A Novel Heterodimeric Cysteine Protease Is Required for Interleukin-1β Processing in Monocytes

Research by N.A. Thornberry, H.G. Bull, J.R. Calaycay, K.T. Chapman, A.D. Howard, M.J. Kostura, D.K. Miller, S.M. Molineaux, J.R. Weidner, J. Aunins, K.O. Elliston, J.M. Ayala, F.J. Casano, J. Chin, G.J.-F. Ding, L. Egger, E.P. Gaffney, G. Limjuco, O.C. Palyha, S.M. Raju, A.M. Rolando, J.P. Salley, Y. Ting-Ting, T.D. Lee, J.E. Shively, M. MacCross, R.A. Mumford, J.A. Schmidt, and M.J. Tocci, Nature ***1992,*** 356, *768*

Condensation and commentary by ***Thomas Dandekar*** *and* ***Patrick Argos,*** *European Molecular Biology Laboratory, Heidelberg*

CONDENSATION OF THE RESEARCH

PURPOSE OF THE STUDY

To characterize the interleukin converting enzyme (ICE) required for interleukin-1β activation

RESEARCHERS' APPROACH

Human ICE was purified. A minimal substrate and inhibitors were defined. ICE cDNA was cloned and expressed, and ICE autoprocessing was analyzed. Finally, specific ICE inhibition in human blood plasma by inhibitors was investigated.

OBSERVATIONS

ICE could be purified from human monocytes (THP.1 cells) by a three-step high performance liquid chromotography procedure. Two subunits of 22 kDa (p20) and 10 kDa (p10) were identified that separated in high dilutions of ICE. On the N-terminal side of the peptide cleavage site recognized by ICE, three amino acids that are followed by an aspartate were required; methylamine was sufficient for the C-terminal side (substitutions reduced catalysis by at least 100-fold).

The best observed peptide substrate was ICE's minimal substrate: Acetyl-Tyr-Val-Ala-Asp-NH-CH_3. Replacing the NH-CH_3 with amino-4-methylcoumarin yielded a fluorogenic substrate. Replacement by diazomethylketone, a well-known cysteine protease inhibitor, led to a potent competitive irreversible inhibitor, designated A. Replacement by an aldehyde group led to a transition-state analog (reversible competitive inhibitor B). Similarly two other inhibitors (C and D) confirmed together with cystamine and other standard inhibitors

CHEMTRACTS—BIOCHEMISTRY AND MOLECULAR BIOLOGY 3:298–300 (1992)
 CCC 1045-2680/92/050298-03$04.00

that ICE is a cysteine protease. ^{14}C-Iodoacetate labeled the peptide with the catalytic cysteine in p20, while inhibitor D enabled the affinity purification of active enzyme.

Degenerate oligonucleotides derived from tryptic peptide sequences led via polymerase chain reaction amplification and short cDNAs to full-length cDNAs from THP.1 cells and human monocytes. Functional expression of ICE in COS cells confirmed the cDNA identity. A constitutively expressed transcript found in low quantity encodes both subunits of the ICE heterodimer. Different 3′ untranslated regions could be isolated; the 5′ untranslated region was extremely short. The resulting ICE amino acid sequence was not found to be homologous to any known protein, but the consensus around Ser 289 and the catalytic Cys 285 aligned with serine and viral cysteine proteases.

The primary translation product of the ICE mRNA, the proenzyme (p45), could be cleaved by ICE to yield p20 and p10; inhibitor B blocked this process. The only other proteins in which the Asp-X bond of the minimal substrate was recognized by this high specific protease were human and murine prointerleukin β. The p45 alone is inactive. Specific inhibition of plasma ICE by inhibitor B resulted in a decrease of processed β-interleukin (mIL1-β) and a corresponding increase in the unprocessed form (pIL1-β). Controls included the weaker effect by the weaker inhibitor C and no effect from other protease inhibitors including pepstatin, neutrophil elastase inhibitor, and phosphoramidon.

COMMENTARY ON THE RESEARCH

Mature interleukin-1β is a major mediator in inflammation and it has also been implicated in septic shock and the healing of wounds.[1] Attempts to identify low–molecular weight antagonists that block the interleukin receptor without eliciting an inflammatory response have been so far unsuccessful. Instead, the authors concentrated on the interleukin converting enzyme and established it to be a cysteine protease of very high specificity. This was not only confirmed by molecular cloning of the gene and biochemical analysis but also pharmacologically with a panel of substrates and inhibitors. Inhibition of ICE in blood plasma was also demonstrated.

A prototype of a very highly specific protease has been identified. Further studies should elucidate what are the exact cues guiding the enzyme specificity and how the three-dimensional structure of the substrate is recognized by the enzyme. It will be interesting to determine which other proteases exhibit this extremely high specificity. ICE could also have additional functions as it is expressed in a wide variety of lymphocytes, as Ceretti et al.[2] report, who independently achieved the cDNA cloning of ICE but did not perform pharmacological studies.

On its own p45 is inactive. This implies that there is yet another protease required for its activation. A whole zymogen cascade, as found in the familiar example of blood clotting, can be envisioned. All members of this cascade would become potential targets for medical intervention. The findings of Thornberry et al. thus open a completely new avenue for immunomodulatory drugs.

As the authors discuss, interleukin-1α and tumor necrosis factor can counterbalance potential effects from modulation of interleukin-1β. This idea has been pursued by Ray et al.[3] who showed that cowpox virus encodes a 38-kDa

serine protease inhibitor protein that also acts as an inhibitor of ICE. However, the modification of this single cytokine response is not sufficient to inhibit inflammatory response and additional cytokine response modifiers seem to be secreted by the virus. Similarly other proteases can erase the effect of ICE inhibition, particularly if they are only concentrated at sites of inflammation.[4] Furthermore, clinical tests to assay any therapeutic payoff still need to be performed. Nonetheless, the many possibilities for intervention in this new highly specific zymogen pathway should lead to successful new immunomodulatory therapies.

REFERENCES

1. Mizel, S.B. *FASEB J.* **1989,** *3,* 2379.
2. Ceretti, D.P., Kozlosky, C.J., Mosley, B., Nelson, N., Van Ness, K., Greenstreet, T.A., March, C.J., Kronheim, S.R., Druck, T., Cannizzaro, L.A., Huebner, K., Black, R.A. *Science* **1992,** *256,* 97.
3. Ray, C.A., Black, R.A., Kronheim, S.R., Greenstreet, T.A., Sleath, P.R., Salvesen, G.S., Pickup, D.J. *Cell* **1992,** *69,* 597.
4. Hazuda, D.J., Strickler, J., Kueppers, F., Simon, P.L., Young, P.R. *J. Biol. Chem.* **1990,** *256,* 6318.