodes and the use of a myeloma partner cell that had been stably transfected with Bcl-2, the founding member of the family of apoptotic regulatory proteins. Bcl-2 plays an important role in lymphoid selection processes, including the establishment of regulation of memory B cells, maintenance of self-tolerance, preventing the expansion of pathogenic B-cell populations, and rescuing cells from apoptosis. Many B cells that do not have sufficient levels of self-reactive surface immunoglobulin fail to receive a survival signal and die. Having the Bcl-2 present in the myeloma fusion partner can enhance their survival.

The RIMMS method works mainly through the innate branch of the immune system. For this reason, it is likely to respond to conformational epitopes better than classical immunizations, which use the adaptive branch of the immune system in which antigens are phagocytized by antigen-presenting cells and presented as fragments to B cells. RIMMS might also offer more epitope diversity in response to a particular antigen, although antibody affinity can be less than with classical immunizations.

REFERENCES

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