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Modeling growth factor activity during proinflammatory stress: Methodological considerations in assessing cytokine modulation of IGF binding proteins released by cultured bovine kidney epithelial cells☆

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Abstract

The present research was conducted to model potential mechanisms through which IGFBPs might be affected by a key proinflammatory response initiating cytokine tumor necrosis factor (TNF-)-α. Madin–Darby bovine kidney epithelial (MDBK) cells, known to release IGFBPs in response to several stimuli, were grown under several conditions and challenged with forskolin (F) or recombinant TNF-α for 24 h. Forskolin increased IGFBP-3 gene expression and media content of BP-3 protein. TNF-α increased basal and augmented F-mediated IGFBP-3 gene expression. However, TNF-α effects on the measurable media content of IGFBPs were influenced by culture conditions; in the absence of added protease inhibitors (PIs) or sufficient media albumin concentration (high BSA, 1 mg/ml), the effect of TNF-α was to decrease ($P < 0.02$) measurable IGFBPs. In the presence of PI and high BSA, media IGFBP-3 levels were shown to be increased by TNF-α consistent with the gene expression data. Changes in media IGFBP-3 protease activity were examined further to explain the observed effects of TNF-α on production and destruction of IGFBPs in media. When recombinant human IGFBP-3 (500 ng/ml) was added to PI-free, low BSA 100 μg/ml) media from TNF-treated MDBK cells, less than 10% of the BP-3 was recognizable by Western blot in 30 min; conversely, inclusion of High BSA and PI in media resulted in attenuation of the protease effect on the IGFBPs. The data suggest that the MDBK model of cellular response to proinflammatory stimulus is affected by culture conditions and that TNF-α affects media content of IGFBPs through effects on IGFBP gene expression coupled with degradation of IGFBPs via enhanced proteolytic enzyme release.
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1. Introduction

The roles for insulin-like growth factors (IGF) as metabolic modifiers have evolved beyond noted endocrine effects to encompass many localized paracrine actions affecting cell survival through antiapoptotic activity [1–3], regulation of blood flow via modulation of nitric oxide synthase activity [4,5], and modification of the intracellular redox milieu [6]. As important as

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the IGF-s are themselves, it is now well recognized that changes in the ubiquitous milieu of IGF binding proteins (IGFBPs) plays a significant role in modifying and modulating specific biochemical and physiological functions of the IGFs [7–10]. Immune stress and the accompanying proinflammatory state has been associated with decreased circulating plasma levels of IGF-1 and altered patterns of IGFBPs [11–13]. It is thought that these changes reflect the capacity to balance and realign metabolic needs with survival and the establishment of a homeostatic environment to thwart the progressive cascade toward multiple organ failure. In fact, de Groof suggested that the positive clinical outcome to severe sepsis correlated with the ability for plasma IGF-1 to be depressed and its distribution to tissues modulated through a differential effect on the pattern of binding proteins [14]. However, IGF-1 mRNA and local tissue concentrations of IGF-1 do not always correlate with measured changes in plasma IGF-1 during immunologically stressful situations [12]. The data suggest that collectively many metabolic features of the proinflammatory response are dictated by the interaction of IGF-1 with the IGFBP-3 complex at the localized cellular level to regulate sepsis-related inhibition of enzyme function [12,15,16], the proteolytic enzymes in particular.

With the onset of recognition of an immunological threat, a cascade of cytokine-driven responses is initiated. A significant first line of defense is the elaboration of initiation response cytokines such as TNF-α and its associated signal transduction elements such as NF-κB [17]. With the progression through the acute phase response, a relative balance between these initiating cytokines [18] and anti-inflammatory cytokines such as IL-10 [19] develops. Contemporary with the development of this proinflammatory cascade, metabolic changes to a more catabolic/less anabolic state, consistent with observed decreases in plasma concentrations and tissue mRNA content of IGF-1 [11,13,14,20]. Evidence exists to suggest that many of the effects on metabolism are mediated through the direct actions of the proinflammatory cytokines themselves on cells and organs via specific receptors [11,18,20,21]. Data further suggest that additional levels of IGF-related metabolic regulation develop, namely, in the capacity for IGF binding protein expression and release into the local environment to modify the distribution of IGF-1 to cells [13]. However, a direct demonstration that the effect of immune stress on IGFBPs can be directed by proinflammatory cytokines like TNF-α is lacking in the literature. The selective modulation of IGF binding proteins directly at the tissue level would permit differential and selective actions of IGFs to be retained while other actions are suspended. Examples of this are suggested in the literature where antiapoptotic tissue repair is driven by a retained localized IGF-1 action downregulating NF-κ-B catabolic pathways. Other examples are in instances where the actions of IGF-1 are modified by the localized change in transgene expression of a particular binding protein or binding protein subunit [3,22,23] while the peripheral circulating plasma concentrations of IGF-1 remain decreased. Similar paracrine effects of deliberate site-directed expression of IGF binding proteins have been shown to modulate some effects of disease states with positive outcomes [7,12,16], a critical feature considering the close interplay between the somatotropic and immune axes during compromised health [24,25].

Unlike many other species, there are few, if any, validated *in vitro* models relevant to the bovine that have application in exploring cellular response to proinflammatory stress. The basic fragility of bovine hepatocytes which results in tremendous loss of viability, and observed laboratory-to-laboratory variability in the functional responsiveness of collagenase-isolated cells has largely hindered their use as culturable cells capable of uniformly reacting to biological response modifiers. To further address this aspect of the endocrine–immune interaction, we conducted the present experiments to define the effects of culture conditions on an *in vitro* model of proinflammatory stress. Specifically, we addressed how culture conditions affect the interpretation of the effects of TNF-α on the pattern of IGFBPs released into media by a line of epithelial cells of bovine origin available to researchers through the American Type Culture Collection (Manassas, VA).

2. Materials and methods

2.1. Cells, general reagents, and supplies

Experiments were performed and replicated separately in three locations: two laboratories within the Growth Biology Laboratory at the USDA (THE and CL), Beltsville and one laboratory at Auburn University (JLS) under similar conditions. Madin Darby bovine kidney epithelial cells were obtained from American Type Culture Collection (Manassas, Va.). Recombinant bovine TNF-α was a generous gift of Novartis Ltd. (formerly Ciba Geigy, St. Aubin, Switzerland). RPMI1640 medium, fetal bovine serum (FBS), trichloroacetic acid, trypan blue, trypsin, forskolin, bovine insulin, transferrin, protease inhibitor cocktail for mammalian cell extracts, selenium and reagents for determination of total urea nitrogen, were procured from Sigma Co. (St. Louis, MO). For poured 1%

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