Concentrating on Cytokines

For our new department, ***BioQ&A****,* Bio/Technology *has assembled an ad hoc panel of practitioners drawn from biotechnology and the biotechnology supply industry to attempt to answer our readers' questions. This month, Alain Artus from Immunotech (Marseille, France) and Richard McGuire from British Biotechnology Products (Abingdon, U.K.) answer questions on cytokines. They will concentrate on generic issues that face researchers adopting new technologies or modifying methods. So readers, send in your queries or problems—or solutions. Next month's column will focus on fermentation, and we welcome your solutions to the questions posed at the end of this column.*

Q. *What sort of factors will influence the outcome of assays of sera for cytokine concentration or activity?*

A. One of the most common problems in assessing cytokine concentrations in complex biological systems is the quality of sample processing. The use of heparin in preparing plasma for assays is one example. Most commercial heparin is contaminated with lipopolysaccharide, a substance frequently used in research for activating macrophages and monocytes. Contaminated heparin thus may stimulate cytokine release as long as the clot and the plasma stay together. What you end up measuring is the actual serum level of cytokine *plus* the amount released on sample processing. We recommend avoiding heparin and using EDTA in the collection of whole blood. EDTA chelates calcium and thereby blocks the active transport of the cytokine from cells and preserves the intracellular cytokine concentration. (A.A.)

Q. *I have been contemplating changing my ELISA. However, different ELISAs can yield a 10-fold difference in cytokine titer. Why?*

A. First, you could check the calibrations of your assay. The calibrating standards should conform to international WHO standards but not all kits do so. For instance, the TNF WHO standard (WHO 87/650) is one unit equal to 40 pg but we have found kits in which one unit appeared to be equivalent to 24 pg. A second factor is that some ELISAs can only measure free cytokine. However, TNF, for instance, has two kinds of soluble receptor with assumed physiological roles in protecting TNF and increasing its physiological turnover. A good ELISA will incorporate monoclonal antibodies whose epitopes on TNF are not masked by receptors. The situation is similar with IL-6. It is the IL-6--IL-6 receptor pair that is recognized by the gp130 membrane receptor. The significant concentration—the one one wants to measure—is thus the sum of free cytokine and soluble receptor-bound cytokine. (A.A.)

Q. *How can I compare antibodies for anti-cytokine activity when they are sold by weight?*

A. It is difficult to compare the activities of different antibodies as the "titer" is hard to define. This can also cause problems when changing from one batch of antibodies to another. In order to standardize batches, some manufacturers will use "neutralizing dose" as a concentration guideline. The "neutralizing dose" is the concentration of antibody required to yield 50 percent of the maximal inhibition of the cytokine when the cytokine is present at five times its normal ED_{50} (five times its normal ED_{50} will normally yield 100 percent of cytokine activity). (R.M.)

Q. *Given the option of either a recombinant or natural tissue-derived cytokine, which is better?*

A. Recombinant proteins from genes expressed in *E. coli* will be free of contamination from other cytokines. They may also be cheaper, since mass production is possible. However, they are not posttranslationally modified in the same way as natural material. Natural cytokines, with their posttranslational modification, will act as they do in tissue. Their disadvantage however, is that other cytokines, copurified at low levels, may have sufficiently high activity to interfere with the system of interest. (R.M.)

Q. *I can buy 10,000 units of IL-6 from one manufacturer for virtually the same price as 100 units from another. How come?*

A. In most cases, there are no internationally recognized standard bioassays for cytokine activity and, therefore, the activities quoted by manufacturers will depend on which bioassay is used. IL-6 activity, for example, can be measured in either a plastocytoma or hybridoma cell line. These systems give different units of activity (1 plastocytoma unit = 100 hybridoma units). (R.M.)

Next Month's Focus: Fermentation

Q. *Antifoam does not affect the productivity of our system or the downstream processing. However, the addition of the antifoam agent does not control the foam as rapidly as we require. What can we do to improve this?*

Q. *Can my existing fermenter be used to culture insect or plant cells?*

Q. *Do I really need to use double sealing systems to ensure containment of recombinant or pathogenic organisms?*

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