Enhanced biological properties resulting from recombinant human cell expression of human cytokines including Interleukin 4 (IL-4)

Recombinant human proteins with human post-translational modifications

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Introduction

Recombinant human proteins expressed in human cells differ from those expressed in non-human systems as they undergo human cell-specific post-translational modifications (PTMs), including glycosylation.

Many cytokines and growth factors are heavily glycosylated, with up to 75% of their mass consisting of carbohydrate moieties. Traditionally, human cytokines have been expressed in non-human cells, including bacteria, yeast and murine expression systems. However, the biological importance of species-specific post-translational modifications, in particular glycosylation, is increasingly being recognised as pivotal to protein function. Distinctly different biological properties between human proteins expressed in human or non-human cells have been identified. Those differences include misfolding, aggregation, non-glycosylation, addition of different sugar structures, and other abnormal post-translational modifications of proteins expressed in non-human cells. Furthermore, it has been proposed that glycosylation is important for secretion, solubility, resistance to proteolysis, immunogenicity, biological recognition, biological activity, *in vivo* stability and clearance of glycoproteins including cytokines and growth factors (Okamoto *et al*, 1991).

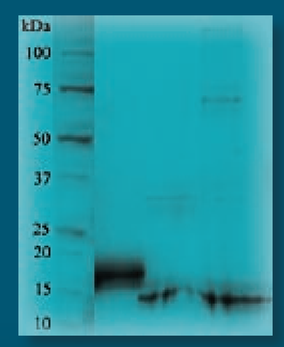
Dendritic-cell (DC) based immunotherapy is a current form of cancer treatment, which was involved in more than 100 clinical trials during 2005. Dendritic cells are usually generated by harvesting the patient's autologous peripheral blood mononuclear cells (MNC), which are then induced to differentiate *ex vivo* using a variety of cytokines including GM-CSF and IL-4. The human cytokines used for generation of DCs for this therapy are often expressed in *E.coli* and not in human cells. However, glycosylation of IL-4, known to be important for biological activity (www.copewithcytokines.de), can be diminished or completely absent in *E.coli* expressed proteins (for further information please visit www.apollocytokineresearch.com).

In vitro comparisons of the biological activity of a number of human cell expressed cytokines, with cytokines derived from other sources revealed that human cell expression confers enhanced bioactivity. These data prompted us to compare the activity of Apollo's human cell expressed GM-CSF^{hcx} and IL-4^{hcx} with other sources of these proteins.

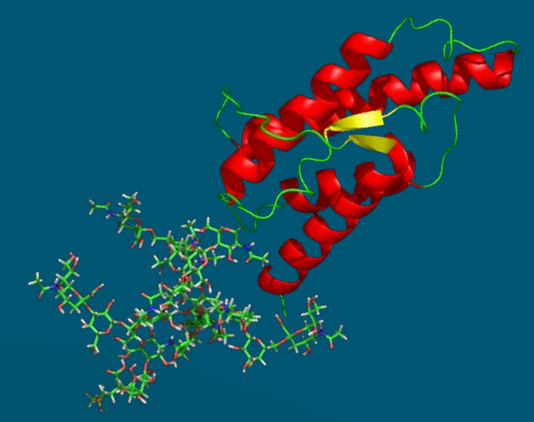
Materials Methods

Bioactivity of IL-4 was measured in cell proliferation assays using a human factor-dependent cell line, TF-1. Cells were incubated in 96-well tissue culture plates with serial dilutions (0-2 ng/ml) of IL-4 for 3-7 days at 37°C, after which cell viability was measured using the MTS assay (CellTiter 96 Aqueous One Solution Cell Proliferation Assay: Promega) according to the manufacturer's instructions. Absorbance was read at 490 nm.

Apollo GM-CSF^{hcx} and *E.coli* GM-CSF (obtained from a commercial source) were compared for colony forming ability using a methylcellulose-based media. Briefly, TF-1 cells were plated in 35mm culture dishes with GM-CSF from 0-10 ng/ml, in triplicate. The cells were incubated for 14 days at 37°C and 5% CO_2 after which colony counts were performed. Colonies were counted using a microscope and a scoring grid. A colony consisted of at least 40 cells.



The 1D gel shows a decrease in molecular weight of human cell expressed IL-4^{hcx} following treatment with PNGase F (thus showing it is N-glycosylated). The lanes are 1) MW markers, 2) untreated IL-4, 3) PNGase F treated IL-4, and 4) glycosidase cocktail treated IL-4. Confirmation of glycosylation at N-38 has also been confirmed by PMF analysis (data not shown).



Molecular representation of IL-4 showing protein chain as ribbon and complex N-glycan structure attached to N-38. Ref www.glycosciences.de (glyprot). File 1hij.pdb.

Bioactivity

Fig. 1

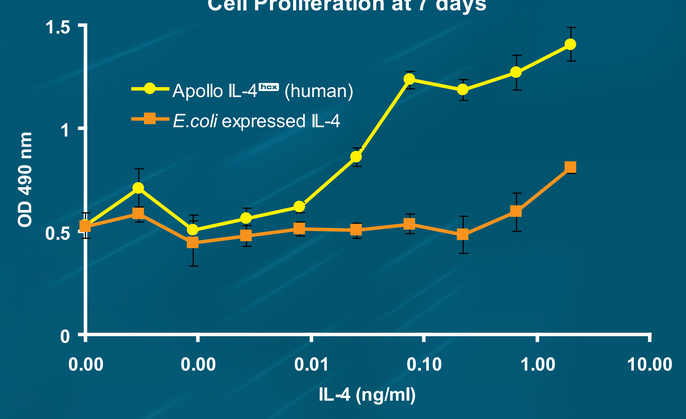


Fig. 2

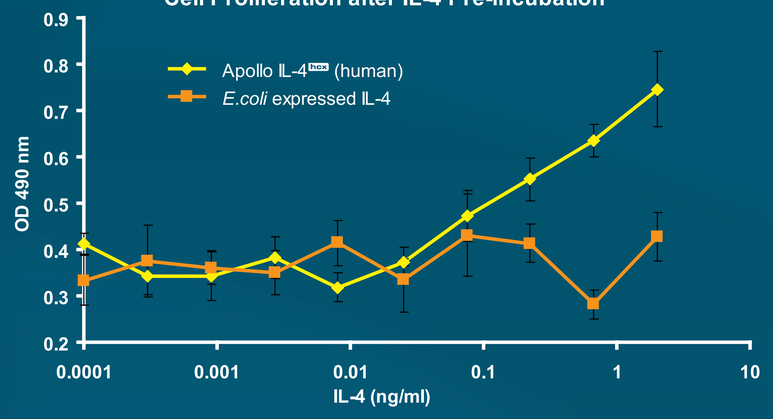
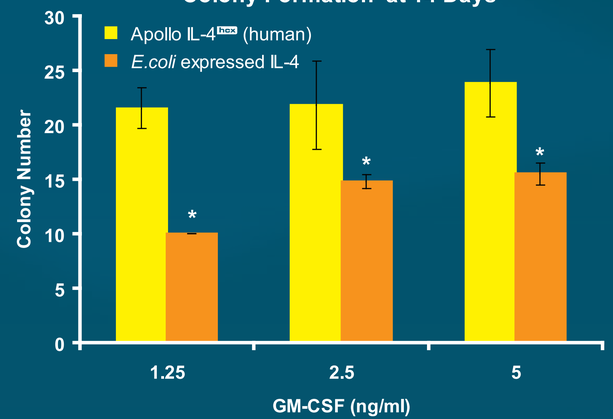


Fig. 3



Results

1. IL-4 Stability

Comparison of IL-4 expressed in *E.coli* or human cells (Apollo Cytokine Research) showed that in an extended cell proliferation assay, Apollo's human cell expressed IL-4^{hcx} induced more cell proliferation after 7 days in culture, suggesting it has a greater biological activity and perhaps a greater half-life (Figure 1). The ED_{50} of human cell expressed IL-4^{hcx} was also lower (0.03 ng/ml) compared to *E.coli* (1 ng/ml). When IL-4 was pre-incubated for 4 days in cell culture medium (RPMI 1640/10% FBS) at 37°C before addition to the assay, Apollo IL-4^{hcx} also induced a much higher level of cell proliferation, demonstrating it has greater stability in cell culture conditions than *E.coli* expressed IL-4 (Figure 2).

2. GM-CSF Stability

Similarly, comparison of human cell expressed GM-CSF^{hcx} with *E.coli* expressed GM-CSF demonstrated that human cell expressed GM-CSF^{hcx} had a significantly higher colony forming ability than *E.coli* expressed GM-CSF ($p<0.005$; Figure 3), re-iterating its enhanced biological potency.

Summary

The data from these preliminary studies demonstrated that human cell expressed GM-CSF^{hcx} and IL-4^{hcx}, with human cell specific glycosylation, have greater biological activity and stability in cell culture than "*E.coli*" expressed proteins.

These data have important implications for *ex vivo* procedures such as derivation of dendritic cells, where GM-CSF and IL-4 in the culture medium must be replenished several times during the process of dendritic cell generation. As this requires up to 14 days in cell culture, the increased stability of human cell expressed proteins could facilitate fewer additions of cytokines. Derivation of dendritic cells using glycosylated human cell expressed cytokines and growth factors, which closely resemble the native proteins, may also enhance the activation and therapeutic efficacy of the cells in terms of their capacity to activate T-cells and their ability to take up, process and present antigens.

References

1. Okamato *et al*. 1991. Arch. Biochem. Biophys. 286, 562-568.