Characterization of Human Immunodeficiency Virus

Since 1984, when the human immunodeficiency virus (HIV) became generally available to research laboratories, the full power of modern molecular biology and genetic analysis has been focused on the 9749 nucleotides which make up the viral genome. The first descriptions of HIV provided evidence for reverse transcriptase activity as well as circumstantial evidence for the existence of a viral proteinase. In 1985, molecular cloning and sequence determination revealed putative gag and pol open reading frames. In the known retroviruses, these open reading frames are first translated into the gag and gag-pol fusion polyproteins, which are subsequently processed to mature proteins, at least in part by a virally encoded proteinase. On the basis of the nucleotide sequence, it was suggested (Ratner et al., 1985) that the pol open reading frame encoded a proteinase analogous to those of other retroviral proteinases.

It was subsequently recognized (Toh et al., 1985) that the highly conserved Asp-Thr-Gly triad in the putative retroviral proteinase sequences was reminiscent of the catalytic center of aspartic proteinases, and it was suggested that the viral enzymes might belong to this family.

Despite this sequence homology, there appeared to be significant differences between the proposed viral enzymes and the classical aspartic proteinases. All of these latter proteinases comprise more than 200 amino acid residues and consist of two homologous domains. The key triad, Asp-Thr-Gly, occurs in each domain, and the two aspartic acids must come together to form the catalytic center. In contrast, the retroviral proteinases contain only around 100 amino acid residues and
a single Asp-Thr-Gly motif. These observations subsequently led to the proposal (Pearl and Taylor, 1987) that the retroviral proteinase must function as a homodimer, with each monomer contributing one aspartic acid residue to the active site.

Before the mechanism of action of the proteinase had been resolved, workers at Roche demonstrated that the proteinase in HIV \textit{pol} was essential for the processing of the \textit{gag} polyprotein (Kramer et al., 1986). Recombinant HIV \textit{gag-pol} could be expressed in yeast, and processing of the \textit{gag} polyprotein was demonstrated. However, when a frame-shift mutation was made in the proteinase region of \textit{pol}, \textit{gag} processing was lost. Thus, at least in yeast cells, the function of HIV proteinase could not be replaced by cellular proteinases and this enzyme was identified as a potential target for HIV therapy. Indeed, subsequent work at Roche showed the proteinase to be responsible for all the cleavages involved in the maturation of both the \textit{gag} and \textit{pol} gene products (Le Grice et al., 1988).

Support for the hypothesis that the proteinase is indeed essential for the viral life cycle came from site-directed mutagenesis experiments in which its active site aspartic acid was mutated (Kohl et al., 1988). Not only did these mutations abolish proteolytic activity, but when the mutant gene was reincorporated into the proviral DNA and used to transfect human colon carcinoma cells, no \textit{gag} processing was observed and the resulting viral particles were noninfectious. Compelling evidence was thus accruing throughout the 1980s that HIV does indeed encode an aspartic proteinase and that this proteinase is mandatory for viral replication.

Development of the Roche AIDS Research Program


Roche's initial commitment to AIDS research was the result of individual interests in basic research, diagnostics, new therapies, and the extended usage of existing therapies. The diagnostics groups in Basel, Switzerland, and Nutley, NJ, recognized the potential for improved clinical diagnostic systems for AIDS, and the Clinical Research and Development Departments began to investigate the use of Roferon A® as a treatment for Kaposi's sarcoma. The potential benefit of IL-2 in AIDS therapy was also being explored. Researchers from the Department of Molecular Genetics and Molecular Oncology in Nutley, in collaboration with the National Cancer Institute (NCI), commenced the studies on \textit{gag} processing in yeast just described. In late 1985, Roche's Antiviral Chemotherapy Group in Welwyn, Hertfordshire, England, initiated a