

Our story of autotaxin: a typical example from discovery to biochemical characterization and inhibitor identification

Jean A. Boutin & Gilles Ferry

*Pharmacologie Moléculaire et Cellulaire, Institut de Recherches Servier
125, chemin de Rond, 78290 Croissy-sur-Seine, France*

Autotaxin is a long known motility factor discovered in the 90's as a protein secreted from cancer cell lines. In the early months of 2000, we purified this protein while attempting to identify the protein responsible for the large secretion of lysophosphatidic acid from adipocyte conditioned medium, a feature previously identified in JS Saulnier-Blache laboratory. After several chromatography columns, we isolated fractions still capable to catalyze the transformation of lysophosphatidylcholine into lysophosphatidyl acid. These fractions were analyzed by mass spectrometry and revealed the presence of several known proteins all but one with no reported catalytic activities. The exception came from a 120 kilodalton protein that was identified as autotaxin. We cloned this protein from adipocytes mRNA, expressed it in several expression systems, and found that autotaxin was indeed catalyzing this phospholipase D activity. Our discovery was confirmed by two reports identifying the same catalytic properties from cancer cells or human plasma. After the cloning of 3 isoforms of autotaxin, expressed in human and in mice, we assessed several new ways of measuring this catalytic activity, including the use of a fluorescent analogue of lysophosphatidylcholine. A complete survey of the enzymatic properties of these isoforms was reported. An initial work of pharmacological characterization was attempted, a autotaxin was reported to catalyze phosphodiesterase activity, using reference compound libraries from either phosphodiesterase or kinase inhibitors. Furthermore, in a more systematic attempt to identify original compounds with high inhibitory ATX potency, a compound from our own chemical library was identified: S 32826. Although extremely potent, this compound has poor *in vivo* properties, particularly a minute clearance from mice blood circulation. Nevertheless, it was used to further validate various hypotheses about the key role of autotaxin in metabolic and cancer diseases. Independently, the construction of transgenic mice expressing a point mutation of autotaxin leading to catalytic impairment, led to the surprising observation that the mutation was lethal (no autotaxin^{-/-} homozygote mice could be obtained), strongly suggesting that autotaxin is the main source of circulating lysophosphatidic acid at least in the earlier stages of development of mice.

Still going in our laboratory are identification of *in vivo* usable inhibitors, construction of transgenic mice and biophysical characterization and possibly crystallization of autotaxin.