ANTIBODIES *IN VITRO*: REALITY AND HYPERBOLE

MIAMI, Fla.—"The application of monoclonal antibodies in immunoassays was indeed always considered obvious to everyone, except perhaps a few scientists who have patents to defend, or lawyers and judges whose definition of obvious goes beyond my scientific logic." So began César Milstein (Medical Research Council, Cambridge, U.K.), delivering this year's Miami *Bio/Technology* Winter Symposium Distinguished Service Lecture. He went on to examine a very recent advance in monoclonal antibody technology—the ability to manipulate immunoglobulin genes *in vitro* and prepare libraries for expression in bacterial or yeast hosts. In this case, however, he thinks the obvious application, recently accorded "molecule of the year" status by segments of the scientific media, "has been oversimplified by the disregard for pages and pages of small print."

After discussing in some detail the way animals produce an immune response, Milstein asked: How easy will it be to mimic in bacteria? His answer: Not very. The major obstacle, he told the audience, is the need to both hypermutate and select the molecules that will constitute the immunoglobulin repertoire. Combining a continuous selection for variants of high affinity with the ability to maintain diversity makes animals such efficient antibody producers. And duplicating this in the laboratory, according to Milstein, is far from obvious.

To begin, he argues, we must mimic a naive immunoglobulin repertoire: If scientists continue to prepare gene libraries from hyperimmunized spleen cells, a considerable portion of the advantage of using bacteria rather than hybridomas as cloning vehicles is already lost. The problems attendant to accomplishing this, while not trivial, are at least tractable. The polymerase chain reaction using general primers and a number of possible combinatorial strategies could produce a naive library of the required diversity and restricted binding affinities. It is when scientists try to *use* the library that the real difficulties begin to emerge.

Researchers must find ways to select high affinity antigen-binding clones. To do this efficiently requires developing new methods that allow cells expressing such antibodies to proliferate at the expense of other cells. Present knowledge of how animals do this is insufficient to provide significant inspiration. And present methods that can detect direct binding to individual cells and then sort them are not necessarily applicable to antibody-producing bacteria or yeast. The above are required to duplicate the primary immune response.

Imitating the critical maturation phase, however, brings another layer of problems to the surface. Producing hypermutated DNA, although possible using current techniques, means manipulating isolated DNA. Thus each round of hypermutation must be followed by retransformation, expression, proliferation, and further selection—efforts that are both technically formidable and cumbersome.

Milstein concluded by noting that "In the end, we may find it convenient to abandon bacteria and return to animals, which are so well equipped to handle complex situations involving cellular interactions."

—Harvey Bialy