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Impact of glycosylation on the effect of cytokines. A special focus on oncology

Abstract

The clinical use of cytokines is largely confined to the field of cancer treatment. Industrial procedures for the preparation of cytokines may or may not affect the glycosylation status of several cytokines. The glycosylation of cytokines may be of importance for the accomplishment of their biological functions, including their biological stability, their interaction with specific receptors and their pharmacokinetic behavior. The purpose of this review is thus to describe and discuss the role of cytokine glycosylation, based on experimental data, and with the clinical perspective on oncology. There is no general rule governing the effects induced by the presence of glycosylation on cytokine activity. Among

different cytokines concerned by glycosylation status, data concerning G-CSF are sufficient to translate experimental findings into clinical evidence. For this cytokine, glycosylation confers advantages for both stability and biological activity. Although at weight equivalence, the glycosylated form confers greater biological activity than the non-glycosylated form, clinical studies have demonstrated that efficacy remains the same when glycosylated and non-glycosylated G-CSF are prescribed at bioequivalent doses.

Full text

INTRODUCTION

Cytokines constitute a heterogeneous family of peptidic compounds, which includes interferons, interleukins, colony-stimulating factors and chemokines, which act as signalling molecules that orchestrate important biological functions in the organism. *In vivo*, most cytokines are found in a glycosylated form. The extensive enzymatic glycosylation machinery of a cell anchors different sugars to N- and O-extremities of the cytokines. The N-linked sites are fitted in the attachment sequence Asn-Xaa-Ser/Thr where Xaa is an intermediate residue. The O-linked sugars are attached to Ser and Thr residues. For a given cytokine, this ability to anchor sugars on the protein induces heterogeneity in terms of different iso-forms. Glycosylation may affect the biological function of cytokines in different ways including: its influence on their intrinsic stability, their solubility and capacity to diffuse through the organism, and on their interaction with specific target cell receptors and their subsequent biological activity. Since the industrial biosynthesis of several cytokines may or may not maintain their glycosylated status, the purpose of this review is to describe and discuss the role of cytokine glycosylation as regards its experimental and clinical aspects. As most cytokines are clinically useful, especially in cancer treatment, concern regarding their activity is of more than merely theoretical interest and may have concrete clinical implications. All data are summarized in table 1 and are detailed as two distinct parts covering experimental and clinical data.

EXPERIMENTAL DATA

Does glycosylation affect cytokine stability?

This question of cytokine stability is of prime importance. Kodama and colleagues have examined the impact of O-linked sugar chains on recombinant human interleukin 5 (rh IL-5) produced by Chinese hamster ovary (CH0) cells using high-performance anion-exchange chromatography in high pH conditions [1]. Sugar chains were digested using various glycosidases and 3 subsets of rh IL-5 were studied: intact rh IL-5, deglycosylated rh IL-5 on the O-site (de-O-glycosylated) and on the N-site (de-N-glycosylated). The authors found that intact rh IL-5 and de-O-glycosylated rh IL-5 produced similar activity when incubated at 70° C for 30 min, while the activity of the de-N-glycosylated rh IL-5 decreased significantly. The authors thus concluded that N-linked sugar chains contribute to the thermostability of rh IL-5. Regarding interferons (IFN_S), Karpusas and colleagues have recently provided experimental evidence which shows that glycosylation of human INF-beta is a determinant factor for the solubility and stability of this compound [2]. It has been known for some time that recombinant human erythropoietin (EPO) possesses N-linked sugars [3]. Tsuda and colleagues have identified the presence of O-linked sugar composed of sialic acid and Gal beta [1-3] Gal NAC [4]. In order to explore the role of these sugars, the authors prepared EPO deglycosylated to different extents using specific glycosidases. Incubation of asialo-EPO and fully deglycosylated EPO at 70° C for 15 min showed a decrease in biological activity ranging between 35 and 11% in comparison with the initial activity measured for the intact EPO. Consequently, EPO resistance to thermal inactivation is largely conferred by the presence of sugars, and terminal sialic acids greatly contribute to the stability of the molecule. In addition, Endo and colleagues have provided experimental results showing that deglycosylation of the N-linked oligosaccharides made EPO remarkably susceptible to aggregation induced by heat treatment [5]. Altogether, these results have strengthened the idea that the carbohydrate chains, both N- and O-linked, are essential to the stability of EPO. Importantly, EPO is used for the treatment of anemia in patients with renal impairment requiring dialysis. Of note, phagocyte activation may occur on the dialysis membrane and can produce large amounts of active oxygen radicals on their surface. Also, the role of N-linked oligosaccharides of EPO in the damaging effect of active oxygen radicals has been recently studied by Uchida and colleagues [6]. *In vitro* generation of oxygen radicals did not result in any specific damage to the carbohydrate moiety of EPO. However, *in vitro* deglycosylation of EPO caused an increase in the susceptibility to oxygen radicals as compared with intact EPO. This observation underlines the role of the oligosaccharide fraction of EPO in protecting this molecule from oxidative damage.

Regarding the granulocyte-colony stimulating factor (G-CSF), Oh-Eda and colleagues showed that the O-linked sugar chain of human recombinant G-CSF (rh G-CSF) contributes to the stability of the molecule by eliminating polymerization [7]. In addition, these authors used circular dichroic and colorimetric analyses to demonstrate that the deglycosylated rh G-CSF is more sensitive to denaturation by heat than the intact form. The *in vitro* stability of both glycosylated and non-glycosylated rh G-CSF was assessed in normal human serum [8]. The authors found that the glycosylated cytokine retained more than 80% of its activity even after 8 hours of incubation. In contrast, the non-glycosylated form was markedly unstable in human serum, but displayed little or no loss of activity when incubated in heat-treated serum for 8 hours. This suggested the involvement of heat-sensitive enzymes, such as proteinases. Thus, to further determine whether non-glycosylated rh G-CSF is degraded by serum proteinases, both forms were incubated with human serum with or without proteinase inhibitors. The non-glycosylated rh G-CSF incubated without inhibitor had a residual activity of about 30% of the control value. When incubated in the presence of proteinase inhibitors, a residual activity of around 70% was observed. It was confirmed that non-glycosylated rh G-CSF rapidly loses its activity in normal serum due to degradation by proteinases. This phenomenon does not occur with the glycosylated rh G-CSF. More recently, another mechanism was advanced to explain why serum may inactivate rh G-CSF [9]. The main causal component was identified as being alpha-2 macroglobulin. The addition of this serum protein to rh G-CSF results in a dramatic loss of biological activity while glycosylation only reduces this loss. It would be of interest to know how alpha-2 macroglobulin and rh G-CSF interact, a point which was not discussed by the authors. It has been hypothesized that the sugar chain of human G-CSF, by being close to Cys-17, may prevent free radicals from attacking the deprotonated sulfhydryl group, by steric hindrance [10]. To further clarify the molecular basis of the stabilisation effect of saccharide moieties on rh G-CSF, the whole glycoprotein was investigated by means of sophisticated NMR analyses and compared with the non-glycosylated form [11]. These NMR data suggest that the carbohydrate moiety reduces the local mobility around the glycosylation site, which could be responsible for the stabilizing effect. More precisely, it was found that glycosylation decreases the flexibility of the loop containing Thr 133 and this loop rigidification could prevent the protein from degrading by proteolysis [11]. To conclude at this stage, *in vitro* studies have demonstrated that glycosylation has a major impact on cytokines' stability, and on their resistance to degradation. These results have practical implications regarding their clinical use; for instance, glycosylated forms are

relatively heat-resistant and can be stored at room temperature whereas non-glycosylated forms need to be stored at 4° C.

Glycosylation and receptor interaction - biological activity

The biological activity mediated by cytokines is governed by their specific interaction with target cell receptors, which trigger an intracellular biochemical cascade. Several investigators have analysed the impact of cytokine glycosylation on receptor binding capacity with subsequent biological activity.

Mancilla and colleagues [12] used a murine hybridoma cell line which specifically proliferates in response to IL-6, in order to investigate the impact of N-glycosylation on the IL-6 and IL-6 receptor interaction. Four sources of rh IL-6 were used: 2 glycosylated proteins were expressed by Chinese hamster ovarian cells while 2 other forms of non-glycosylated proteins were expressed by *Escherichia coli*. The study demonstrated that glycosylated forms of IL-6 exhibit a greater ability to stimulate hybridoma cells than non-glycosylated forms. It is surprising that glycosylated IL-6 binding to hybridoma cells is similar to that of non-glycosylated cytokine. However, the glycosylated form can displace the non-glycosylated cytokine whereas the reverse is not possible, suggesting a preferential binding of the glycosylated IL-6 to its receptor. In addition, the authors provided evidence that the ability of glycosylated IL-6 to stimulate hybridoma cell proliferation is reduced after N-glycanase treatment. More recently, Nabeshima and colleagues coupled a galactose monosaccharide with a C9 spacer to recombinant IL-1alpha in order to study the impact on its activities and to develop IL-1 with less deleterious effects [13]. In this case, the glycosylated cytokine exhibited reduced affinity to type I and type II receptors. In this study, the authors examined a variety of IL-1 activities *in vivo*, and observed that they were uniformly reduced by about 10 to 20-fold as compared with untreated IL-1alpha. In addition to the impact on cytokine stability, the role of sugar chains in the *in vitro* activity of recombinant human IL-5 was examined by Kodama and colleagues [1]. After digestion by glycosidases, the removal of N-linked sugar chains resulted in a 2- to 8-fold increase in activity, and de-O-glycosylation showed 10-fold higher activity. These results indicate that the presence of linked sugar chains reduces the activity of IL-5 and that O-linked sugars decrease activity more effectively than N-linked ones. One can regret that in this latter study no information on the interaction with receptors was provided.

Human EPO is characterized by its large carbohydrate chains which represent close to 40% of its total mass. Mature EPO possesses three N-linked oligosaccharides (at positions 24, 38 and 83) and one O-linked (at position 126) [3]. EPO constitutes an excellent model for investigating the role of sugar chains on glycoprotein since its gene and its multiple glycoforms are available. EPO-alpha and -beta are the two forms of recombinant human EPO both synthesized by Chinese hamster cells. The pharmaceutical compound is EPO-alpha. Storring and colleagues compared several batches of these EPOs and found differences in a range of lectin-binding assays and in activities, by *in vivo* and *in vitro* mouse bioassays and by immunoassay [14]. They showed that the differences in effects related to EPO isoform composition were accounted for by differences in glycosylation. In addition, Sytkowski and colleagues [15] examined the role of N-linked oligosaccharides of EPO using quantitative enzymatic deglycosylation. They found that N-deglycosylated cytokine exhibits full biological activity and potency *in vitro*. The importance of the EPO glycosylation pattern was also examined by Yamaguchi and colleagues [16] who found that removal of N-glycosylation affects the binding affinity of EPO to the receptor but does not play a major role in the *in vitro* activity, while the *in vivo* activity decreased significantly. Furthermore, Higuchi and colleagues analysed the roles of O-and N-glycosylation of EPO [17]. They confirmed that N-linked sugar chains are not required for *in vitro* activity but are strong determinants of the *in vivo* activity where they act as anchors for the essential terminal sialic acids. A linear relationship between the *in vivo* bioactivity and the sialic acid content has been observed by Morimoto and colleagues [18]. N-glycosylation of EPO may have a role *in vivo* by optimizing the maintenance of protein in the circulation. It has been shown that the limited activity of asialo-EPO is related to a rapid accumulation in the liver as a result of specific binding [19]. In contrast to N-glycosylation, the O-linked sugar chain was found to have no essential role in the biological activity of the cytokine *in vivo* or *in vitro*. This impact could be explained by interaction with the receptor [17].

Jagerschmidt and colleagues studied thrombopoietin (TPO) using site-directed mutagenesis and identified functionally important residues [20]. In various investigations they demonstrated that the O-glycosylation site located at position 110 of TPO is not necessary for *in vitro* cytokine bioactivity. However, they did not examine whether the interaction with TPO, the specific receptor, was affected by O-glycosylation or not.

Regarding G-CSFs and their clinical importance, it is surprising to note that the literature provides relatively few data about the impact of glycosylation on the binding of the cytokine to its specific receptor. Of note, Lowenberg and coworkers observed, from Scatchard analysis, that G-CSF receptor binding affinity of the glycosylated form was 3-fold higher than for the non-glycosylated form [21]. The role of G-CSF glycosylation has, however, been thoroughly explored for its impact on biological activity. Decleva and colleagues [22] compared the effects of lenograstim (glycosylated recombinant human G-CSF) and its deglycosylated counterpart (filgrastim) on superoxide production by polymorphonuclear leukocytes (PMN). Considering the triggering activity of the cytokine, no O_2 release was elicited from PMN treated with either preparation of G-CSF. Instead, a clear potentiation of TNF-induced respiratory burst was more markedly produced when preincubating the cells with glycosylated versus deglycosylated preparation. This suggests that the sugar moiety of the molecule could be of importance in improving the priming activity exerted by G-CSF on the metabolic response of PMN. A study compared the biological potency of the two recombinant human G-CSFs by performing neutrophil colony assay in normal bone marrow [23]. Neutrophil colonies were blindly scored at day 14. Of note, Lenograstim stimulated neutrophil colony formation at doses 16 times lower than non-glycosylated G-CSF. In the concentration range of 4 to 128 ng/ml, half to two-thirds of the assays with lenograstim resulted in the formation of "fairly large" (50 to 100 cells) or "large" colonies with more than 100 cells, whereas with filgrastim, such colonies were identified in just one-third of plates at concentrations of 32 and 64 ng/ml only. More recent data confirmed this potency advantage conferred by the glycosylation status of G-CSF [24]. The authors used leukapheretic products without further processing and purified $CD34^+$ cells. In the presence of stem cell factor and IL-3, the stimulating capacity of the two forms of G-CSF was tested at 1 and 10 ng/ml. For both unpurged blood and $CD34^+$ cells, lenograstim was significantly more effective than filgrastim. *In vitro* data provided by Querol and colleagues [25] suggest that the significantly greater biological activity (nucleated cell generation, $CD34^+$ cell expansion, CFU-GM production), observed when using glycosylated G-CSF could be compensated by a 10 times higher concentration of deglycosylated G-CSF. In addition, Plard and coworkers investigated the *in vivo* potency of filgrastim and lenograstim in an animal model of cyclophosphamide-induced neutropenia [26]. They found that lenograstim produced an increase in absolute neutrophil count which was consistently and significantly higher than the response observed with identical doses of filgrastim. As mentioned above, an increase in G-CSF stability has already been established with glycosylated forms [9-11]. Recently,

data were provided suggesting that the stability of the G-CSF molecule has a predominant effect on *in vitro* biological activity [25]. The continuous presence of cytokines, using either form of G-CSF, significantly increased the rate of à $CD34^+$ expansion, but the glycosylated form produced higher activity than its deglycosylated counterpart. The authors concluded that the greater biological activity was due to the better stability of the G-CSF molecule in its glycosylated form. The *in vivo* increase in activity for glycosylated forms of G-CSF may be due to higher intrinsic activity and/or higher resistance to degradation. The respective impact of both causes remains unknown. In total, there are concordant data demonstrating that glycosylation confers greater biological activity to G-CSF.

The induction of different genes by human interferon-beta (IFN-beta) leads to diverse effects through antiviral, antiproliferative and immunomodulatory activities. Two different recombinant IFN-beta products have been approved for clinical use, an N-glycosylated form with the predicted natural amino acid sequence (IFN-beta-1a), and a non-glycosylated form that carries a Met-1 deletion and a Cys-17 to Ser mutation (IFN-beta-1b). *In vitro* activity assays were performed to compare the effects of the two forms: antiviral activity, antiproliferative activity and immunomodulatory function [27]. IFN-beta-1a was at least 10 times more potent than IFN-beta-1b. The authors infer that the greater *in vitro* potency of IFN-beta-1a results from a stabilizing effect of the carbohydrate on structure. It must be emphasized that the methods used did not address whether the results were due to a difference in stability or in protein folding.

Above all, the relation between glycosylation and its biological activity appears to be inconsistent in the cytokine arena and no general rule can be inferred. For example, vascular endothelial growth factor (VEGF) can act as a real cytokine for endothelial cells as it triggers a mitogenic signal via specific receptors present on these cells. Tests were conducted to determine to what extent glycosylation (N-linked carbohydrate on asp 75) modifies the angiogenic properties of VEGF *in vitro* (microvascular endothelial cell assay) and *in vivo* (unilateral hind limb ischemia in rabbits) [28]. Interestingly, both *in vitro* and *in vivo* results led to the conclusion that the potential of VEGF to stimulate angiogenesis is unaffected by the glycosylated state of the molecule. In summary, for G-CSF and IFN-beta, glycosylation confers a clear advantage as demonstrated by various *in vitro* experiments [22-24, 27]. In contrast, for other cytokines such as TPO and

especially VEGF, the presence of grafted oligosaccharides does not affect the bioactivity of the proteins. For EPO, the results are more contrasted, with an absence of any impact of glycosylation observed *in vitro* whereas, in the *in vivo* situation, glycosylation confers an advantage to the molecule. At the other extreme, the biological activity of cytokines such as IL-5 may be hampered by the presence of glycosylation.

CLINICAL DATA

Does glycosylation modify the pharmacokinetic behaviour of cytokines?

Considering that glycosylation may affect the affinity of cytokines for their specific receptors, some pharmacokinetic differences are to be expected when administering cytokines either in the glycosylated or in the deglycosylated form. Watts and colleagues examined the pharmacokinetic behavior of lenograstim and filgrastim (non-glycosylated G-CSF) in healthy male volunteers [29]. Elimination half-lives for filgrastim and lenograstim were 2.75 hours and 2.95 hours respectively, thus providing no evidence of statistical difference. In contrast, there was a higher area under the curve with filgrastim in comparison with lenograstim (> 20%, $p < 0.05$). A recent meta-analysis underlines the fact that EPO reduces the odds of transfusion for cancer patients undergoing therapy [30]. Unfortunately, there are no comparative data available concerning the metabolism of the two cytokines which seem to have opposing effects as concerns their respective biological activity. This suggests that the difference in bio-efficacy in favor of the glycosylated form is not related to pharmacokinetic changes.

Halstenson and colleagues [31] undertook a randomised, double-blind, crossover study in which the pharmacokinetics and pharmacodynamics of intravenous and sub-cutaneous administration of EPO-alpha and EPO-beta were compared in normal male volunteers. After intravenous administration, the steady-state volume of the distribution of EPO-beta was 7.7% greater than with the alpha form ($p < 0.05$). The terminal elimination half-life after intravenous administration was 20% longer for EPO-beta as compared with EPO-alpha. After subcutaneous administration, EPO-beta showed delayed drug absorption as compared with the alpha form ($p < 0.005$). These pharmacokinetic differences were reflected in differences in pharmacodynamics since a significantly greater absolute reticulocyte response was obtained after subcutaneous EPO-beta.

One can consider that pharmacokinetic and pharmacodynamic differences between the two EPO forms were caused by differences in their glycosylation. Storring and colleagues undertook a comparison between EPO-alpha and EPO-beta [14] focussing particularly on differences in glycosylation. Epoetin was found to differ from epoetin alpha since it showed: a greater proportion of more basic isoforms, a greater proportion of EPO binding to *Erythrina cristagalli* agglutinin (which binds N-glycans with nonsialylated outer Gal beta1-4GlcNAc moieties), and isoforms with higher *in-vivo*: *in vitro* bioactivity ratios. Epoetin also displayed slightly more EPO binding to *Lycopersicon esculentum* agglutinin (which binds N-glycans containing repeating Gal beta1-4GlcNAc sequences), to the leucoagglutinin of *Phaseolus vulgaris* (which binds tetraantennary and 2,6-branched triantennary N-glycans) and to *Agaricus bisporus* agglutinin (which binds Gal beta1-3GalNAc containing O-glycans) than epoetin alpha.

Does the glycosylation of cytokines affect their clinical activity?

The impact of glycosylation on the clinical efficacy of cytokines has been almost exclusively explored for G-CSF, both in healthy volunteers and in cancer patients. The biological end point of most comparative studies was obtaining of circulating $CD34^+$ cells which is an indicator of G-CSF action on bone marrow.

Two published studies concern healthy volunteers. A randomised study was undertaken, in which the same dose of G-CSF was given either in the glycosylated (lenograstim) or in the non-glycosylated form (filgrastim) [32]. The study involved 5 consecutive days of treatment with G-CSF (10 mug/kg/d) followed by a washout period of 4 weeks. A crossover design was scheduled after this first step. The $CD34^+$ and CFU-GM peaks observed on day 5-6 were significantly higher in the lenograstim group (104 $CD34^+$ cells/mul ± 38 and 14.6 CFU-GM/mul ± 8.4) as compared with the filgrastim group (82 $CD34^+$ cells/mul ± 35 and 10.2 CFU-GM/mul ± 4.6). Watts and colleagues undertook a similar study at a different dose level (5 mug/kg/d) [29]. Again, the production of $CD34^+$ was higher in the lenograstim group, but in this case the difference did not reach statistical significance. At this stage, clearly, the two G-CSFs are not bioequivalent and, given at weight equivalence doses, lenograstim is superior to filgrastim.

A non-randomised study was conducted in 101 patients with lymphoma using a peripheral blood progenitor cell mobilisation procedure based on

cyclophosphamide administration (1,500 mg/m^2 day 1) and r G-CSF (starting day 2). The end point was to reach 5,000 WBC/mm^3 [33]. Forty-three patients received filgrastim at a dose of 10 mug/kg/d and 58 received lenograstim at a dose of 263 mug/d, which corresponds to 3.5 mug/kg/d. The efficacy of deglycosylated G-CSF, given at a higher ponderal dose (x 3), was found to be similar to that of the glycosylated form. As confirmation, De Arriba and colleagues [34] studied 30 patients with breast cancer in a prospective study comparing the *in vivo* priming effect of lenograstim and filgrastim given at bioequivalent doses: 15 patients received filgrastim at a mean dose of 0.84 ± 0.01 mU/kg/d, and lenograstim was administered to 15 patients at a mean dose of 0.82 ± 0.02 mU/kg/d. The results clearly showed non-significant differences in the release of WBC, monocytes, total $CD34^+$ cells and immature subfractions of $CD34^+$. No significant differences between the two recombinant molecules were found either as regards the content of total $CD34^+$, $CD34^+/38^-$ and $CD34^+/DR^-$ cell subsets in the leukapheresis product or the cost of the mobilisation and collection procedures. Altogether, these results suggest that priming with equivalent biological doses of glycosylated or non-glycosylated G-CSF formulated for clinical use has an identical impact on peripheral blood progenitor cells. However, further studies with a larger number of subjects treated at bioequivalent doses are needed to draw a definitive conclusion concerning clinical differences between glycosylated and non-glycosylated G-CSF [35].

CONCLUSION

From examination of experimental data, one can conclude that no general rule can be inferred concerning the effects induced by the presence of glycosylation on cytokine activity. In most experimental studies, when glycosylation affects cytokine behavior and its subsequent biological activity, the influence is evident with a 2- or 3-fold difference in favour of the glycosylated form. Nonetheless, for many cytokines, several different *in vitro* assays have been used and clear-cut conclusions are difficult to draw on these bases. The use of specific or general glycosylation pathway inhibitors should be helpful to further define glycosylation as a parameter of cytokine regulation.

The use of cytokines has definitively proven clinical benefits, particularly in oncology. Only data concerning G-CSF are available to translate experimental data into clinical evidence regarding glycosylation status of cytokines. Clearly, glycosylation confers advantages for both stability and biological activity.

Although, at weight equivalence, the glycosylated form confers greater biological activity than the non-glycosylated form, clinical studies have demonstrated that efficacy remains the same when glycosylated or non-glycosylated G-CSF are prescribed at bioequivalent doses.

Glycolysation definitely emerges as an important factor for the modulation of cytokine activity, but no definitive general conclusion can be drawn regarding the clinical impact of cytokine glycolysation.

References

1. Kodama S, Tsujimoto M, Tsuruoka N, Sugo T, Endo T, Kobata A. 1993. Role of sugar chains in the *in vitro* activity of recombinant human interleukin 5. *Eur. J. Biochem.* 211: 903.

2. Karpusas M, Whitty A, Runkel L, Hochman P. 1998. The structure of human interferon beta: implications for activity. (Review) *Cell. Mol. Life Sci.* 54: 1203.

3. Lai P H, Everett R, Wang F F, Arakawa T, Goldwasser E. 1986. Structural characterization of human erythropoietin. *J. Biol. Chem.* 261: 3116.

4. Tsuda E, Kawanischi G, Ueda M, Masuda S, Sasaki R. 1990. The role of carbohydrate in recombinant human erythropoietin. *Eur. J. Biochem.* 188: 405.

5. Endo Y, Nagai H, Watanabe Y, Ochi K, Takagi T. 1992. Heat-induced aggregation of recombinant erythropoietin in the intact and deglycosylated states as monitored by gel permeation chromatography combined with a low-angle laser light scattering technique. *J. Biochem.* (Tokyo) 112: 700.

6. Uchida E, Morimoto K, Kawasaki N, Isaki Y, Abdu Said A, Hayakawa T. 1997. Effect of active oxygen radicals on protein and carbohydrate moieties of recombinant human erythropoietin. *Free Rad. Res.* 27: 311.

7. Oh-Eda M, Hasegawa M, Hattori K, Kuboniwa H, Kojima T, Orita T, Tomonou K, Yamazaki T, Ochi N. 1990. O-linked sugar chain of human granulocyte colony-stimulating factor protects it against polymerisation and denaturation allowing it to retain its biological activity. *J. Biol. Chem.* 265: 11432.

8. Kishita M, Motojima H, Oh-Eda M, Kojima T, Kamachi S, Fukushima M, Ono M. 1992. Stability of granulocyte colony stimulating factor in serum. *Clin. Report* 26: 221.

9. Mire-Sluis A R, Abernethy H A, Dolman C, Thorpe R. 1999. The effects of environmental influences and post-translational modification on the biological activity of granulocyte colony-stimulating factor. *Pharm. Pharmacol. Commun* 5: 45.

10. Hasegawa M A. 1993. Thermodynamic model for denaturation of granulocyte colony-stimulating factor: O-linked sugar chain suppresses not the triggering deprotonation but the succeeding denaturation. Biochem. *Biophys. Acta* 1203: 295.

11. Gervais V, Zerial A, Oschkinat H. 1997. NMR investigations of the role of the sugar moiety in glycosylated recombinant human granulocyte - colony - stimulating factor. *Eur. J. Biochem.* 247: 386.

12. Mancilla J, Schlinder R, Dinarello CA. 1990. Importance of glycosylation for receptor binding and biological activity of IL-6. *Molecular and Cellular Biology of cytokines,* Wiley-Liss, p. 51-56.

13. Nabeshima S, Chiba T, Takei Y, Ono A, Moriya K, Onozaki K. 1998. Development of glycosylated human interleukin-1 alpha, neoglyco IL-1alpha coupled with D-galactose monosaccharide: biological activities *in vivo*. *Glycoconj. J.* 15: 491.

14. Storring P L, Tiplady R J, Gaines Das R E, Stenning B E, Lamikanra A, Rafferty B, Lee J. 1998. Epoietin alpha and beta differ in their erythropoietin isoform compositions and biological properties. *Br. J. Haematol.* 100: 79.

15. Sytkowski A J, Feldman L, Zurbuch D J. 1991. Biological activity and structural stability of N-deglycosylated recombinant human erythropoietin. *Biochem. Biophys. Res. Commun.* 176: 698.

16. Yamaguchi K, Akai K, Kawanishi G, Ueda M, Masuda S, Sasaki R. 1991. Effects of site-directed removal of N-glycosylation sites in human erythropoietin on its production and biological properties. *J. Biol. Chem.* 266: 20434.

17. Higuchi M, Oh-Eda M, Kuboniva H, Tomonoh K, Shimanoka Y, Ochi N. 1992. Role of sugar chains in the expression of the biological activity of human erythropoietin. *J. Biol. Chem.* 267: 7703.

18. Morimoto K, Tsuda E, Said A A, Uchida E, Hatakeyama S, Ueda M, Hayakawa T. 1996. Biological and physicochemical characterization of recombinant human erythropoietins fractionated by Mono Q column chromatography and their modification with sialytransferase. *Glycoconj. J.* 13: 1013.

19. Fukuda M, Sasaki H, Lopez L, Fukuza M. 1989. Survival of recombinant erythropoietin in the circulation: the role of carbohydrates. *Blood* 73: 84.

20. Jagerschmidt A, Fleury V, Anger-Leroy M, Thomas C, Agnel M, O'Brien D P. 1998. Human thrombopoietin structure - function relationships: identification of functionally important residues. *Biochem. J.* 333: 729.

21. Lowenberg B. 1997. Proliferative activity of rG-CSFs in cell line bioassays. *Int. J. Hematol.* 96: 418.

22. Decleva E, Cramer R, Zabucchi G. 1995. Glycosylation improves the priming effect exerted by recombinant human granulocyte colony - stimulating factor (lenograstim) on human neutrophil superoxide production. *Int. J. Tissue React.* 17: 191.

23. Nissen C, Dalle Carbonare V, Moser Y. 1994. *In vitro* comparison of the biological potency of glycosylated versus nonglycosylated r G-CSF. *Drug Invest.* 7: 346.

24. Pedrazzoli P, Gribelli N, Pavesi L, Preti P, Piolini M, Bertolini F, Robustelli Della Cuna G. 1996. Effects of glycosylated and non-glycosylated G-CSFs, alone and in combination with other cytokines, on the growth of human progenitor cells. *Anticancer Drugs* 16: 1781.

25. Querol S, Cancelas J A, Amat L, Capmany G, Garcia J. 1999. Effect of glycosylation of recombinant human granulocytic colony - stimulating factor on expansion cultures of umbilical cord blood $CD34^+$ cells. *Haematologica* 84: 493.

26. Plard J P, Nohynek G J, Wells M Y, Zerial A. 1996. Comparison of the potency of glycosylated and non - glycosylated recombinant granulocyte colony - stimulating factors (rHuG - CSF) in neutropenic (cyclophosphamide - treated) CD rats. *Br. J. Haematol.* 93: 360.

27. Runkel L, Meier W, Pepinsky R B, Karpusas M, Whitty A, Kinball K, Brickelmaier M, Muldowney C, Jones W, Croelz S E. 1998. Structural and functional differences between glycosylated and non-glycosylated forms of human interferon-beta (IFN-beta). *Pharm. Res.* 15: 641.

28. Walter D H, Hink U, Asahara T, Van Belle E, Horowitz J, Tsumuri Y, Vandlen R, Heinsohn H, Keyt B, Ferrara N, Symes J F, Isner J M. 1996. The *in vivo* bioactivity of vascular endothelial growth factor/vascular permeability factor is independent of N-linked glycosylation. *Lab. Invest.* 74: 546.

29. Watts M J, Addisson I, Long S G, Hartley S, Warrington S, Boyce M, Linch D C. 1997. Crossover study of the haematological effects and pharmacokinetics of glycosylated and non-glycosylated G-CSF in healthy volunteers. *Br. J. Haematol.* 98: 474.

30. Seidenfeld J, Piper M, Flamm C, Hasselblad V, Armitage J O, Bennett C L, Gordon M S, Lichtin A E, Wade J L 3rd, Woolf S, Aronson N. 2001. Epoetin treatment of anemia associated with cancer therapy: a systematic review and meta-analysis of controlled clinical trials. *J. Natl. Cancer Inst.* 93: 1204.

31. Halstenson C E, Macres M, Katz S A, Schnieders J R, Watanabe M, Sobota J T, Abraham P A. 1991. Comparative pharmacokinetics and pharmacodynamics of epoetin alpha and epoetin beta. *Clin. Pharmacol. Ther.* 50: 702.

32. Höglund M, Smedmyr B, Bengtsson M, Tötterman T H, Cour-Chabernaud V, Yver A, Simonsson B. 1997. Mobilization of $CD34^+$ cells by glycosylated and nonglycosylated G-CSF in healthy volunteers - a comparative study. *Eur. J. Haematol.* 59: 177.

33. Watts M J, Sullivan A M, Jamieson E, Pearce R, Fielding A, Devereux S, Goldstone A H, Linch D C. 1997. Progenitor-cell mobilization after low-dose cyclophosphamide and granulocyte colony-stimulating factor: an analysis of

progenitor-cell quantity and quality and factors predicting for these parameters in 101 pretreated patients with malignant lymphoma. *J. Clin. Oncol.* 15: 535.

34. de Arriba F, Lozano M L, Ortuno F, Heras I, Moraleda J M, Vicente V. 1997. Prospective randomized study comparing the efficacy of bioequivalent doses of glycosylated and nonglycosylated rG-CSF for mobilizing peripheral blood progenitor cells. *Br. J. Haematol.* 96: 418.

35. Martin-Christin F. 2001. Granulocyte colony stimulating factors: how different are they? How to make a decision? (Review) *Anticancer Drugs* 12: 185.

Figures

Table
Impact of glycosylation on cytokine effects
– synthetic view –

	Experimental data				Clinical data			
	Stability	Ref	Biological activity	Ref	Pharmacokinetics	Ref	Clinical activity	Ref
IL-1			O-deglycosylation ↑↑ N-deglycosylation ↑	[1]				
IL-5	O-deglycosylation ↔ N-deglycosylation ↓	[1]						
IL-6			Deglycosylation ↓	[12]				
INFβ	Deglycosylation ↓	[2]	N-deglycosylation ↓	[27]				
EPO	N-and O-deglycosylation ↓	[4, 5]	N-deglycosylation ↓ O-deglycosylation ↔	[17]	Terminal $t_{1/2}$ longer for EPOβ/EPOα	[31]		
G-CSF	Deglycosylation ↓	[8]	deglycosylation ↓	[22-26]	– similar terminal $t_{1/2}$ for glycosylated and deglycosylated forms – higher serum exposure for the deglycosylated form	[29]	Deglycosylation ↓	[32-34]
TPO			O-deglycosylation ↔	[20]				
VEGF			Deglycosylation ↔	[28]				

IL: interleukin; IFN: interferon; EPO: erythropoietin; G-CSF: granulcoyte colony-stimulating factor; TPO: thrombopoietin; VEGF: vascular endothelial growth factor. ↑: increased function; ↔: unchanged function; ↓: loss function. $t_{1/2}$: elimination half-life.

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thioredoxin, monocytes, MCP-1.

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