Glycoconjugate Journal 15, 491–498 (1998)

Development of glycosylated human interleukin-1*α*, neoglyco IL-1*α*, coupled with D-galactose monosaccharide: biological activities *in vivo*†

Sachi Nabeshima, Taku Chiba, Yutaka Takei, Asako Ono, Kayoko Moriya, and Kikuo Onozaki*

Department of Hygienic Chemistry, Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabe, Mizuho, Nagoya 467 Japan

In our previous study, a galactose monosaccharide with C9 spacer was chemically coupled to recombinant human interleukin 1*α* (rhIL-1*α*) in order to study the effect of glycosylation on its activities, and to develop IL-1 with less deleterious effects. The glycosylated IL-1*α* exhibited reduced activities *in vitro* by 10 to 10 000-fold depending upon different aspects of activities addressed. The affinity to type I and II IL-1 receptors were also reduced. In this study we examined a variety of IL-1 activities *in vivo*, including upregulation of serum levels of IL-6, *α*1-acid glycoprotein, NOx, corticosterone, downregulation of serum level of glucose, and recovery of peripheral white blood cells (WBCs) from myelosuppression in 5-fluorouracil-treated mice. In contrast to the biological activities *in vitro*, these activities *in vivo* were uniformly reduced by only about 10 to 20-fold compared to untreated IL-1*α*.

***Keywords*: neoglycoprotein, interleukin 1, cytokine**

Introduction

Carbohydrates function as a ligand for specific binding proteins present in serum and lectins on the cell surface [1]. Carbohydrates also play a crucial role in the biological activity of glycosylated growth factors, hormones and cytokines, including erythropoietin and gonadotropin [2, 3]. Carbohydrates in several cytokine receptors, such as interferon and IL-1 receptors (IL-1R), contribute to their ligand binding affinity [4, 5]. Pharmacokinetic dynamics of the macromolecules, including proteins, is known to depend on their molecular size and carbohydrate moieties [6]. Compounds with less than 20 kDa exhibit large urinary clearances which are close to the glomerular filtration rate, while macromolecules larger than 70 kDa are eliminated in the circulation by hepatic uptake which is mediated through the carbohydrate moiety. It therefore seems possible to alter or modify the biological activities through modifications of clearance and tissue distribution by the introduction of various carbohydrates into bioactive proteins. Although several methods have been developed to construct neoglycoproteins [7–9], few attempts have been made in their application to biological active proteins [10, 11].

Interleukin-1 (IL-1) is a cytokine involved in immune and inflammatory responses, hematopoiesis, and homeostatic reactions [12]. Although IL-1 has beneficial effects in treatment of cancer patients through its direct antiproliferative effect on some tumours and indirect effects through augmenting host defence and haematopoiesis, its therapeutic use is limited by the toxicity, including hypotension, fever, anorexia and diarrhea. Human IL-1 is nonglycosylated. To develop neoIL-1 with less deleterious effects while preserving its beneficial effects, we have chemically introduced carbohydrate moieties to human recombinant (rh) IL-1α [13–17].

In our previous study, we conjugated chemically synthesized α-D-Man-1-6-D-Man[Man_2α(1-6)] into rhIL-1α. Man_2α(1-6) IL-1α exhibited reduced biological activities *in vitro* by 10 to 1000-fold [13], however, it preserved some activities *in vivo* [14]. In addition, in mice Man_2α(1-6) IL-1α distributed more to liver and less to kidney than untreated IL-1α [15].

Lectins specific to galactose/*N*-acetyl galactosamine (Gal/GalNAc) are present in hepatocytes [18, 19] and macrophages [20]. It has also been reported that Gal/GalNAc-specific C-type lectin is expressed on the cell surface of inflammatory macrophages and on activated tumoricidal macrophages [21]. It was, therefore, expected that an introduction of Gal into IL-1 may alter the activity through its interaction with Gal/Gal-specific lectin *in vivo*. In the previous studies we synthesized D-Gal-conjugated rhIL-1α

* To whom correspondence should be addressed. Tel.: 81-52-836-3419; Fax: 81-52-835-3419; E-mail: konozaki@phar.nagoya-cu.ac.jp
† Refer also to Chiba *et al.*, 1998 *Glycoconjugate J.* **15**, 63–67 and Nabeshima *et al.*, 1998 *Glycoconjugate J.* **15**, 69–74

[16]. IL-1α coupled with averagely 9.1 Gal molecules exhibited reduced activities by 10 to 10 000-fold compared with untreated IL-1α in the activities performed *in vitro* [17]. Its binding affinities to type I and type II IL-1 receptors were also decreased by 500 and 100-fold, respectively. In this study we determined the biological activities of Gal-IL-1α *in vivo*.

Materials and methods

Animals

ICR female mice (6 weeks old) were purchased from the Japan SLC Co. (Hamamatsu, Japan) and fed ad libitum and housed in temperature- and light-controlled (12 h day) rooms. Mice were used in experiments after 1 week of acclimatization.

Reagents

RPMI 1640 was purchased from Sigma Chemical Co. (St Louis, MO) and fetal bovine serum (FBS) from JRH Biosciences (Lenexa KS). PBS was purchased from Nissui Seiyaku Co. (Tokyo, Japan). rhIL-1α (2×10^7 U mg^{-1}) was provided by Dr Yamada (Dainippon Pharmaceutical Co., Osaka, Japan). Human recombinant IL-6 was provided by Dr Y. Akiyama (Ajinomoto Co., Yokohama, Japan).

Cell culture

A murine hybridoma clone MH60·BSF2 provided from Dr T. Hirano (University of Osaka) was maintained in culture medium (RPMI 1640, 100 U ml^{-1} of penicillin G 100 μg ml^{-1} of streptomycin, and 10% heat-inactivated FBS) containing 1 U ml^{-1} of rhIL-6 [22].

Synthesis of galactosylated IL-1α

Acyl azide derivative of galactose with C9 spacer was synthesized and introduced to rhIL-1α as described previously [16]. The galactosylated IL-1α was purified by anion-exchange chromatography, and the galactose-coupling was confirmed by *R. communis* lectin blotting. Based on the molecular weight, the average number of carbohydrate molecules introduced was 9.1 per molecule IL-1α. By endotoxin test using *Limulus amoebocyte* assay (sensitivity limit, 0.1 ng ml^{-1}), the endotoxin contamination of the galactosylated IL-1α was negative.

Measurement of serum levels of IL-6, glucose, α1-acid glycoprotein, NOx and corticosterone

IL-1αs were diluted to the desired concentration with sterilized PBS and administered to mice intraperitoneally. Although the samples were endotoxin negative, to prevent the effect of an undetectable amount of endotoxin, polymyxin B was added at 5 μg ml^{-1}. Mice were fasted after the administration. At the times indicated for the experiment the mice were bled. IL-6 activity in serum was measured by proliferation assay with IL-6-dependent MH60·BSF2 cells [22]. The amount of IL-6 was expressed as the equivalent amount of rhIL-6.

The glucose levels in serum was determined using a glucose B-test kit (Wako Pure Chemical Industries, Ltd, Osaka, Japan).

α1-acid glycoprotein (α1-AGP) concentration in serum was measured using a mouse IAP kit (Sanko Jyunyaku Co., Ltd., Tokyo, Japan).

NOx levels in serum were measured by the method described by Misko TP *et al.* [23]. Briefly, 30 μl of each sample were incubated for 15 min at 37 °C with 10 μl of the nitrate reductase (2.5 U ml^{-1}; Boehringer Mannheim) and 10 μl nicotinamide-adenine dinucleotide phosphate (2 mM; Sigma Chemical Co.). After incubation, 50 μl of Griess reagent and 50 μl of TCA (10% aqueous solution) were added. Protein precipitates were removed by centrifugation at 15 000 rpm for 5 min and 50 μl of each supernatant were transferred to a 96-well plate (Falcon) and the O.D.595 nm was measured using an ELISA autoreader (Bio-Rad Laboratories, Richmond, CA).

Corticosterone level in serum was measured by using a Rat corticosterone [^{125}I] assay system with magnetic separation (Amersham, Aylesbury, UK).

Haematology

Peripheral blood samples (10 μl) of mice obtained through the tail vein were added to 90 μl of Türk solution (Wako Pure Chemical Industries, Ltd, Osaka, Japan). The white blood cell (WBC) number was counted microscopically using a haemacytometer.

Determination of protein content

The amount of protein was determined using a protein assay kit (Bio-rad, Richmond, CA) with bovine serum albumin as a standard.

Statistical analysis

Differences between group means were assessed by *t* test.

Results

Ability of IL-1 to induce serum IL-6 in mice

Mice were injected intraperitoneally with untreated IL-1α or galactosylated IL-1α (Gal-IL-1α) and serum IL-6 level was determined. Mice injected with untreated IL-1α exhibited a sharp increase in IL-6 levels with a maximum level 2 h after IL-1 treatment (Figure 1A). The elevation of serum IL-6 was statistically significant with an injection of 0.1 μg untreated IL-1α per mouse (Figure 1B). A dose experiment indicated that Gal-IL-1α exhibited about 1/20 activity of untreated IL-1α.

Effect of IL-1 treatment on serum glucose level in mice

The effect of IL-1 on serum glucose level was examined. Mice were injected intraperitoneally with untreated IL-1α

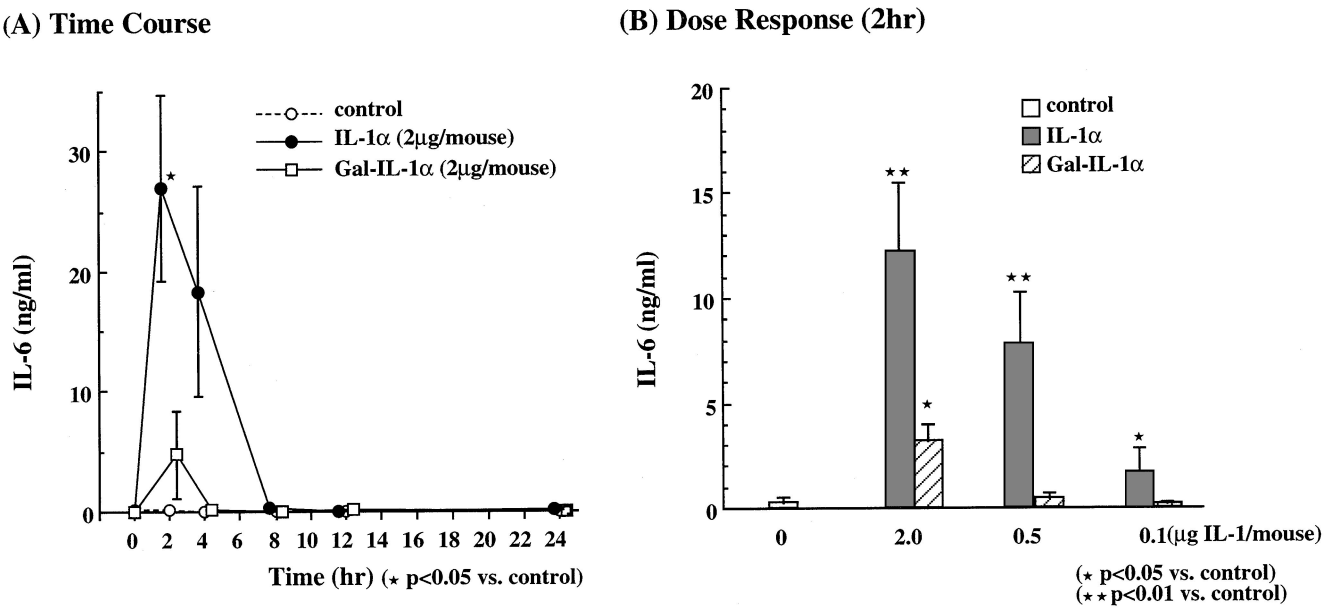

Figure 1. Effect of IL-1 on serum IL-6 level in mice. IL-1αs were intraperitoneally administered and bled at the time indicated (A) or 2 h later (B). Serum IL-6 level was determined by MH60·BSF-2 bioassay. Each point represents the mean ± SD of determinations from ten (A) and five (B) animals.

or Gal-IL-1α, and then fasted. In control mice the serum glucose level decreased with duration of fasting (Figure 2A). Untreated IL-1α caused a statistically significant reduction at 2 h and 4 h after treatment. Gal-IL-1α also caused a reduction at 2 h after treatment, but at 4 h after treatment the glucose level was not significantly different from the control level. A dose-response experiment confirmed the weak potency of Gal-IL-1α–about 1/20 activity of untreated IL-1α (Figure 2B).

Ability of IL-1 to induce serum α1-AGP in mice

As we expected that Gal-IL-1α may accumulate in the liver through the Gal-specific lectin and exhibit increased activity, mice were injected intraperitoneally with untreated IL-1α or galactosylated IL-1α, and their ability to induce α1-AGP, a major product of hepatocytes in response to IL-1, was examined. After a single injection of 2.0 μg per mouse, untreated IL-1α increased the α1-AGP level 8 h after injection, and the increase continued up to 24 h, whereas Gal-IL-1α only slightly increased the level (Figure 3A). A dose-response experiment indicated that Gal-IL-1α exhibited 1/10 to 1/20 activity of untreated IL-1α (Figure 3B).

Ability of IL-1 to induce serum NOx in mice

IL-1 induces nitric oxide (NO) synthesis in a variety of cell types. The nitric oxide generated reacts with molecular oxygen and water, and subsequently nitrite and nitrate accumulated in biological fluids [23]. To examine the ability of IL-1 to induce NO, serum nitrate was converted to nitrite by nitrate reductase, and then the amount of total nitrite was determined. Untreated IL-1α injected mice exhibited an increase in serum NOx level with a maximum level 8 h after IL-1 treatment, and the level returned to control level after 24 h (Figure 4A). On the other hand, Gal-IL-1α exhibited a very weak potency of the induction 4 and 8 h after injection. After 12 h the level was not different from that of the control. A dose–response experiment after 4 h treatment showed that Gal-IL-1α exhibited about 1/20 activity of untreated IL-1α (Figure 4B). Although the data was not shown, a similar reduction in the activity of Gal-IL-1α was observed after 8 h.

Effect of IL-1 treatment on serum glucocorticoid level in mice

IL-1 stimulates the hypothalamus–pituitary–adrenal axis which results in an increase of glucocorticoid [24]. Corticosterone is the major element of glucocorticoid in mice, therefore, we examined the corticosterone level of IL-1 treated mice. Mice injected with untreated IL-1α exhibited a sharp increase in serum corticosterone level with a maximum 2 h after IL-1 treatment (Figure 5A). The elevation was statistically significant with an injection of 0.1 μg untreated IL-1α per mouse. On the other hand, Gal-IL-1α exhibited a very weak potency of induction, and a dose–response experiment showed about 1/20 activity of untreated IL-1α (Figure 5B).

Recovery of myelosuppression as a result of IL-1α treatment in 5-fluorouracil-treated mice

We determined the ability of Gal-IL-1α to recover the peripheral white blood cell (WBC) counts in 5-fluorouracil

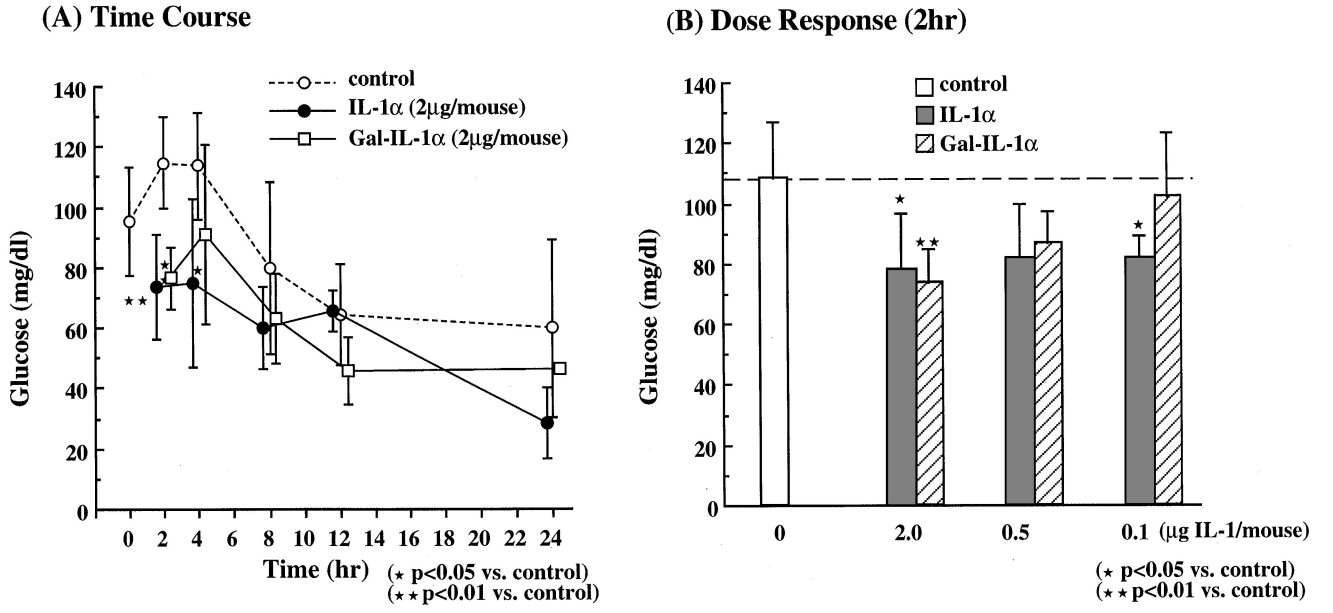

Figure 2. Effect of IL-1 on serum glucose level in mice. IL-1*a*s were intraperitoneally administered and bled at the time indicated (A) or 2 h later (B). Serum glucose level was determined using the glucose B-test (Wako). Each point represents the mean ± SD of determinations from ten (A) and five (B) animals.

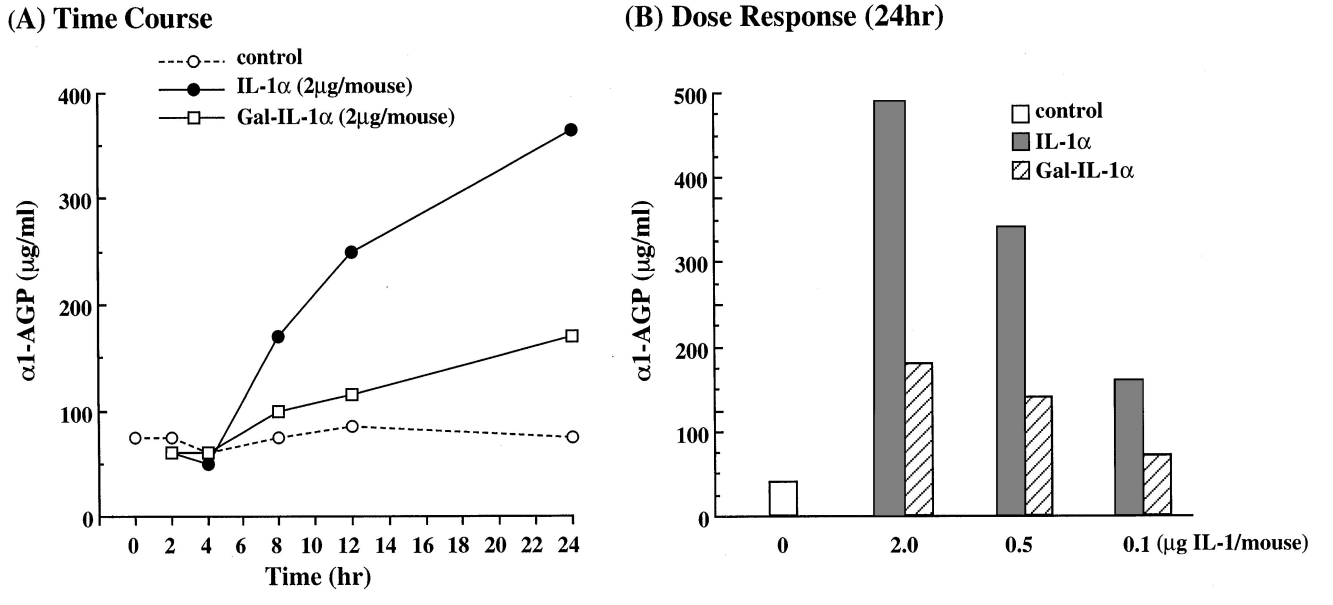

Figure 3. Effect of IL-1 on serum *a*1-AGP level in mice. IL-1*a*s were intraperitoneally administered and bled at the time indicated (A) or 24 h later (B). Serum *a*1-AGP level was determined using a mouse IAP kit. Each point represents the value in pooled sera from five animals.

(5-FU) treated mice. Mice were injected with 5-FU (12 mg per mouse) as a single dose intravenously on day 0. From day 1, IL-1αs were administered intraperitoneally or intravenously twice a day (400 or 40 ng per day). In every mouse, a decrease in WBCs in peripheral blood occurred 1–3 days after 5-FU treatment (data not shown). At day 10 after administration of PBS into mice (control), the number of WBCs in peripheral blood was $8.46 \pm 1.4(\times 10^3$ cells per $nm^3)$. As shown in Figure 6A, when IL-1αs were injected intraperitoneally, untreated IL-1α at either dosage caused a recovery in the level of the WBC count. Untreated IL-1α at 400 ng per day increased the level over control. Gal-IL-1α at 400 ng per day also caused the recovery over PBS administration in 5-FU treated mice. Although it was not

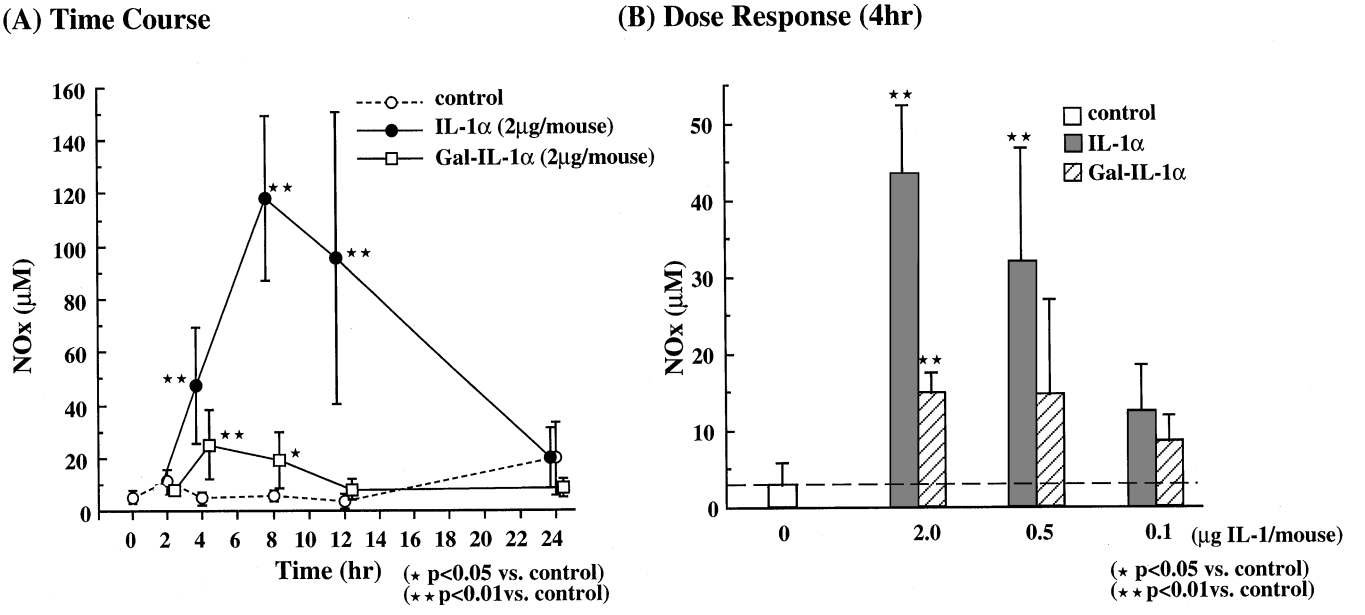

Figure 4. Effect of IL-1 on serum NOx level in mice. IL-1*a*s were intraperitoneally administered and bled at the time indicated (A) or 4 h later (B). Serum NOx level was determined as described in materials and methods. Each point represents the mean ± SD of determinations from five animals.

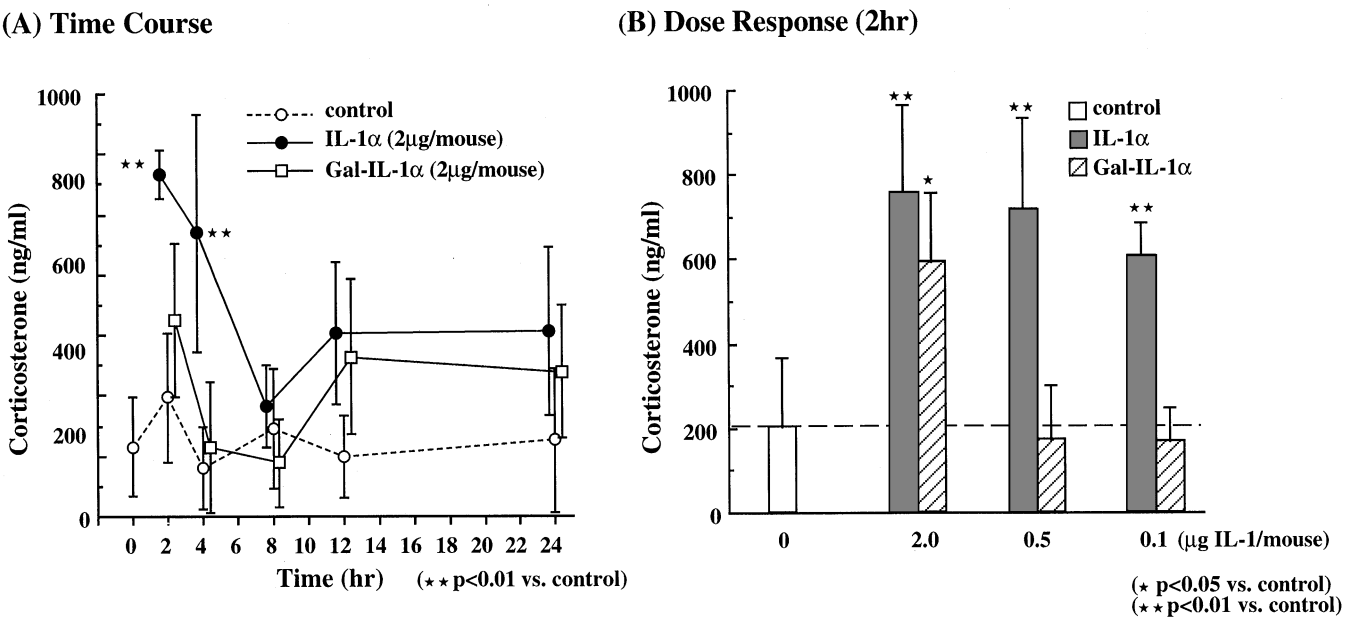

Figure 5. Effect of IL-1 on serum corticosterone level in mice. IL-1*a*s were intraperitoneally administered and bled at the time indicated (A) or 2 h later (B). Serum corticosterone level was determined using rat corticosterone [^{125}I] assay system (Amersham). Each point represents the mean ± SD of determinations from five animals.

significant, Gal-IL-1α at 40 ng ml^{-1} tended to cause the recovery of WBC level. When IL-1αs were injected intravenously, the IL-1 effect was more evident (Figure 6B). Gal-IL-1α at either dosage caused the recovery over PBS administration in 5-FU treated mice, and showed about 1/10 activity of untreated IL-1α.

Discussion

In the present study, Gal-IL-1α appeared to exhibit uniformly decreased activities *in vivo* by about 10 to 20-fold compared to untreated IL-1α. Compared to the *in vitro* system, that of *in vivo* seems to be more complex. Lectins

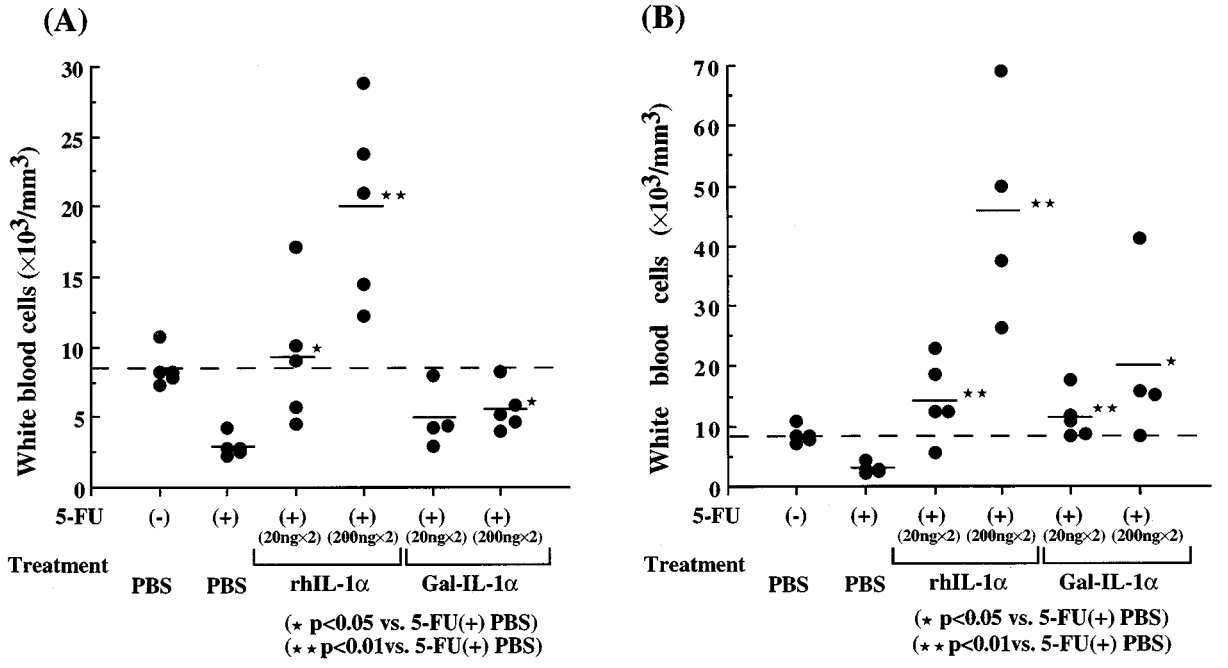

Figure 6. Recovery of myelosuppression as a result of IL-1*a* treatment in 5-fluorouracil (5-FU)-treated mice. Mice were received 5-FU (12 mg per mouse) as a single dose intravenously on day 0. IL-1*a*s (400 or 40 ng day^{-1}) were administrated intraperitoneally (A) or intravenously (B) twice daily from day 1 to day 10. PBS was administrated intraperitoneally in the same manner. Each group consisted of five mice. On day 10, animals were bled through the tail vein, blood was suspended to the Türk solution, and the number of WBCs was counted. The mouse receiving Gal-IL-1*a* (40 ng day^{-1}, i.p.) accidentally died on day 1. Two mice, each receiving 5FU + PBS or Gal-IL-1*a* (400 ng day^{-1}, i.v.) died on day 7 from wasting.

specific to galactose/*N*-acetyl galactosamine (Gal/GalNAc) are present in hepatocytes and macrophages [18–21]. Therefore, we expected that the introduction of galactose may affect the biological activities *in vivo*, especially in the activities where hepatocytes or macrophages are involved. Gal-conjugated superoxide dismutase (Gal-SOD) is reported to distribute more in the liver and exhibits an augmented protective effect against hepatic injury induced by ischemia/reperfusion in rats [11]. In this study, among the activities examined *in vivo*, induction of IL-6, α1-AGP and glucocorticoid and decrease of serum glucose are related to hepatocyte function. IL-1 alone or in synergy with IL-6 or glucocorticoid induces acute phase proteins [25]. α1-AGP is the representative produced by hepatocytes in response to these stimuli. The activity of Gal-IL-1α in induction of α1-AGP, however, appeared to be weak compared to original IL-1. Prolongation of the IL-1 effect was not observed. Probably it was due to the weak ability in induction of IL-6 and glucocorticoid. Our findings are in contrast to those of Gal-SOD, probably because of the differences in the requirement of specific receptors; activity of IL-1 is mediated through interaction with IL-1R, while that of SOD is not. In addition, it is possible that uptake of Gal-IL-1α into hepatocytes is enhanced by Gal/GalNAc-specific lectin present in the hepatocytes, and the endocytosed IL-1 may be trapped in the endosome, subsequently its degradation is enhanced. The possibility is also not ruled out that the difference is due to the different route of administration; Gal-SOD and Gal-IL-1α were administrated intravenously and intraperitoneally, respectively.

In response to IL-1, serum level of glucose decreases. Whether insulin is involved in the IL-1 dependent serum glucose reduction is controversial [26]. A hypothesis is postulated that IL-1 induces an accumulation of platelets in the liver, then serotonin is released from the platelets, and subsequently serotonin decreases the serum glucose level [27]. Gal-IL-1α exhibited the weak activity. In contrast to Gal-IL-1α, Man_2α(1-6) IL-1α exerted the same potency as untreated IL-1α in the activity [14]. Although the reason is not known, it may be due to the different distribution of these IL-1s in the liver. As described modification with galactose may enhance the uptake of Gal-IL-1α by hepatocytes, thus its degradation may be enhanced. While Man_2α(1-6) IL-1α may resist them through interaction with Man-specific lectin present in macrophages.

Nitric oxide is an important mediator in neurotransmission, vasodilation and host defence against microorganism and tumour cells. IL-1 alone or in synergy with tumour necrosis factor and interferon augments the production of NO from many cell types, including macrophages, hepatocytes, vascular endothelial cells and smooth muscle cells [28]. This is especially true of NO produced by smooth muscle cells which in conjunction with PGI_2 produced by endothelial cells, is implicated in the hypotension caused by

IL-1 [29]. Gal-IL-1α appeared to be weak in the induction of serum NOx level. This finding would be useful in the therapeutic use of Gal-IL-1α because Gal-IL-1α would be weak in the induction of hypotension. However, a disadvantage is that Gal-IL-1α will be weak in host defence against infection of microorganisms and tumours.

IL-1 stimulates the hypothalamus–pituitary–adrenal axis which results in an increase of glucocorticoid [24]. IL-1 also induces glucocorticoid by directly stimulating the adrenal cortex [30]. Glucocorticoid, thus induced plays an important role in the feedback system against IL-1 activity by inhibiting both production and function of IL-1. Serum glucocorticoid inducing activity was also weak in Gal-IL-1α.

One of the beneficial effects of IL-1 is the enhancement of recovery of peripheral WBCs in chemotherapeutic drug-treated animals [31]. IL-1 is known to increase the survival of early progenitor cells and to enhance multipotential colony formation through the induction of several haematopoietic growth factors, such as granulocyte- and granulocyte-macrophage colony stimulating factors, IL-3, and IL-6 [12, 32]. IL-1 also enhances the sensitivity of progenitor cells to the growth factors through upregulation of their receptors [33]. Although it was weak compared to untreated IL-1α, Gal-IL-1α enhanced the recovery of peripheral WBCs reduced by 5-FU. The activity, however, was especially remarkable when administrated intravenously. As untreated IL-1α also exhibited more potent activity when administrated intravenously than intraperitoneally, it is possible that IL-1α either untreated or glycosylated is trapped in the route from peritoneal cavity to bone marrow. Gal-IL-1α may be more efficiently trapped in the route, especially in the liver, than untreated IL-1α.

Although Gal-IL-1α exhibited decreased activities *in vivo*, the magnitude of the decrease was relatively small and there was not much difference between the activities. This is in contrast to the activities *in vitro*. As previously reported, the magnitude of the reduction of *in vitro* activities varied from 10 to 10 000-fold depending on the assays [17]; proliferative effect on mouse T cells (1/30), antiproliferative effect on mouse myeloid leukemic cells (1/100) and human melanoma cells (1/100), stimulatory effects on IL-6 synthesis by human melanoma cells (1/10) and PGE_2 synthesis by human fibroblast cells (below 1/10 000). A major factor contributing to IL-1 activity is its binding affinity to IL-1 receptors. Type I IL-1R(IL-1RI) functions to deliver IL-1 signals into cells [34]. In contrast, type II IL-1R(IL-1RII) is unable to transduce the IL-1 signal, but it works as a regulator of IL-1 action by functioning as a decoy receptor in a cell-associated form or in a released form from the cell surface upon stimulation with a variety of stimuli [35, 36]. As reported previously, receptor binding affinities of Gal-IL-1α to IL-1RI, and IL-1RII decreased by about 500 and 100-fold, respectively [17]. Therefore, the difference in the magnitude of the decrease in *in vitro* activities is not simply explained by the affinity to IL-1R. The first possibility is that each cell type expresses IL-1R with a differential ratio in the number of IL-1RI and IL-1RII. Thus, the magnitude of IL-1 activity may be regulated by the balance between the number of IL-1R1 and IL-1RII. The second possibility is that the carbohydrate moieties of IL-1R is responsible for the variety. As it is reported that carbohydrate moieties of IL-1R contribute to the binding affinity of IL-1 to IL-1R [5], each cell type may express IL-1R with different carbohydrates. The third possibility is an involvement of second subunit of IL-1RI. It is suggested that the IL-1R accessory protein (IL-1RAcP) is recruited to the IL-1/IL-1RI complex, subsequently the IL-1 signal is transduced into cells [37]. Thus, galactose-coupling of IL-1 may affect the recruitment of IL-1RAcP to the IL-1/IL-1RI complex. Collectively glycosylation of IL-1 may affect IL-1 binding to IL-1R by multiple mechanisms.

Finally, another important issue presented here is that even if the activity of IL-1 is weak *in vitro*, it does not necessarily mean that its activity *in vivo* is comparably weak. Although this should be interpreted cautiously because human IL-1α activities were evaluated in mice, this should be borne in mind in the development of neoIL-1 for therapeutic use. The finding also encourages us to develop neoIL-1 with other species of carbohydrates.

Acknowledgments

We thank Drs H. Hayashi and T. Takii for valuable suggestions. This work was supported in part by a grant from the Ministry of Education, Science and Culture of Japan.

References

1 Ashwell G, Harford J (1982) *Annu Rev Biochem* **51**: 531–54.
2 Tsuda E, Kawanishi G, Ueda M, Masuda S, Sasaki R (1990) *Eur J Biochem* **188**: 405–11.
3 Goverman JM, Parsons TF, Pierce JG (1982) *J Biol Chem* **257**: 15059–64.
4 Fischer T, Thoma B, Scheurich P, Pfizenmaier K (1990) *J Biol Chem* **265**: 1710–17.
5 Mancilla J, Ikejima T, Dinarello CA (1992) *Lymphokine Cytokine Res* **11**: 197–205.
6 Hashida M, Nishikawa M, Takakura Y (1995) *J Control Release* **36**: 99–107.
7 Goebel WF, Avery OT (1929) *J Exp Med* **50**: 521–31.
8 Krantz MJ, Lee YC (1976) *Anal Biochem* **71**: 318–21.
9 Hoffman J, Larm O, Scholander E (1983) *Carbohydr Res* **117**: 328–31.
10 Fujita T, Nishikawa M, Tamaki C, Takakura Y, Hashida M, Sezaki H (1992) *J Pharmacol Exp Ther* **263**: 971–8.
11 Fujita T, Furitsu H, Nishikawa M, Takakura Y, Sezaki H, Hashida M (1992) *Biochem Biophys Res Commun* **189**: 191–6.
12 Dinarello CA (1996) *Blood*: **87** 2095–147.
13 Takei Y, Wada K, Chiba T, Hayashi H, Ishihara H, Onozaki K (1994) *Lymphokine Cytokine Res* **13**: 265–70.

14 Takei Y, Chiba T, Wada K, Hayashi H, Yamada M, Kuwashima J, Onozaki K (1995) *J Interferon Cytokine Res* **15**: 713–19.
15 Takei Y, Yang D, Chiba T, Nabeshima S, Naruoka M, Wada K, Onozaki K (1996) *J Interferon Cytokine Res* **16**: 333–36.
16 Chiba T, Nabeshima S, Takei Y, Onozaki K (1997) *Glycoconjugate J*: *in press.*
17 Nabeshima S, Chiba T, Takei Y, Watanabe S, Okuyama H, Onozaki K (1997) *Glycoconjugate J*: *in press.*
18 Drickamer K, Mamon JF, Binns G, Leuhg JO (1984) *J Biol Chem* **259**: 770–8.
19 Spiess M, Lodish HF (1985) *Proc Natl Acad Sci USA* **82**: 6465–9.
20 Oda S, Sato M, Toyoshima S, Osawa T (1989) *J Biochem (Tokyo)* **105**: 1040–3.
21 Sato M, Kawakami K, Osawa T, Toyoshima S (1992) *J Biochem (Tokyo)* **111**: 331–6.
22 Matsuda T, Hirano T, Kishimoto T (1988) *Eur J Immunol* **18**: 951–6.
23 Misko TP, Schilling RJ, Salvemini D, Moore WM, Currie MG (1993) *Anl Biochem* **214**: 11–16.
24 Besedovsky H, Rey A, Sorkin E, Dinarello CA (1986) *Science* **233**: 652–4.
25 Baumann H, Morella KK, Wong GHW (1993) *J Immunol* **151**: 4248–57.
26 Rey AD, Besedovsky H (1989) *Proc Natl Acad Sci USA* **86**: 5943–7.
27 Endo Y, Nakamura M (1992) *Br JPharmacol* **105**: 613–19.
28 Nathan C (1992) *FASEB J* **6**: 3051–64.
29 Beasley D, Schwartz JH, Brenner BM (1991) *J Clin Invest* **87**: 602–8.
30 Tominaga T, Fukata J, Naito Y, Usui T, Murakami N, Fukushima M, Nakai Y, Hirai Y, Imura H (1991) *Endocrinology* **128**: 526–31.
31 Moore MAS, Marren DJ (1987) *Proc Natl Acad Sci USA* **84**: 7134–8.
32 Rennick D, Yang G, Gemmell L, Lee F (1987) *Blood* **69**: 682–91.
33 Kitamura T, Takaku F, Miyajima A (1991) *Inter Immunol* **3**: 571–7.
34 Sims JE, Acres RB, Grubin CE, McMahan CJ, Wignall JM, March CJ, Dower SK (1989) *Proc Natl Acad Sci USA* **86**: 8946–50.
35 McMahan CJ, Slack JL, Masley B, Cosman D, Lupton SD, Brunton LL, Grubin CE, Wignall JM, Jenkins NA, Brannan CI, Copeland NG, Huebner K, Croce CM, Cannizzarro LA, Benjamin D, Dower SK, Sproggs MK, Sims JE (1991) *EMBO J* **10**: 2821–32.
36 Re F, Muzio M, Rossi MD, Polentarutti N, Giri JG, Mantovani A, Colotta F (1994) *J Exp Med* **179**: 739–43.
37 Greenfeder SA, Nunes P, Kwee L, Labow M, Chizzonite RA, Ju G (1995) *J Biol Chem* **270**: 13757–65.

Received 7 July 1997, revised 26 August 1997, accepted 26 August 1997