Elicitation of Monoclonal Antibodies using Lipoparticles

Antibodies against Membrane Proteins

MAbs targeting the native structure of membrane proteins have been challenging to derive by conventional means. The most specific and potent MAbs against such proteins target conformationally-complex epitopes formed by the three-dimensional structure of the proteins, and therefore cannot be derived using peptides or misfolded proteins. Structurally-intact membrane proteins expressed in whole cells or membrane preparations are typically used to elicit MAbs against these targets. However, these inoculation formats contain low concentrations of the target antigen and often fail to generate a strong immune response against the membrane protein. A source of highly concentrated, structurally-intact membrane proteins can improve the success rate for generating high-quality MAbs.

The Lipoparticle

Lipoparticles are virus-like particles that incorporate high concentrations of target membrane proteins in their native conformation. Target membrane proteins are incorporated into Lipoparticles at concentrations 10 to 100-fold higher than in cells or membrane preparations. Because membrane proteins within Lipoparticles are derived directly from the cell surface without mechanical disruption or detergents, the native structure and orientation of the membrane proteins are retained. Lipoparticles are approximately 150 nm in diameter, so are readily suspended in aqueous solutions that can be used for inoculation. Unlike traditional sources of membrane proteins, Lipoparticles do not contain cytoplasmic proteins or inverted membrane proteins that can result in an unfocused immune response. As a result, Lipoparticles represent a high quality immunogen.

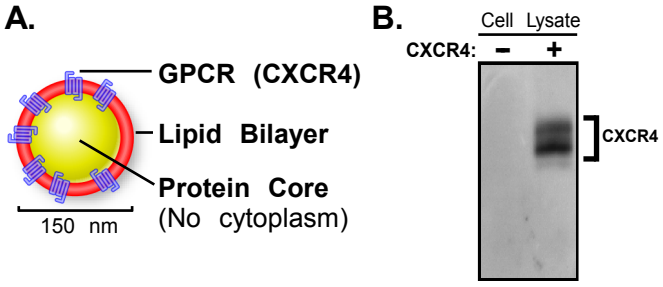

Figure 1. Specific, high-titer antibody production against the GPCR CXCR4 using Lipoparticles. **A.** Lipoparticles contain high concentrations of a target membrane protein in a virus-like particle assembled around a core protein (Gag). Cytoplasmic proteins are not present within Lipoparticles. **B.** Western blot reactivity using anti-CXCR4 Lipoparticle serum against cells prepared with or without CXCR4. Different cell types were used for Lipoparticle production and CXCR4 detection.

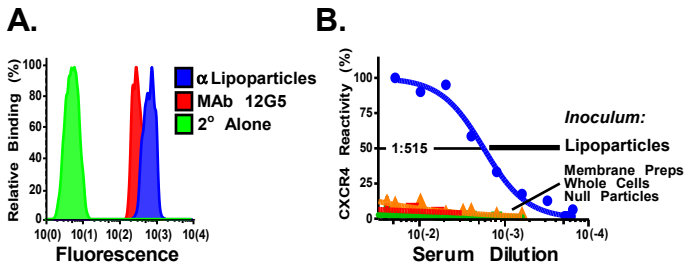

Figure 2. CXCR4 Lipoparticles produce high titer sera. **A.** Staining of CXCR4-positive cells with CXCR4-Lipoparticle antiserum (1:200), as analyzed by flow cytometry. For comparison, staining of cells with the high affinity, conformation-dependent MAb 12G5 (50 ng/ml) is also shown. **B.** CXCR4-Lipoparticle antiserum (blue curve) exhibits high-titer binding to CXCR4-positive cells, as analyzed by flow cytometry. Antiserum against Null Lipoparticles, CXCR4-membrane preps, or CXCR4-expressing cells exhibited little or no detectable reactivity in the same experiment.

Use of Lipoparticles as Immunogens

To generate antibodies targeting the GPCR CXCR4, purified Lipoparticles incorporating the receptor were used to immunize naïve mice. Sera from these mice demonstrated strong immunoreactivity against CXCR4, as demonstrated by denaturing Western Blot using cell lysates expressing the receptor (**Figure 1**). Serum against CXCR4-Lipoparticles also reacted against the native structure of CXCR4 on the cell surface (**Figure 2**). To quantify the immune response, sera dilutions were tested by flow cytometry using CXCR4-positive cells. The 50% reactivity titer of CXCR4-Lipoparticle antiserum was 1:515, representing a highly reactive immune response against the membrane protein. By comparison, sera derived from mice that were immunized with Null Lipoparticles (not containing CXCR4), CXCR4-expressing cells, or CXCR4-containing membrane preparations demonstrated little or no reactivity to CXCR4. The cells and membrane preparations used for comparison were prepared from cells expressing the same high levels of CXCR4 used to prepare the Lipoparticles, so their relative immune responses in mice reflect the 10 to 100-fold higher concentrations of CXCR4 found in Lipoparticles. These data demonstrate that Lipoparticles can be used to generate specific high-titer immune responses to conformationally-intact membrane proteins.

Contact Us

Lipoparticles are produced with customized membrane proteins, and are validated for use with specific applications. Evaluation materials are available for preliminary testing. For more information contact us at:

215.966.6061
info@integralmolecular.com
www.integralmolecular.com