Immunoreactivity Differences between Human Cytokines and Growth Factors Expressed in Human and Non-Human Cell Systems

Leaders in human cell expressed recombinant proteins

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Introduction

Recombinant human proteins expressed in human cells are distinct from those produced by non-human cell systems. In particular, human proteins undergo a variety of highly specific post-translational modifications (PTMs), glycosylation being one of the best known examples. The cells of non-human species do not glycosylate their proteins in the same way that human cells do. In many cases, the differences are profound, especially in species that are phylogenetically distant to humans (Reviewed in Brooks, S. A., 2004) [1].

There is a lack of research identifying how these PTM differences affect ELISA immunoreactivity. Commercially available ELISA kits use non-human cell expressed protein standards, particularly *E. coli*, and antibodies derived against these proteins. While some kits use alternatives to *E. coli* standards, such as insect and CHO derived proteins, few, if any, ELISA kits utilise human cell expressed recombinant proteins.

This work identifies differences in the immunoreactivity of human cytokines and growth factors expressed in human cells, compared to the same proteins expressed in non-human cells.

Methods & Materials

Apollo Cytokine Research has produced a range of human cell expressed proteins by recombinant methods. These have been purified by a variety of chromatographic techniques. Following purification, the proteins are quantitated using several different methods.

Commercially available ELISA kits were obtained and used according to the manufacturer's directions. Additional tests were performed by substituting protein standards from alternate sources.

A glycoprotein showing post-translational modifications. The oligosaccharide chains are shown in spacefill (balls) and the polypeptide is in ORANGE (ribbon). The Asn residues to which the the oligosaccharides are attached are shown in BLUE. The oligosaccharide sequence starts with two N-acetylglucosamine residues (YELLOW) and has several other monosaccharide residues (RED) attached.

Results

A representative selection of summary graphs reporting comparative ELISA results for Apollo's human cell expressed proteins and commercially available ELISA standards is provided here.

Figures 1 – 4 demonstrate the immunoreactivity differences that were found.

Summary

The data obtained from these studies indicate that cytokine quantity can be mis-estimated by a factor as high as ten. Differences were demonstrated in the immunoreactivity of eight proteins, using standard commercial ELISA kits: EPO, IL-2, IL-3, IL-4, IL-6, IL-10, TNF-alpha and VEGF-165. Separate studies confirm the bioactivity of Apollo's human cell expressed proteins. The changes that were found in the biological properties of the cytokines and growth factors may be related to the glycosylation and/or other post-translational modifications unique to human cells.

These results suggest implications for the accuracy of measurement of native human proteins in serum and laboratory samples. This may also have implications for the effectiveness of diagnosis and research of patho-physiological cytokine levels in clinical settings.

Immunoreactivity

Fig. 1

ELISA Results: CHO vs Human Cell Expressed Human EPO

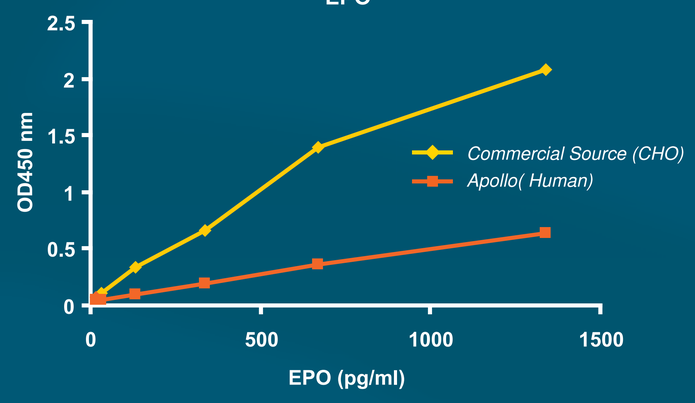


Fig. 2

ELISA Results: *E.coli* vs Human Cell Expressed VEGF

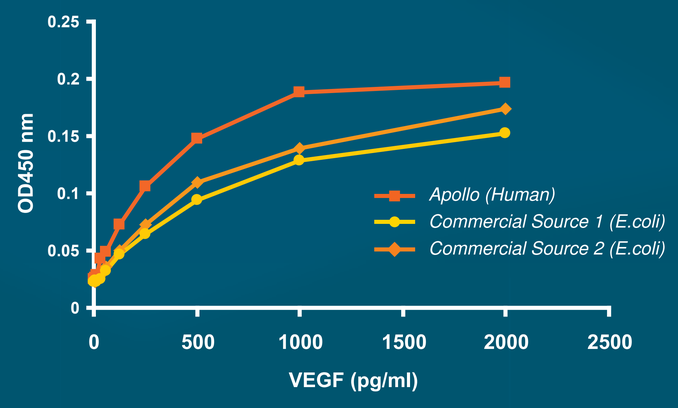


Fig. 3

ELISA Results: *E.coli* vs Human Cell Expressed Human IL-6

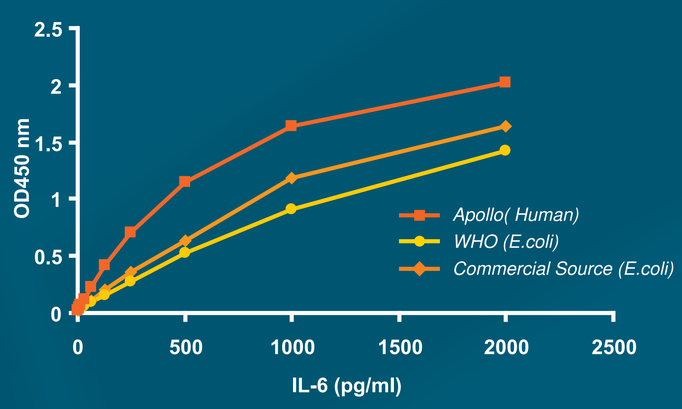
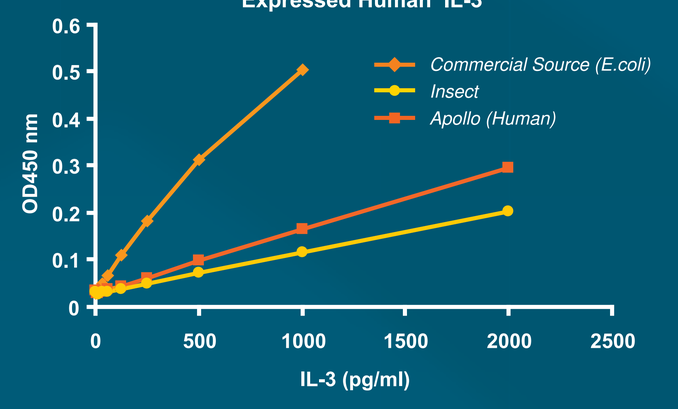


Fig. 4

ELISA Results: Human Expressed vs *E.coli* & Insect Expressed Human IL-3



References

1. Brooks, S. A. (2004) *Mol. Biotechnol.* 28, 241-255).

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