Pergamon

Biochemical Pharmacology, Vol. 47, No. 5, pp. 853–858, 1994.

0006-2952/94 $6.00 + 0.00

0006-2952(93)E0032-3

TARGETED DELIVERY OF SUPEROXIDE DISMUTASE TO MACROPHAGES VIA MANNOSE RECEPTOR-MEDIATED MECHANISM

YOSHINOBU TAKAKURA, SADA MASUDA, HIDEAKI TOKUDA, MAKIYA NISHIKAWA and MITSURU HASHIDA*

Department of Basic Pharmaceutics, Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan

(*Received* 11 *June* 1993; *accepted* 28 *September* 1993)

Abstract—Human recombinant superoxide dismutase (SOD) was modified into a mannosylated form (Man-SOD), and its cellular uptake and inhibitory effect on superoxide anion release were studied *in vitro*, using cultured mouse peritoneal macrophages. [^{111}In]Man-SOD was taken up by the macrophages to a great extent, whereas no significant uptake was observed for native and galactosylated SOD. The uptake of Man-SOD was inhibited significantly at a low temperature and by the presence of mannan, mannose and colchicine, demonstrating the targeted delivery of Man-SOD via mannose receptor-mediated endocytosis. Man-SOD exhibited a superior inhibitory effect on superoxide anion release from inflammatory macrophages stimulated by phorbol-myristate acetate. The present study suggested the potential of Man-SOD as a therapeutic agent for the inflammatory disease mediated by superoxide anions generated by macrophages.

Key words: mouse peritoneal macrophages; mannose receptor; receptor-mediated endocytosis; cell culture; superoxide dismutase; superoxide anion; targeting

A number of studies have suggested the important pathophysiological role of reactive oxygen species in various diseases such as inflammation and ischemia/reperfusion injuries [1–4]. Superoxide dismutase (SOD)† can be used as a therapeutic agent since it is an antioxidant enzyme capable of eliminating superoxide anion, which exists in the upper stream of the reactive oxygen metabolism cascade [5]. However, the experimental and therapeutic potentials of SOD are greatly restricted due to its rapid elimination from the circulation by glomerular filtration in the kidney [6, 7].

In our previous study, we demonstrated that targeted delivery of human recombinant SOD could be achieved by chemical modification with mono- and polysaccharides [8]. In particular, Gal-SOD and Man-SOD were selectively delivered to the liver parenchymal and nonparenchymal cells, respectively, by receptor-mediated endocytosis. In addition, these glycosylated derivatives showed superior therapeutic effects on hepatic injury induced by ischemia/reperfusion in rats [9]. In this study, we evaluated uptake characteristics of Man-SOD at a cellular level, using cultured mouse peritoneal macrophages, and its potency to eliminate superoxide anions generated from the elicited macrophages, a model of inflammatory macrophages.

* Corresponding author. Tel. (81) 75-753-4525; FAX (81) 75-453-4575.

† Abbreviations: SOD, superoxide dismutase; Gal-SOD, galactosylated SOD; Man-SOD, mannosylated SOD; BSA, bovine serum albumin; Gal-BSA, galactosylated BSA; Man-BSA, mannosylated BSA; Cyt *c*, ferricytochrome *c*; PMA, photobol-12-myristate-13-acetate and TGC, thioglycolate.

MATERIALS AND METHODS

Chemicals. Recombinant human SOD (111-Ser) was supplied by the Asahi Chemical Industry Co., Shizuoka, Japan. Bovine serum albumin (Fraction V, BSA), horse heart ferricytochrome *c* (Type VI, Cyt *c*), phorbol-12-myristate-13- acetate (PMA) and colchicine were purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A. Thioglycolate (TGC) broth was obtained from the Nissui Pharmaceutical Co., Tokyo, Japan. D-Galactose, D-mannose and yeast mannan were obtained from Wako Pure Chemical, Osaka, Japan. ^{111}Indium chloride ([^{111}In]-Cl_3) was supplied by the Nihon Medi-Physics Co., Takarazuka, Japan. All other chemicals were of the finest grade available.

Synthesis and characterization of SOD and BSA derivatives. Glycosylated SOD and BSA were synthesized as reported previously [8]. In brief, Man- and Gal-SOD or Man- and Gal-BSA were synthesized by reacting SOD or BSA with 2-imino-2-methoxyethyl 1-thioglycoside, prepared by the method of Lee *et al.* [10], in 50 mM borate buffer (pH 10.0) for 5 hr at room temperature. The purity of glycosylated SODs was confirmed by affinity chromatography with Con A-Sepharose (Man-SOD, Man-BSA) or agarose-peanut lectin (Gal-SOD, Gal-BSA). The degree of modification of amino groups was determined by measuring the amount of free amino groups with trinitrobenzene sulfonic acid using glycine as a standard [11]. Enzymatic activity of SOD was determined by the nitroblue tetrazolium reduction method using an SOD test kit (Wako Pure Chemical). The molecular weight of SOD derivatives was estimated by HPLC (LC-6A, Shimadzu, Japan) using a Shim-pack Diol-300 column (i.d.

Table 1. Physicochemical properties of SOD and BSA derivatives tested in this study

Compound	Number of NH_2 groups*	Molecular weight†	Enzymatic activity‡ (U/mg)
SOD	24.0	32,000	3200 (100)
Gal-SOD	2.7	35,000	2720 (85.0)
Man-SOD	3.8	34,000	2730 (85.3)
BSA	60.0	67,000	
Gal-BSA	16.0	68,000	
Man-BSA	15.8	68,000	

* The degree of modification of amino groups was determined by measuring the amount of free amino groups with trinitrobenzene sulfonic acid using glycine as a standard [11].

† The molecular weight of SOD and BSA derivatives was estimated by HPLC. The apparent molecular weight of SOD and BSA derivatives was determined by the calibration curve obtained from the marker proteins.

‡ Enzymatic activity of SOD was determined by the nitroblue tetrazolium reduction method using an SOD test kit. Numbers in parentheses are retained enzymatic activity expressed as the percentage of native enzyme.

7.5 mm × 50 cm) eluted with 20 mM phosphate buffer (pH 7.0) containing 0.2 M sodium sulfate. The apparent molecular weight of SOD derivatives was determined by the calibration curve obtained from the marker proteins (Gel Filtration Kit, Pharmacia, Uppsala, Sweden). The physicochemical properties of SOD and BSA derivatives are summarized in Table 1. Native and glycosylated proteins were radiolabeled with ^{111}In using the bifunctional chelating agent diethylenetriaminepentaacetic acid anhydride, by the method of Hnatowich *et al.* [12]. This radiolabeling method was selected to evaluate cellular uptake of proteins separate from metabolic degradation, since ^{111}In is accumulated in the cells by being converted to an iron-binding protein after intracellular degradation [13].

Harvesting and culture of macrophages. Male ICR mice, weighing 20–25 g, were obtained from the Shizuoka Agricultural Cooperative Assocation for Laboratory Animals, Shizuoka, Japan. Resident macrophages were collected from the cavity of the mouse with RPMI 1640 medium. Washed cells were suspended in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS, Flow Laboratories, Irvine, U.K.), penicillin G (100 U/mL), and streptomycin (100 μg/mL) and were plated on 6- or 12-well culture plates (Falcon, Becton Dickinson, Lincoln Park, NJ, U.S.A.) at a density of 1×10^5 cells/cm^2. After incubation for 2 hr at 37° in 5% CO_2–95% air, adherent macrophages were washed with culture medium three times and then cultured under the same conditons. Elicited macrophages were harvested from the mice 4 days after intraperitoneal injection of 1 mL of 2.9% of TGC medium. These cells were processed and cultured as described above.

Cellular uptake study. Uptake studies were carried out using cultured macrophages in 12-well plates. Cells were washed three times with 1 mL of warm RPMI 1640 medium, and 0.5 mL of the medium containing ^{111}In-labeled proteins (1 μg/mL) was added. The cells were washed three times with 2 mL of cold medium at appropriate time intervals and were solubilized with 0.5 mL of 0.3 N NaOH with 0.1% Triton X-100. Aliquots were taken for the determination of ^{111}In radioactivity counting and protein content. The radioactivity was counted in a well-type NaI scintillation counter (ARC-500, Aloka Co., Tokyo, Japan), and protein was measured by the modified Lowry method [14].

Inhibitory effect of Man-SOD on superoxide anion generation. Elicited macrophages in culture plates were washed quickly with pH 7.4 Hanks' balanced salt solution (HBSS) without phenol red. In continuous exposure experiments, cells were incubated with 2.2 mL of HBSS containing SOD or Man-SOD (2.4 to 300 U/mL) together with Cyt *c* (70 μM) and PMA (3 μg/mL). At an appropriate time, the reaction mixture was removed, placed into iced centrifuge tubes, and promptly centrifuged at 200 *g*. The optical density of the supernatants was determined spectrophotometrically at 550 nm using mixtures from plates without cells as blanks, and the concentration of reduced Cyt *c* was determined using an extinction coefficient of 2.1×10^4 M^{-1} cm^{-1} [15]. In pretreatment experiments, cells were incubated with HBSS containing SOD or Man-SOD (60 and 300 U/mL) in the absence or presence of colchicine (50 μg/mL) for 1 or 3 hr at 37° in 5% CO_2–95% air. After washing with vigorous swirling with HBSS, 2.2 mL of reaction mixture containing Cyt *c* (70 μM) and PMA (3 μg/mL) in HBSS was added to the plates at 37°. After 30 min, the reaction mixture was collected as described above and then subjected to assay.

RESULTS

Uptake by cultured macrophages. Figure 1 shows the time courses of uptake of ^{111}In-labeled SOD and BSA and their glycosylated derivatives by cultured mouse resident peritoneal macrophages. Rapid uptake was observed for [^{111}In]Man-SOD and [^{111}In]Man-BSA, whereas uptake of native and galactosylated proteins was significantly lower. Table 2 summarizes the effects of various conditions on the uptake of [^{111}In]Man-SOD. The uptake varied depending on the culture period of macrophages: macrophages cultured for 1 day showed no significant uptake, whereas marked uptake was observed for 2- and 3-day-old macrophages. The uptake of [^{111}In]-Man-SOD was greatly inhibited at a low temperature (4°) and by the presence of excess amounts of mannan and mannose. Colchicine, an endocytosis inhibitor, also significantly reduced the uptake of [^{111}In]Man-SOD by macrophages.

Inhibitory effect on superoxide anion release. In continuous exposure experiments, both native SOD and Man-SOD inhibited superoxide generation from macrophages in a concentration-dependent manner to a similar extent (Fig. 2). Pretreatment experiments were carried out at a concentration of 300 U/mL at which both SOD and Man-SOD showed almost

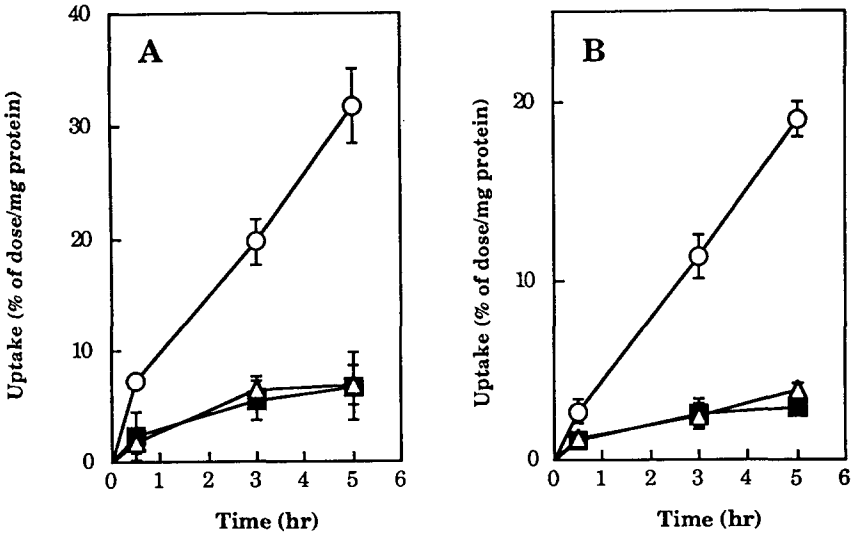

Fig. 1. Uptake time courses of [^{111}In]SOD (A) and [^{111}In]BSA (B) derivatives by cultured mouse peritoneal macrophages at 37°. Resident macrophages were incubated with [^{111}In]SOD or [^{111}In]BSA (△), [^{111}In]Man-SOD or [^{111}In]Man-BSA (○), and [^{111}In]Gal-SOD or [^{111}In]Gal-BSA (■) at 1 μg/mL, and associated radioactivity and protein content were measured. Results are expressed as means ± SD (N = 3).

Table 2. Effects of various experimental conditions on uptake of [^{111}In]Man-SOD (1 μg/mL) by cultured mouse peritoneal macrophages

Condition			Uptake (% of dose/mg protein/5 hr)
Temperature (°)	Culture period (days)	Inhibitor	
37	1		5.04 ± 0.97*
37	2		29.83 ± 3.89
37	3		31.80 ± 3.29
4	3		7.26 ± 1.91*
37	3	Mannan (1 mg/mL)	2.11 ± 0.35*
37	3	Mannose (50 mM)	1.06 ± 0.55*
37	3	Colchicine (50 μg/mL)	5.38 ± 0.89*

Results are expressed as means ± SD (N = 3).
* Significantly different ($P < 0.001$) from the control value (37°, 3 days, without inhibitor).

complete inhibition of superoxide generation from the macrophages in the continuous exposure experiments (Fig. 3). Man-SOD showed a significant inhibitory effect on PMA-stimulated production of superoxide anions by the macrophages compared with native SOD. A marked effect was observed in the case of the 3-hr pretreatment (Fig. 3, Expt. 2). Figure 4 illustrates the results of pretreatment experiments performed in the presence of colchicine. The endocytosis inhibitor abolished the inhibitory effect of man-SOD on superoxide anion release from the macrophages.

DISCUSSION

Phagocytes involving macrophages and neutrophils generate reactive oxygen species, which contribute to both host defense and inflammation, when exposed to certain stimuli. Therefore, controlled manipulation of SOD at a cellular level would be of importance from the viewpoint of therapy for inflammatory diseases. The purpose of this *in vitro* study was to clarify the potential of Man-SOD for cell-specific delivery to macrophages through the mannose receptor [16].

Uptake experiments demonstrated that Man-SOD can be targeted to macrophages via mannose receptor-mediated endocytosis (Fig. 1, Table 2). Based on this finding, the inhibitory effect of Man-SOD on superoxide production was studied using TGC-elicited macrophages, since these macrophages have an increased capacity compared with resident

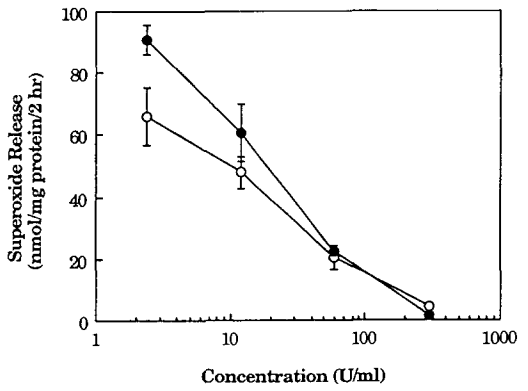

Fig. 2. Inhibitory effect of SOD and Man-SOD on superoxide anion release from cultured mouse peritoneal macrophages in continuous exposure experiments. Elicited macrophages cultured for 3 days were incubated with SOD (○) and Man-SOD (●) (2.4 to 300 U/mL) together with Cyt *c* (70 μM) and PMA (3 μg/mL); the reaction mixture was removed after 2 hr of incubation. The amount of superoxide anions released into the medium from the macrophages was determined spectrophotometrically by Cyt *c* reduction, and cellular protein in the plate was measured. Superoxide release from control macrophages incubated in SOD-free medium was 87.7 nmol/mg protein/2 hr. Results are expressed as means ± SD (N = 3).

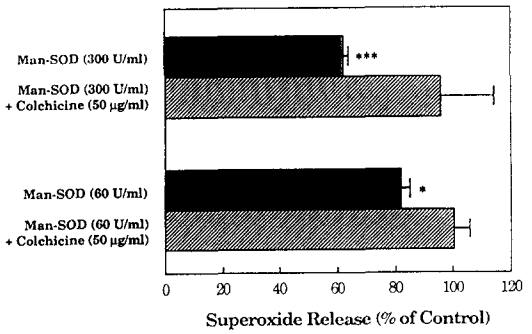

Fig. 4. Inhibitory effect of Man-SOD on superoxide anion release from cultured mouse peritoneal macrophages in pretreatment experiments in the presence or absence of colchicine. Elicited macrophages cultured for 3 days were incubated with Man-SOD at concentrations of 60 and 300 U/mL in the absence and presence of colchicine (50 μg/mL) for 3 hr. After extensive washing, reaction mixture containing Cyt *c* (70 μM) and PMA (3 μg/mL) was added to the plates. After 30 min, the reaction mixture was collected and subjected to superoxide anion release assay, and cellular protein in the plate was measured. Superoxide release from control macrophages incubated in SOD-free medium was 24.9 and 23.3 nmol/mg protein/30 min in the absence and presence of colchicine, respectively. Results are expressed as means ± SD (N = 3). Key: (*) $P < 0.05$, and (***) $P < 0.001$ vs control.

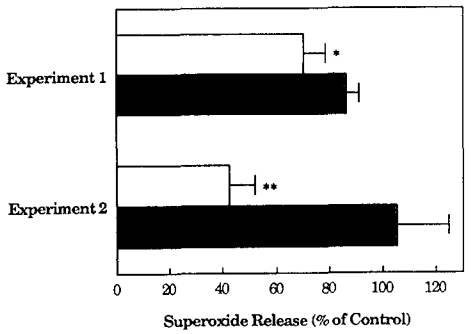

Fig. 3. Inhibitory effect of SOD and Man-SOD on superoxide anion release from cultured mouse peritoneal macrophages in pretreatment experiments. Elicited macrophages cultured for 3 days were incubated with SOD (shaded bars) and Man-SOD (open bars) for 1 hr (Expt. 1) and 3 hr (Expt. 2). After extensive washing, reaction mixture containing Cyt *c* (70 μM) and PMA (3 μg/mL) was added to the plates. After 30 min, the reaction mixture was collected and subjected to superoxide anion release assay, and cellular protein in the plate was measured. Superoxide release from control macrophages incubated in SOD-free medium was 13.1 and 6.8 nmol/mg protein/30 min in Expts. 1 and 2, respectively. Results are expressed as means ± SD (N = 3). Key: (*) $P < 0.05$, and (**) $P < 0.01$ vs control.

macrophages to generate superoxide anion upon stimulation with PMA [15]. TGC-elicited macrophages cultivated for 3 days also effectively took up [^{111}In]Man-SOD at a rate of 31.6% of the dose/mg protein/5 hr (data not shown), which was almost identical to that observed in resident macrophages (Fig. 1A). This result is consistent with the finding that resident and TGC-elicited mouse peritoneal macrophages cultivated for 60 hr showed similar significant uptake activity for Man-BSA [17].

In experiments in which macrophages were exposed continuously to enzyme solutions during PMA stimulation, we found that both native and mannosylated SOD had essentially the same potency for inhibition of superoxide generation in a concentration range of 2.4 to 300 U/mL (Fig. 2). However, when macrophages were incubated with these enzymes for 1 or 3 hr and then washed extensively to remove enzyme-containing medium, Man-SOD showed a superior inhibitory effect (Fig. 3). Remarkable inhibition was observed in the 3-hr pretreatment experiment. In addition, the inhibitory effect of pretreated Man-SOD on superoxide anion release was impaired in the presence of colchicine, an endocytosis inhibitor (Fig. 4). Consequently, the inhibitory effect should be attributed to the enzyme taken up by the cells during the incubation period as observed in the cellular uptake study. Pretreatment experiments more closely resemble the *in vivo* condition, in which Man-SOD is effectively incorporated into the cells via receptor-mediated endocytosis, whereas native SOD undergoes rapid clearance. Therefore, we can expect a beneficial effect of Man-SOD on superoxide production by inflammatory macrophages *in vivo*.

In our previous study on the prevention of hepatic ischemia/reperfusion injury [9], the most superior therapeutic effect was obtained with Man-SOD

targeted to the liver nonparenchymal cells including Kupffer cells, the predominant source of reactive oxygen species [18]. That result is in good agreement with observations in the present study. However, it is suspected that Man-SOD may undergo lysosomal degradation in the macrophages after rapid internalization [19]. Furthermore, superoxide anions are generated by membrane-bound NADPH-oxidase in macrophages [20]. These conditions seem not to be advantageous for Man-SOD to suppress superoxide production of macrophages. For effective inhibition of superoxide release into the extracellular space, Man-SOD should exist on or in the vicinity of the cell surface. Tietzse *et al.* [21] reported that intact ligand–mannose receptor complexes can return from the intracellular pool to the cell surface in alveolar macrophages, which may account for the present beneficial effect of Man-SOD. The detailed mechanism of the inhibition of superoxide generation by Man-SOD remains to be elucidated.

Macrophage mannose receptor expression is known to be decreased by various conditions, such as activation by *Bacillus* Calmette-Guérin [22], treatment with lipopolysaccharide and PMA [23], and infection with *Leishmania donovani* [24]. Special attention must be paid to these effects in the case of targeted drug delivery to macrophages through the mannose receptor.

In the present study, culture age-dependent uptake of Man-SOD by the macrophages was observed (Table 2). This phenomenon may involve mannose receptor expression as reported for cultured bone marrow macrophages [25, 26]. Dexamethasone has been reported to increase expression of the manose receptor in macrophages [27, 28]. Therefore, it would be interesting to use dexamethasone in order to improve the efficacy of Man-SOD. Further studies are ongoing in our laboratory to evaluate the effect of dexamethasone.

In conclusion, the present study has demonstrated the possibility of targeting of SOD to macrophages via a mannose receptor-mediated mechanism. Man-SOD may thus have potential for therapy of inflammatory diseases mediated by superoxide anions generated by macrophages.

REFERENCES

1. McCord JM, Free radicals and inflammation: Protection of synovial fluid by superoxide dismutase. *Science* **185**: 529–531, 1974.
2. Ando Y, Inoue M, Araki S and Morino Y, Inhibition of carrageenin-induced paw edema by a superoxide dismutase derivative that circulates bound to albumin. *Biochim Biophys Acta* **1073**: 374–379, 1991.
3. Kawamoto S, Inoue M, Tashiro S, Morino Y and Miyauchi Y, Inhibition of ischemia and reflow-induced liver injury by an SOD derivative that circulates bound to albumin. *Arch Biochem Biophys* **277**: 160–165, 1990.
4. Jaeschke H, Reactive oxygen and ischemia/reperfusion injury on the liver. *Chem Biol Interact* **79**: 115–136, 1991.
5. Fridovich I, Superoxide radical: An endogenous toxicant. *Annu Rev Pharmacol Toxicol* **23**: 239–257, 1983.
6. Pyatak PS, Abuchowski A and Davis FF, Preparation of a polyethylene glycol: superoxide dismutase adduct, and an examination of its blood circulating life and anti-inflammatory activity. *Res Commun Chem Pathol Pharmacol* **29**: 113–127, 1980.
7. Odline B, Appelgren L-E, Baytai A and Wolgast M, Tissue distribution of ^{125}I-labelled bovine superoxide dismutase (SOD) in the rat. *Pharmacol Toxicol* **62**: 95–100, 1988.
8. Fujita T, Nishikawa M, Tamaki C, Takakura Y, Hashida M and Sezaki H, Targeted delivery of human recombinant superoxide dismutase by chemical modification with mono- and polysaccharide derivatives. *J Pharmacol Exp Ther* **263**: 971–978, 1992.
9. Fujita T, Furitsu H, Nishikawa M, Takakura Y, Sezaki H and Hashida M, Therapeutic effects of superoxide dismutase derivatives modified with mono- or polysaccharides on hepatic injury induced by ischemia/reperfusion. *Biochem Biophys Res Commun* **189**: 191–196, 1992.
10. Lee YC, Stowell CP and Krantz M, 2-Imino-2-methoxyethyl 1-thioglycolate: New reagents for attaching sugars to proteins. *Biochemistry* **15**: 3956–3963, 1976.
11. Habeeb AFSA, Determination of free amino groups in proteins by trinitrobenzenesulfonic acid. *Anal Biochem* **14**: 328–336, 1966.
12. Hnatowich D, Layne WW and Childs R, The preparation and labeling of DTPA-coupled albumin. *Int J Appl Radiat Isot* **12**: 327–332, 1982.
13. Brown BA, Comeau RD, Jones PL, Liberatore FA, Neacy WP, Sands H and Gallagher BM, Pharmacokinetics of the monoclonal antibody B72.3 and its fragments labeled with either ^{125}I or ^{111}In. *Cancer Res* **47**: 1149–1159, 1987.
14. Wong C-S and Smith RL, Lowry determination of protein in the presence of Triton X-100. *Anal Biochem* **63**: 414–417, 1975.
15. Johnston RB Jr, Godzik CA and Cohn ZA, Increased superoxide anion production by immunologically activated and chemically elicited macrophages. *J Exp Med* **148**: 115–127, 1978.
16. Stahl PD, Rodman JS, Miller MJ and Schlesinger PH, Evidence for receptor-mediated binding of glycoproteins, glycoconjugates, and lysosomal glycosidases by alveolar macrophages. *Proc Natl Acad Sci USA* **75**: 1399–1403, 1978.
17. Ezekowitz RAB, Austyn J, Stahl PD and Gordon S, Surface properties of bacillus Calmette-Guérin-activated mouse macrophages: Reduced expression of mannose-specific endocytosis, Fc receptors, and antigen F4/80 accompanies induction of Ia. *J Exp Med* **154**: 60–76, 1981.
18. Jaeschke H, Bautista AP, Spolarics Z and Spitzer JJ, Superoxide generation by neutrophils and Kupffer cells during *in vivo* reperfusion after hepatic ischemia in rats. *J Leukoc Biol* **52**: 377–382, 1992.
19. Stahl PD, Schlesinger PH, Sigardson E, Rodman JS and Lee YC, Receptor-mediated pinocytosis of mannose glycoconjugates by macrophages: Characterization and evidence for receptor recycling. *Cell* **19**: 207–215, 1980.
20. Babior BM, The respiratory burst of phagocytes. *J Clin Invest* **73**: 599–601, 1984.
21. Tietze C, Schlesinger PH and Stahl PD, Mannose-specific endocytosis receptor of alveolar macrophages: Demonstration of two functionally distinct intracellular pools of receptor and their roles in receptor recycling. *J Cell Biol* **92**: 417–424, 1982.
22. Imber MJ, Pizzo SV, Johnson WJ and Adams DO, Selective diminution of the binding of mannose by murine macrophages in the late stages of activation. *J Biol Chem* **257**: 5129–5135, 1982.
23. Shepherd VL, Abdolrasulnia R, Garrett M and Cowan HB, Down-regulation of mannose receptor activity in

macrophages after treatment with lipopolysaccharide and phorbol esters. *J Immunol* **145**: 1530–1536, 1990.
24. Basu N, Set R and Das PK, Down-regulation of mannose receptors on macrophages after infection with *Leishmania donovani*. *Biochem J* **277**: 451–456, 1991.
25. Clohisy DR, Bar-Shavit Z, Chappel JC and Teitelbaum SL, 1,25-Dihydroxyvitamin D_3 modulates bone marrow macrophage precursor proliferation and differentiation: Up-regulation of the mannose receptor. *J Biol Chem* **262**: 15922–15929, 1987.
26. Clohisy DR, Chappel JC and Teitelbaum SL, Bone-marrow-derived mononuclear phagocytes autoregulate mannose receptor expression. *J Biol Chem* **264**: 5370–5377, 1989.
27. Shepherd VL, Konish MG and Stahl PD, Dexamethasone increases expression of mannose receptors and decreases extracellular lysosomal enzyme accumulation in macrophages. *J Biol Chem* **260**: 160–164, 1985.
28. Cowan HB, Vick S, Conary JT and Shepherd VL, Dexamethasone up-regulates mannose receptor activity by increasing mRNA levels. *Arch Biochem Biophys* **296**: 314–320, 1992.