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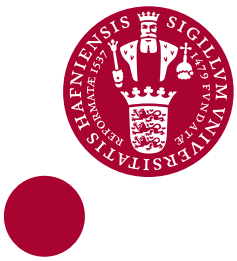

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Couchman, John R

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COMMENTARY

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Transforming growth factor-β activation in cell-free extracellular matrix preparations. Commentary

John R. Couchman

Biotech Research & Innovation Centre, University of Copenhagen, Denmark

Abstract
Transforming growth factor-β (TGF-β) is an important regulator of many cellular and immunological functions. It is often deposited in extracellular matrices in a latent form. This commentary is to draw attention to the likelihood that preparing cell-free matrices from tissue cultures by high pH buffers, such as ammonium hydroxide, can activate the TGF-β. Therefore, cells subsequently seeded onto such matrices may respond to the presence of active TGF-β in addition to interactions with macromolecular extracellular matrix components. *(Folia Histochemica et Cytobiologica 2019, Vol. 57, No. 4, 157–158)*

Key words: TGF-β; latent TGF-binding protein; cell free ECM; high pH buffers

As far back as the 1980s the roles of extracellular matrix (ECM) components in regulating cell behaviour became increasingly popular. In addition to reductionist work in isolating and carefully characterising individual matrix macromolecules, efforts were made to assess whole matrices. Gospodarowicz's group identified several methods to grow cell monolayers to confluency, allowing them to assemble extracellular matrices, then removing the cells to leave the matrix for analysis, or to seed other cells on them [1–3]. Among these techniques, low concentrations of detergent, such as Triton X-100 together with hypotonic solutions containing ammonium hydroxide were developed. This high pH method is effective, and little cell debris remains attached to the matrices that are left largely intact.

At that time, little was known about transforming growth factor-β (TGF-β), but in the succeeding few years it became apparent that this important growth factor is expressed in a latent form. Activation of TGF-β could be achieved in various ways, and early experiments with soluble, medium-derived growth factor showed that extremes of pH were highly effective [4–6]. Subsequently, a number of more physiologically relevant mechanisms to activate TGF-β have been characterised, including proteases, thrombospondin-1, reactive oxygen species and several integrin receptors [5, 7–10].

Further developments in the field showed that latent TGF-β could be inserted into the extracellular matrix through its interaction with large latent TGF-binding proteins (LTBPs), of which four distinct types are now known in mammals [11]. In this way the ECM serves as a reservoir of latent TGF-β by virtue of its association with a structural matrix component, *i.e.* LTBP [12, 13].

What appears to have escaped widespread attention is that preparation of cell-free matrices from cell cultures through the use of ammonium hydroxide can activate matrix-associated TGF-β. Therefore, cells subsequently seeded onto matrices prepared in this way will encounter not only matrix components, interacting with integrins, syndecans, CD44 *etc.*, but also activated TGF-β. Many years ago we found that matrices prepared from PF-HR9 mouse endodermal cells by alkali treatment would inhibit subsequently seeded mink lung epithelial cells (Mv1Lu) (M. Austria & J. Couchman, unpublished results). It is well known that TGF-β inhibits the proliferation of these cells [14], and in our case the inhibition could be overcome by TGF-β-specific blocking antibodies.

Even today current methods describe the use of 20 mM ammonium hydroxide (sometimes in PBS with

Correspondence address: John R. Couchman
Biotech Research & Innovation Centre,
University of Copenhagen, Denmark
Biocentre 1.3.16, Ole Maaløes Vej 5,
2200 Copenhagen N, Denmark
e-mail: john.couchman@bric.ku.dk

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detergent [15, 16], or more usually in hypotonic conditions without detergent [17]), to prepare cell-free matrices, but there is no mention of the possibility that latent TGF-β can be activated by this method. I have often encountered young scientists who use this method and many are surprised when I advise them that it can result in the activation of the matrix-associated TGF-β. Therefore, the purpose of this commentary is to bring this aspect of matrix biology to scientists' attention. Where this is a key issue, other methods at neutral pH can be used, such as 2 M urea together with non-ionic detergents and hypotonic buffers [3, 17].

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