What role is the iliac lymph node method for production of monoclonal antibodies?

Yoshikazu Sado, Ph.D.
Division of Immunology
Shigei Medical Research Institute
2117 Yamada, Okayama 701-0202, Japan

Dear colleagues;

We have reported a novel method, the mouse iliac lymph node method, to make hybridomas for producing mouse monoclonal antibodies in Acta Biochemica et Cytochemica 39: 89-94, 2006*. The key advantages over the conventional method (Table 1) are:

1) Only a single injection is enough for immunization.

2) It takes only 2 weeks to obtain sensitized B cells in mouse iliac lymph nodes.

3) It is about 10-times more efficient than the conventional method using the spleen as a source of B cells.

4) It is also a more humane method from the view point of animal care.

Table 1.

Comparison of the two methods

(Per a cell-fusion attempt; antigen, OVA)

	The conventional spleen method	The iliac lymph node method
Minimum number of mice	1 mouse	2 mice
Number of immunization	3 times	1 time
Minimum antigen amount needed	150 μg	100 μg
Minimum immunization period	34 days	14 days
Number of positive wells in ELISA	about 10 wells	about 100 wells
Efficiency of cloning	low	high

For immunization, an antigen-adjuvant (FCA) emulsion is injected once intramuscularly into the mouse tail base of both sides (Fig. 1). The volume injected is 50 micro liters per site. The injection never induces unnatural behavior, weight loss, suppuration, or ulceration in the mice during the period of immunization.

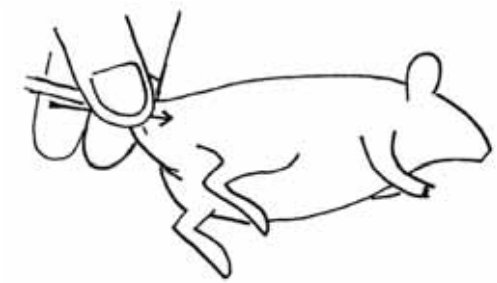

Fig. 1. Intramuscular injection into the tail base

This method also can be used in rats, although 100 micro liters of the antigen-adjuvant (FCA) emulsion is injected per site. The rats given the emulsion seem to have no particular pain after the injection. We are able to have the same result without using the hind footpad injection which we do not want to use.

The mouse iliac lymph node method is a Japanese patent (No.4098796), but not a worldwide one. It means that the method can be used freely outside Japan. The rat lymph node method reported by Kishiro et al., 1995** is not a patent.

I strongly recommend the use of the mouse and rat iliac lymph node method to produce monoclonal antibodies in place of the conventional spleen method.

* Sado Y, Inoue S, Tomono Y, Omori H. Lymphocytes from enlarged iliac lymph nodes as fusion partners for the production of monoclonal antibodies after a single tail base immunization attempt. Acta Histochem Cytochem. 39: 89-94, 2006.

**Kishiro Y, Kagawa M, Naito I, Sado Y. A novel method of preparing rat-monoclonal antibody-producing hybridomas by using rat medial iliac lymph node cells. Cell Struct Funct. 20: 151-156, 1995.

(These papers are available from the PubMed.)