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MATTERS ARISING

Physiological relevance of erythroid-potentiating activity or TIMP

WE note the unusual story unfolding in *Nature* concerning the molecular biology of a glycoprotein that acts as a tissue inhibitor of metalloproteinases (TIMP) and has erythroid-potentiating activity (EPA)[1,2]. While the occurrence of two such disparate activities within one molecule would be interesting, the physiological relevance of these data should evoke some caution.

TIMP is a well described entity capable of blocking the action of vertebrate collagenases as well as numerous other neutral metalloproteinases, that is, gelatinases, proteoglycanases and type-specific collagenases. This inhibitor forms a tight ($K_i < 10^{-9}$) enzyme/inhibitor complex and appears to serve as a general tissue anti-proteinase[3]. TIMP is almost ubiquitous in human tissues. Connective tissue fibroblasts of multiple origins synthesize this product[4,5] as do several cell types of haematopoietic origin including alveolar macrophages, HL-60 and platelets[6–8].

In vivo plasma concentrations of TIMP are on the order of 17 nM with a fairly narrow (~25%) standard deviation[5]. TIMP concentrations in amniotic fluid are even higher, averaging 68–102 nM. It is unlikely that the concentrations of this glycoprotein in tissue and bone marrow are significantly different; and these levels are at least 1,000-fold higher than the maximally stimulating concentrations of EPA (10–20 pM)[9]. Although it may be the case that *in vivo* conditions require significantly higher EPA concentrations than the *in vitro* assays, these data suggest that the marrow is saturated with respect to the activity of this modulator.

Thus, the dual role of TIMP/EPA appears incongruous in several respects. This glycoprotein is almost ubiquitous in human tissues and seems to be a common housekeeping product of numerous cell types, perhaps in keeping with its role as a protective agent of the extracellular matrix. While purified EPA demonstrates maximal activity at biologically anticipated concentrations, these are far exceeded by ambient levels of the glycoprotein. It may well be that the erythroid-potentiating activity of TIMP represents a coincidental property of this molecule. Alternatively, it might be best to re-examine the properties of this glycoprotein using both purified natural products and a purified recombinant-derived protein.

GEORGE P. STRICKLIN
Department of Medicine,
Veterans Administration,
Medical Center,
and University of Tennessee, Memphis,
1030 Jefferson Avenue,
Memphis, Tennessee 38104, USA
HOWARD G. WELGUS
Department of Medicine,
Jewish Hospital of St Louis at
Washington University Medical Center,
216 S. Kingshighway,
St Louis, Missouri 63110, USA

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GASSON AND GOLDE REPLY—The description of a single glycoprotein with both protease inhibitor and erythroid-potentiating activity is indeed intriguing. Erythroid-potentiating activity was originally purified using a sensitive bioassay which measures the enhanced growth of normal human erythroid progenitors in semi-solid medium[1]. The purified 28,000-dalton protein was subsequently sequenced, and oligonucleotide probes were generated and used to obtain complementary DNA clones[2]. Both the purified natural and biosynthetic (recombinant) proteins stimulate the growth of primitive and more mature erythroid precursors *in vitro*. In addition, the partially purified EPA and the purified biosynthetic material have direct effects on colony formation by human cell lines[3,4]. The availability of human cell lines as homogeneous target cells has made it possible to pursue the mechanism of action of human erythroid-potentiating activity. Our recent studies using radioiodinated human EPA have demonstrated the presence of specific cell surface receptors on responsive cell lines, but not on other types of primary human cells or cell lines (unpublished observation). It seems that EPA has many of the properties of a haematopoietic growth factor, in that it stimulates primary cells, responsive cell lines, and appears to bind to a specific cell surface receptor which could mediate these biological responses. We doubt whether hormonal potentiation of cellular growth observable at picomolar concentrations on target cells with specific binding sites could be considered "coincidental".

The role of the EPA *in vivo* is, of course, a difficult question to address. Using the cDNA clone as a probe, as well as a sensitive radioimmunoassay to look for the synthesis of EPA messenger RNA and protein, we had arrived at similar conclusions to those reported for TIMP. In other words, EPA appears to be synthesized in many cell types; this is not necessarily unusual for haematopoietic growth factors (for example, colony-stimulating factor, CSF-1 or M-CSF, is produced by most, if not all, tissues examined in the mouse). Our estimate of the circulating EPA concentration in human serum was somewhat lower than 17 nM more in the range of 5–10 nM, although these levels are also high relative to the concentration of EPA required for a maximum growth-promoting effect *in vitro*. As we do not know the site of EPA action *in vivo* nor, as pointed out, do we know the actual concentration required for biological activity *in vivo*, it is hard to interpret these findings in a physiological framework.

Because of the availability of cell culture techniques, it has been possible to purify this erythroid growth factor. Molecular biological approaches have allowed us to produce sufficient material to investigate the effects on responsive target cells at the molecular level *in vitro* and at the organismic level *in vivo*. Ultimately it should be possible to reconcile the enzymatic inhibitory effects and the growth-promoting properties of the molecule. We feel it would be unwise at this point to regard the hormonal properties of this molecule as 'coincidental' or trivial.

JUDITH C. GASSON
DAVID W. GOLDE
Division of Hematology-Oncology,
Department of Medicine,
UCLA School of Medicine,
Los Angeles, California 90024, USA

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