BIOCHEMICAL JOURNAL LETTERS

Is raf-1 the 74 kDa signalling molecule in neutrophils?

The paper by Dusi et al. [1] reports the tyrosine phosphorylation of a number of proteins associated with neutrophil oxidase activation. Although the identity of MAP (mitogen-activated protein) kinase as a tyrosine-phosphorylation substrate in neutrophils may be secure, and in agreement with that reported by Thompson et al. [2], the identity of the 75 kDa protein as raf-1 (molecular mass 72–74 kDa) must remain questionable.

Activation of neutrophils by a number of agents, including many 'priming' agents such as TNF-α (tumour-necrosis factor α) and GMCSF (granulocyte-monocyte colony-stimulating factor) (which do so without any change in cytosolic free Ca^{2+} concentration), induces tyrosine phosphorylation of a number of proteins (reviewed in [3]). A common tyrosine-phosphorylated protein in a number of studies has a reported molecular mass of 72–76 kDa [3–5]. We have therefore postulated that this protein plays a key role in activation of the oxidase, by acting at a cross-talk site between tyrosine phosphorylation and Ca^{2+} signalling via serine/threonine phosphorylation [3–5]. Proteins, such as raf-1, whose activity is regulated by both serine/threonine and tyrosine phosphorylation are therefore attractive candidates. However, Grinstein et al. [6] report an inability to immunoprecipitate raf-1 from neutrophils. We also have been unable to immunoprecipitate raf-1 from neutrophils, using either of two commercially available antibodies [4], one a monoclonal raised against the whole molecule (Oncogene Science, Uniondale, NY, U.S.A.) and the other a polyclonal raised against a peptide from raf-1 (TCS Biologicals, Buckingham, U.K.). Johnson's group [7,8], who have reported detection of MEK-1 (MAP kinase kinase) phosphorylation activity in anti-raf-1 immunoprecipitates in neutrophil lysates (using a polyclonal antibody raised against a peptide, from Santa Cruz Biotech), do not demonstrate that the immunoprecipitates contain 74 kDa protein. Dusi et al. [1] also fail to immunoprecipitate raf-1 from neutrophils (using the Santa Cruz Biotech antibody). Instead, they base their preference for identifying the 75 kDa protein as raf-1 on the 'exact coincidence' of superimposed blots for tyrosine phosphorylation and raf-1 [1]. Because of the similarity in molecular masses, superimposition would be expected to occur, regardless of whether raf-1 was the tyrosine-phosphorylation target. This can therefore provide no evidence that raf-1 becomes tyrosine-phosphorylated during neutrophil activation.

Furthermore, the question arises whether 74 kDa raf-1 is expressed in mature neutrophils. Unlike Dusi et al. [1], we cannot detect 74 kDa raf-1 in mature neutrophils by Western blotting, under conditions in which it can be readily identified in fibroblasts and the promyeloid cell line HL60 [4,9]. A cross-reacting protein of 56 kDa, however, was present. As HL60 cells are induced to differentiate towards granulocytes, there is a striking loss of detectable 74 kDa raf-1, but an increase in 56 kDa raf-like protein [9]. Despite the loss of 74 kDa raf-1 during this time, signal coupling to the oxidase and to tyrosine phosphorylation of the 74 kDa target increases [9]. There was, however, a correlation between the increase in the 56 kDa raf-like protein and signal coupling.

The evidence is thus accumulating that a 74 kDa protein is an important signalling molecule in neutrophils, potentially a cross-talking point between tyrosine phosphorylation and Ca^{2+}-induced serine-phosphorylation pathways which may underlie the mechanism of 'priming' of these cells [3]. We therefore agree with Dusi et al. [1] that tyrosine phosphorylation of a 74–75 kDa protein plays a key role in neutrophil activation, but suggest the identification of this protein as raf-1 may be, at this stage, premature.

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