IL-2 CRYSTALS, TNF TESTS, AND AIDS VACCINES

NEW ORLEANS—While discussing Takeda Chemical Co.'s "Attempts to Overcome Difficulties in the Production of Some Recombinant Proteins," Yukio Sugino described the first crystallization of *E. coli*-synthesized interleukin-2 (IL-2). Sugino, director of Takeda's Biotechnology Laboratories in Osaka and a member of *Bio/Technology*'s Scientific Advisory Board, spoke at the magazine's conference, *Bio/Technology Looks to the Next Decade*—a conclave of some 350 researchers and research managers who met here at the end of January.

The recombinant IL-2, which accumulates in inclusion bodies in the bacterial host (see photos), is denatured with guanidine hydrochloride as a first step in the purification. This is, Sugino pointed out, rather ironic: such treatments had long been considered the "untouchables of protein chemistry."

Crystallization is achieved from highly concentrated reoxidized material dissolved in an almost saturated sodium-chloride solution. The Takeda system yields two forms of IL-2—one with an N-terminal methionine and one without. Crystals of the former have primary structure and specific activity almost identical to the natural protein. X-ray diffraction studies are underway. Investigators hope this work will illuminate structure-function relationships that could allow further engineering of the IL-2 molecule.

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Left: **Electron micrograph of an ultra-thin section of a recombinant *E. coli* synthesizing IL-2. The protein accumulates in the dense inclusion bodies at either end of the cell.** ***Right:*** **A crystal of IL-2 purified from these inclusion bodies.**

Interferon Promotes TNF Activity

David Goeddel, director of molecular biology at Genentech and *Bio/Technology* board member, reported on recent experiments that show synergy between two other biological response modifiers—tumor necrosis factor (TNF) and gamma interferon. Adding small amounts of TNF to gamma interferon preparations induced substantial cytotoxicity in a murine melanoma cell line normally insensitive to either protein. Anti-TNF antibodies specifically inhibit the effect.

Goeddel speculated that the synergy might stem from an observed, gamma-interferon-induced two- to three-fold increase in the number of TNF receptors on the target cells. Goeddel also showed that TNF can act as a positive growth regulator in a number of normal fibroblast lines.

Towards an AIDS Vaccine

Bernard Moss of the U.S. National Institutes of Health described NIH's first attempts to construct an AIDS vaccine. The trial vaccine consists of the HTLV-III *env* gene inserted into the thymidine kinase sequences of vaccinia virus DNA. The vaccine induces correctly processed HTLV gp160 gene product and elicits antibodies to the gp120 protein in mice. Purified gp120 is apparently ineffective as an antigen; its activity in this case indicates the importance of correct presentation. In the vaccinia system, the protein is synthesized intracellularly and is presumably delivered to the cell surface and presented in a biologically meaningful way.

NIH researchers are now doing tissue-culture tests to find out whether the recombinant vaccinia provokes protective antibodies. And others are evaluating the vaccine's immunogenicity in primates.

HTLV-III envelope proteins are variable and, Moss noted, the neutralizing activity of gp120 antibodies from the sera of AIDS patients is indeed low. The antibodies are, however, highly cross-reactive, he pointed out.

Synthetic Anti-Idiotype Vaccines

Perhaps one tactic for enhancing the neutralization activity of these immunoglobulins will come from the once-esoteric realm of anti-idiotypes. Mark Greene of the University of Pennsylvania Medical School reported on his most recent work with anti-idiotypic antibodies to reovirus hemagglutinin. His analysis of the variable region of the anti-idiotypic antibody's kappa light chain uncovered a sequence with strong homology to a globular domain of the original viral protein. The sequence apparently lies on the outside of the protein shell.

Based on these observations, Greene synthesized a 13-mer peptide representing the homologous sequence. He then tested its immunogenicity. Even without adjuvant, this peptide specifically induces an *in vivo* cellular immune response about 50 percent of that achieved by immunization with whole virus. Although the generality of these observations remains to be tested, such strategies might also potentiate the activity of other antigens. **—Harvey Bialy**