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Auron P E, Webb A C, Rosenwasser L J, Mucci S F, Rich A,Wolff S M & Dinarello C A. Nucleotide sequence of human monocyte interleukin 1 precursor cDNA. *Proc. Nat. Acad. Sci. USA* 81:7907-11, 1984.
[Biol. Dept., Massachusetts Inst. Technol.; Harvard-MIT Div. Health Sci. Technol., Cambridge; Dept. Med., New England Med. Ctr.. Tufts Univ. Sch. Med., Boston: and Dept. Biol. Sci., Wellesley Coll., MA]

A cDNA which coded for the molecule termed interleukin-1b was isolated from stimulated human blood monocytes. The cDNA was the first reported for this polypeptide, which affects the functions of many cells, particularly during infection or inflammation. The cDNA coded for a precursor which lacked a secretory signal pre-peptide. This and other characteristics reported in the paper have opened new fields of inquiry into the uniqueness of IL-1. [The *SCI*® indicates that this paper has been cited in more than 780 publications.]

Molecular Cloning of Human Interleukin-1b

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Because of the lack of definitive information about the molecular nature of interleukin-1 (IL-1), C.A. Dinarello and S.M. Wolff, who had been studying IL-1, approached A. Rich in December 1981 with a proposal to clone human IL-1. The collaborative project began on February 2,1982, and included P.E. Auron, a member of Rich's lab who was interested in the regulation of inducible genes. Endotoxin-stimulated human blood monocytes were chosen as a source of mRNA because of the absence of an appropriate cell line. Unfortunately, these ceils contain abundant nucleases which resulted in low yields of mRNA. However, new techniques which became available at the time allowed us to overcome this problem and increase our chances of deriving a full-length cDNA clone. In the course of cloning IL-1 over the next two years, we extracted RNA from blood cell concentrates equivalent to 240 liters of human blood.

The project gained two more members who were instrumental in achieving our goal. A.C. Webb, a developmental biologist who was interested in monocyte activation as a model for cellular differentiation, provided expertise in mRNA expression using *Xenopus laevisoocyte* injection. L.J. Rosenwasser, who was studying the immune functions of IL-1, provided the T-cell proliferation assay specific for IL-1. Rosenwasser and Dinarello had previously observed that the neutral species of human endogenous pyrogen and IL-1 were likely the same molecule.

We began by deriving a fractionated, enriched mRNA preparation which specifically coded for IL-1 activity. We then generated a cDNA library from stimulated monocytes which was then screened using a multipronged approach involving "plus-minus" screening and hybrid released translation. This scheme increased the probability of deriving a stimulation-specific clone and did not require that the clone be full-length in order for us to derive diagnostic IL-1 activity. Thus, by May 1983, we had isolated a partial IL-1 cDNA clone which was then used to obtain full-length cDNAs.

The results generated by our studies presented us with several unexpected findings, each of which demonstrated the uniqueness of IL-1. First, IL-1 mRNA could be translated in vitro into a protein with an apparent molecular weight of 35 kD on SDS PAGE, whereas IL-1 purified from human monocytes was 15-18 kD. There were reports of larger (38 kD) IL-1 activity[1] but the relationship between these two forms was not clear at the time. Auron had postulated that IL-1 is first synthesized as a larger precursor. The cDNA sequence verified this by revealing a 31 kD open reading frame. Also surprisingly, this precursor protein did not contain an obvious secretory signal pre-peptide. This finding was consistent with earlier reports of intracellular IL-1 and focused attention on the question of how the precursor (pro-IL-1) was processed and transported out of the cell to accumulate as a smaller protein. Another unique finding was the fact that during the preparation of non-(endotoxin) stimulated monocytes, which was required for the "plus-minus" screening, Dinarello discovered that simple adherence of monocytes to glass resulted in the same amount of mRNA coding for IL-1 as present in endotoxin-stimulated cells. This was later shown to be a characteristic of IL-1b, that is, an increase in cytoplasmic mRNA in the absence of translation into protein.[2] We therefore used nonadherent, unstimulated monocyte mRNA as a source of reverse transcribed "minus" probe.

Consequently, this seminal paper provided important information crucial to studies which have and continue to shed new light on a fascinating molecule in medicine and biology.

1. **Dinarello C A, Goldin N P & Wolff** S **M.** Demonstration and characterization of two distinct human leukocytic pyrogens. *J. Exp. Med.* 139:1369-81. 1974. (Cited 160 times.)
2. **Schindler R, Clark B D** & **Dinarello** C A. Dissociation between inlerleukin-lb mRNA and protein synthesis in human peripheral blood mononuclcar cells. *J. Biol. Chem.* 265:10232-7. 1990.
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