Florida State University
Immunization Routes for Antibody Production

There are a number of variables that will eventually dictate the "best" route for administering antigen during antibody production. Among these are the species of animal being used, the adjuvant and multiple characteristics of the antigen. To enhance antibody production. the antigen should be widely distributed to lymphoid tissue. Selection of the ideal route for antigen injection must also take into consideration the possibility of any subsequent inflammatory reaction. It is the responsibility of the investigator to minimize any discomfort or pain to the animal which may be a result of antigen administration.

Four things to keep in mind when considering the route of antigen administration:

1. Inject the smallest possible volume per site.
2. Use multiple injection sites when necessary.
3. Space injection sites far enough apart to avoid coalescing of inflammatory lesions. Failure to do so may result in significant tissue necrosis.
4. No single route of administration is ideal.

For further assistance and training in the techniques listed below, please contact the LAR veterinarian.

Possible routes of administration include: intravenous, intradermal, subcutaneous, intramuscular, intraperitoneal, intranodal and intrasplenic. These routes are discussed below.

Intravenous: Delivers antigen to the spleen and secondarily to lymph nodes. A good choice for soluble antigens without any adjuvant. Not recommended for adjuvated antigens due to the risk of embolism. Adjuvants that may be used intravenously include liposomes, properly prepared water-in-oil double emulsions (single emulsions are not appropriate) and dispersed alum. Disadvantages include a lack of antigen depot effect, poorer response in titer when used as the primary immunization, increased risk of tolerance or anaphylactic reactions. Is often used for booster injections following primary immunization with an antigen-adjuvant given via another route.

Intradermal: Frequently used route. Low rate of absorption into the bloodstream but rapid uptake into the lymphatic system. Presence of large numbers of Langerhan's dendritic cells in the dermis will transport intact as well as processed antigen to draining lymph nodes. Disadvantage of this route is the tendency of the injection sites to ulcerate if the antigen preparation is inflammatory. Proper site preparation is absolutely necessary to perform this correctly (hair must be clipped in order to observe proper needle placement).

Subcutaneous: Probably the most frequently used route due to the ease of injection technique. Larger volumes maybe administered per site. Slower rate of absorption as the antigen is introduced primarily through the lymphatic system. Rate of absorption is dependent upon the blood flow in the area (dependent upon skin temperature), activity of underlying muscles and contact area. Areas of loose skin will allow further spread of the antigen-adjuvant preparation, thereby increasing the contact area. Good choice for antigens likely to induce anaphylactic reaction if absorbed into the bloodstream. Disadvantage is inflammatory reactions are less likely to stay at the site of injection (migration may occur with the development of fistulous tracts).

Intramuscular: Often relied upon to provide rapid uptake into the bloodstream and lymphatics. However, this is dependent upon the size of the antigen. Good site for small molecular weight drugs that are irritant as they are rapidly absorbed into the blood and the inflammatory response distributed. Large molecules are more likely to be absorbed primarily by the lymphatic system which lie only in the fascial planes. Advantage is that larger amounts can be injected intramuscularly. Disadvantage is the more prolonged absorption and spread of antigen-adjuvant preparation along the fascial planes. Such spread (and accompanying inflammation) may cause problems especially where nerves are encountered. Major disadvantage is the inability to monitor the injection site. Use of this method in rodents is discouraged due to the small muscle mass available. Use of complete Freund's via this route is controversial.

Intraperitoneal: Used frequently for rodents, less often in other species. Antigen is taken up by the lymphatic system rapidly and transferred to draining nodes, the thoracic duct and hence the vascular system. Advantages include the relatively larger volume of preparation that can be injected, several different types of adjuvants can be used and the antigen is widely distributed to lymphoid tissue. Disadvantage is the risk of anaphylactic shock if boosters are rapidly absorbed into the vascular system. The use of complete Freund's adjuvant is not permitted for intraperitoneal administration without scientific justification and approval by the ACUC due to it's inflammatory capacity.

Intranodal: Allows for direct delivery of antigen to lymphoid tissues. Is useful when only small quantities of antigen are available. Not suitable for use with adjuvants that are inflammatory such as complete Freund's adjuvant. Moderate to severe inflammatory reactions secondary to adjuvant injection may destroy the lymphoid tissue.

Intrasplenic: Similar to intranodal above. Frequently surgery is required in order to properly place antigen-impregnated nitrocellulose.

Note on footpad injections: Is primarily a combination of the intradermal and subcutaneous routes. There may be some intravenous route if the injection is not performed properly. Use of the route must be scientifically justified and approved by the ACUC due to the

high probability of severe inflammation.

References:

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