In vitro analytical based

This type of assay is probably the hardest to develop and justify to regulatory agencies as replacements for in vivo assays. The mechanism of action needs to be fully understood to ensure that the in vitro assay mimics what occurs biologically. For this reason regulatory agencies frown upon purely analytical techniques such as chromatographic analysis since it may not be possible to ensure that the measured changes always coincide with the biological response. As an example although it has been proposed to replace the bioassay with a chromatographic assay for evaluation of hGH potency, this approach has not been accepted by regulatory agencies.

If the mechanism of action of the protein drugs is well understood, it may be possible to develop an in vitro assay, which mimics the biological response. This may require development of specific reagents that are actually components of the biological pathway. As an example the tissue-based and whole animal model bioassays for human relaxin have been replaced with a cell-based assay that uses endometrial cells, which produce cAMP upon stimulation with relaxin (Fei, Gross, Lofgren, Mora-Worms, & Chen, 1990).

A common target for mAbs are transmembrane proteins on cell surfaces which act as receptors that result in cellular responses that are linked to a disease. The region of the transmembrane protein that is exposed on the surface is termed the cytoplasmic domain. This cytoplasmic domain, which is soluble, can be used to develop a binding assay to determine the effectiveness of binding of a mAb to the exposed receptor. However, even though the binding may not be altered, the biological response may be changed using a degraded form of the protein drug.

Development of a potency assay for a mAb

The potential problem using a simple binding assay is demonstrated by studies using murine monoclonal IgG1 antibody that binds to the growth receptor p185HER2. This mAb was heated at 40 °C, which resulted in the generation of irreversible aggregates. Mixtures of heated samples with one that was not heated resulted in mAb solutions with different percent of irreversible aggregate. The expected percent of aggregate and that determined by SEC were similar. These samples were then assayed using two binding assays to the soluble cell target receptor. One of the assays used an enzyme-linked immunosorbent assay (ELISA) and the other a radioimmunoassay approach using radionuclide-labeled reagents to determine binding to the extracellular domain (ECD) of the p185HER2 target. The samples were also assayed using an ex vivo cell-based assay that assesses the ability of the mAb to inhibit growth of human SK-BR-3 breast carcinoma cells. Although both binding assays are in good agreement, the decrease in determined concentration for the response ("active concentration") as a function of irreversible aggregate is less than measured by the ex vivo antiproliferation cell-based assay (Figure 2.6). This example clearly shows that a determination of binding to target may not be sufficient to assess the potency of a mAb.

Although binding to an ECD of a target may not be useful as a potency assay, it is possible to develop such an assay if the mechanism of action is well understood. In the case of a mAb that binds to IgE for the treatment of asthma the mechanism of action is fairly well understood. On exposure to an antigen, B-lymphocytes transform to plasma cells that produce an IgE specific to that allergen, i.e., the Fab regions interact with the