Scavenger-receptor-mediated delivery of daunomycin elicits selective toxicity towards neoplastic cells of macrophage lineage

Amitabha MUKHOPADHYAY, Bratati MUKHOPADHYAY, Rajendra K. SRIVASTAVA and Sandip K. BASU*
Institute of Microbial Technology, Sector 39A, Chandigarh 160 014, India

Transformed cells of macrophage lineage such as J774A.1, P388D1 and IC21 take up and degrade a conjugate of the antineoplastic drug, daunomycin, with maleylated BSA with high efficiency and saturation kinetics through the scavenger receptors expressed on the surface of these cells. By contrast, transformed cells of non-macrophage lineage, namely L929, EL4, Bowes melanoma and CHO (Chinese-hamster ovary), do not take up and degrade the conjugate, indicating that these cells are scavenger-receptor-deficient. In the conjugated form, about 0.1 μM -daunomycin cause 50% inhibition in the uptake of [^3H]thymidine by the receptor-bearing J774A.1 cells, whereas the receptor-deficient Bowes-melanoma cells are not affected. Free daunomycin (0.1 μM) does not significantly affect the uptake of [^3H]thymidine by either cell type. Treatment of cells derived from intraperitoneal tumours induced in BALB/C mice by J774A.1 cells with 0.4 μM -daunomycin in the conjugated form for 5 h abolished their ability to form tumours in BALB/C mice. By contrast, transplantation of untreated cells or cells treated with free daunomycin under identical conditions led to tumour formation and subsequent death of the BALB/C mice. These results indicate that this modality for selective elimination of scavenger-receptor-bearing neoplastic cells may be useful for the treatment of histiocytic malignancies in which cells of macrophage lineage turn malignant.

INTRODUCTION

The numerous toxic side reactions arising out of non-specific drug uptake by normal cells complicate successful chemotherapy of cancer. In order to minimize the toxicity of the antitumour agents, attempts have been made for selective delivery of such drugs exclusively to the tumour cells, using specific antibodies coupled with anticancer drugs, toxins, or liposomes loaded with such agents [1–3]. The major drawbacks of antibody-mediated drug-delivery approaches (reviewed in [4]) are: (a) the majority of the so-called tumour-associated antigens are also often found on normal cells; (b) tumour-cell populations are heterogeneous in terms of expression of tumour-associated antigens; and (c) most of the cell-surface antigens are not efficiently internalized when antibodies are bound, thereby limiting the intracellular availability of the drug.

We have been exploring the alternate possibility of exploiting the cell-type-specificity and high efficiency of the scavenger-receptor-mediated endocytic pathway [5] for selective delivery of drugs to tumour cells bearing such receptors. This receptor system: (i) recognizes polyanionic macromolecules such as maleylated BSA (MBSA); (ii) consists of several distinct molecular species; and (iii) is present only on the surface of macrophages and to a lesser extent on monocytes and endothelial cells [6–8]. After high-affinity binding with the cell-surface receptors, MBSA is internalized and degraded in the lysosomes. We have previously shown the feasibility of using MBSA as a carrier of drugs to non-proliferating macrophages both *in vitro* and *in vivo* for the elimination of intracellular microbial pathogens causing leishmaniasis and tuberculosis [9–12]. In the present investigation we demonstrate that a conjugate of MBSA with daunomycin (DNM) can bring about selective elimination of the scavenger-receptor-bearing proliferating cells of macrophage origin, whereas the receptor-deficient cells remain unaffected. This approach, if

successful *in vivo*, might be helpful in combating malignancies such as monocytic leukaemia, histiocytosis etc., in which cells of macrophage lineage turn malignant.

MATERIALS AND METHODS

Cells

J774A.1, P388D1 and IC21 are mouse tumour-cell lines of monocyte-macrophage origin obtained from the American Type Culture Collection. Bowes melanoma, a human cancer-cell line, was kindly provided by Dr. Desirée Collen of the University of Leuven, Leuven, Belgium. EL4 (mouse T-cell lymphoma) and L929 (mouse fibroblast) cell lines were obtained from Dr. Peter H. Krammer of the German Cancer Research Centre, Heidelberg, Germany and Indira Nath of the All-India Institute of Medical Sciences, New Delhi, India, respectively.

These cells were maintained in appropriate media, that is, Dulbecco's modified Eagle medium (DMEM) (J774A.1 and L929), Roswell Park Memorial Institute (RPMI) 1640 (P388D1, IC21, and EL4), minimal essential medium (MEM) (Bowes melanoma) and Ham's [Chinese-hamster ovary (CHO)] with 10%-(v/v)-fetal-calf serum (FCS), 2 mM-L-glutamine and gentamycin (50 $\mu\text{g}/\text{ml}$) and were grown at 37 °C in an air/CO₂ (19:1) atmosphere.

Maleylation of BSA

BSA was maleylated by allowing the protein to react with maleic anhydride at pH 8.0 as described in [13]. Electrophoretic mobilities of the chemically modified protein was tested by 0.7%-agarose-gel electrophoresis at pH 8.4 [14].

Preparation of the drug conjugate

MBSA was coupled to DNM by using glutaraldehyde, as described in [15]. Briefly, 3 mg of MBSA was mixed with 400 μg

Abbreviations used: BCA, bichinchonic acid; CHO, Chinese-hamster ovary; DNM, daunomycin; FCS, fetal-calf serum; MBSA, maleylated BSA; PBS, phosphate-buffered saline (0.02 M-sodium phosphate/0.15 M-NaCl, pH 7.4); Cl₃Ac, trichloroacetic acid; DMEM, Dulbecco's modified Eagle Medium; MEM, minimal essential medium; RPMI, Roswell Park Memorial Institute.

* To whom correspondence should be addressed.

of DNM in 1 ml of phosphate-buffered saline (PBS). Glutaraldehyde (0.1 %) was added dropwise to the reaction mixture to achieve a final concentration of 0.01 % and the reaction mixture was stirred for 15 min at room temperature. The reaction was stopped by adding 50 μ l of 1 M-lysine, and the conjugate was recovered by Sephadex G-50 column chromatography. Void-volume fractions containing the MBSA-DNM conjugate were pooled and subjected to extensive dialysis against PBS. The DNM content of the conjugate was determined by measuring the absorbance at 495 nm [15]. MBSA showed no absorbance at 495 nm. Protein was measured by the bicinchoninic acid (BCA) reagent [16]. Stoichiometric estimation revealed that the conjugate contained 3–4 mol of DNM/mol of MBSA.

Radioiodination of MBSA-DNM

MBSA-DNM was radioiodinated with Na^{125}I by the ICl_2 -catalysed reaction [17]. More than 99 % of the radioactivity was trichloroacetic acid (Cl_3Ac)-precipitable, and the specific activity of the radiolabelled drug conjugate was 120 c.p.m./ng of protein.

Assay of uptake and degradation of ^{125}I -MBSA-DNM

For these experiments, suspensions containing 5×10^5 cells in 1 ml of medium A [media such as DMEM, RPMI 1640 etc., appropriate for the particular cell line as described above, supplemented with 10 % heat-inactivated FCS, 2 mM-L-glutamine and gentamycin (50 $\mu\text{g}/\text{ml}$)] were placed in each well of 24-well tissue-culture plates and incubated at 37 °C in an air/ CO_2 (19:1) atmosphere. After 16 h, the cells were washed with FCS-free medium A. Each well then received 1 ml of medium B (medium A without FCS, but with 1 mg of BSA/ml) containing indicated concentrations of ^{125}I -MBSA-DNM, and the monolayers were incubated at 37 °C for 5 h (Fig. 1 below) or various other periods (Fig. 2 below). The amounts of Cl_3Ac -soluble (non-iodide) radioactivity released in the medium were determined as previously described [17]. The monolayers were washed three times with ice-cold PBS containing BSA (1 mg/ml) and once with PBS. The cells were dissolved in 1 ml of 0.1 M-NaOH and the cell-associated radioactivity was measured by placing an aliquot in a γ -radiation counter. Cellular protein was estimated by the BCA method [16]. Each monolayer contained 80–90 μg of protein.

Cytotoxicity assay *in vitro*

The cytotoxicity of the drug conjugate was assayed by the [^3H]thymidine-uptake assay [18]. Briefly, 1×10^5 cells (J774A.1 or Bowes melanoma)/well were incubated overnight in 1 ml of medium A at 37 °C. The monolayers were washed twice with 1 ml of medium A. Each well received 1 ml of medium A containing indicated concentrations of DNM in free or conjugated form and the monolayers were incubated for 30 min at 37 °C. The cells were then washed thrice with 1 ml of medium A and incubated in 1 ml of drug-free medium A at 37 °C. After 20 h (or at indicated time), [^3H]thymidine (5 $\mu\text{Ci}/\text{ml}$) was added to each well. After 3 h, the cells were washed four times with 1 ml of medium A to remove unincorporated radioactivity. The cells in each well were dissolved in 0.5 ml of 0.1 M-NaOH and the radioactivity associated with the cells were determined.

Ability of the drug-treated cells to form tumours in mice

Cells from the peritoneal cavity of donor BALB/C mice bearing J774A.1 intraperitoneal tumours were collected by lavage with 10 ml of PBS per mouse, pooled, washed and suspended in medium A at a concentration of 1×10^6 cells/ml. These cells were incubated in the presence of 0.4 μM -DNM, either in the free or the conjugated form, for 5 h at 37 °C in a humidified incubator with an air/ CO_2 (19:1) atmosphere. The cell suspension was

washed three times with PBS and 5×10^6 cells in 0.1 ml of PBS were transplanted subcutaneously in BALB/C mice. Mean survival times of eight to ten mice in each group were determined.

RESULTS

Uptake and degradation of ^{125}I -MBSA-DNM by J774A.1 cells

When J774A.1 cells were incubated with increasing concentrations of ^{125}I -MBSA-DNM at 37 °C for 5 h, the cell-associated radioactivity increased in a saturable fashion (Fig. 1a). During this period, radioactivity was also released in the medium as Cl_3Ac -soluble material, indicating proteolytic degradation of the drug conjugate (Fig. 1b). Half-maximal values for both uptake and degradation of ^{125}I -MBSA-DNM at 37 °C were achieved at a concentration of about 6 μg of protein/ml. The saturability of both the uptake and the degradation processes becomes even more explicit when the values for uptake and degradation of ^{125}I -MBSA-DNM in the presence of excess MBSA (300 $\mu\text{g}/\text{ml}$) are

Fig. 1. Relationship between the concentration of ^{125}I -MBSA-DNM and the extent of uptake (a) or degradation (b) of MBSA-DNM by J774A.1 cells

The monolayers were incubated for 5 h at 37 °C with the indicated amounts of ^{125}I -MBSA-DNM in the absence or presence of excess MBSA (300 $\mu\text{g}/\text{ml}$) and processed as described in the Materials and methods section. Receptor-mediated uptake (●) or degradation (▲) was obtained by subtracting the values for non-saturable uptake (□) or degradation (▽) from total uptake (○) or degradation (△). Results are expressed as ng of MBSA-DNM associated with the cells or degraded per mg of cell protein (means $\pm$ S.D. for three independent experiments).

Fig. 2. Effect of monensin (3 μM) on the rate of accumulation (●, control; ○, with monensin) and degradation (▲, control; △, with monensin) of ^{125}I -MBSA-DNM (6 μg protein/ml)

Results are means $\pm$ S.D. for three independent determinations.

Table 1. Degradation of ^{125}I -MBSA-DNM by J774A.1 cells: competition by negatively charged macromolecules

Abbreviation used: IC_{50} , concentration ($\mu\text{g}/\text{ml}$) required for 50% inhibition of degradation of ^{125}I -MBSA-DNM ($6\text{ }\mu\text{g}/\text{ml}$).

Effective competitor	IC_{50}	Ineffective competitor	IC_{50}
MBSA	6	Fetuin	600
Fucoidin	3	Chondroitin sulphate A	200
Dextran sulphate	4	Heparin	80
Polyinosinic acid	1	Polyadenylic acid	60
Polyguanylic acid	3	Polycytidylic acid	75

Table 2. Degradation of ^{125}I -MBSA-DNM by cell lines of macrophage and non-macrophage origin

Each monolayer of the indicated cell lines received 1 ml of medium B containing ^{125}I -MBSA-DNM ($6\text{ }\mu\text{g}/\text{ml}$) in the absence or presence of MBSA ($300\text{ }\mu\text{g}/\text{ml}$). After incubation at 37°C for 5 h, the medium in each well was processed to determine the amount of ^{125}I -MBSA-DNM degraded by the cells as described in the Materials and methods section. Results are means $\pm$ S.D. (to the nearest integer) of three independent determinations.

Cell line	Degradation (ng/5 h per mg of cellular protein)	
	^{125}I -MBSA-DNM	^{125}I -MBSA-DNM + MBSA
Macrophages		
J774A.1	3568 ± 115	505 ± 31
P388D1	2247 ± 114	434 ± 37
IC-21	1878 ± 46	289 ± 23
Non-macrophages		
L929	437 ± 28	59 ± 12
EL-4	361 ± 6	71 ± 10
Bowes melanoma	121 ± 4	33 ± 2
CHO	41 ± 3	13 ± 2

Fig. 3. Uptake of ^3H thymidine by J774A.1 (●) and Bowes-melanoma cells (○) in the presence of DNM in free (a) or conjugated form (b)

The results are expressed as percentages of the uptake of ^3H thymidine by untreated cells and are means $\pm$ S.D. for three independent experiments.

subtracted from the respective total amounts of ^{125}I -MBSA-DNM associated with the cells or degraded.

When J774A.1 cells were incubated with $6\text{ }\mu\text{g}$ of ^{125}I -MBSA-DNM/ml at 37°C for various times, the cell-associated radio-

activity reached a steady-state plateau after about 60 min, but the Cl_3Ac -soluble radioactivity continued to appear in the medium linearly, reflecting continuing uptake and degradation of the drug conjugate (Fig. 2). Approx. 5 times as much ^{125}I -MBSA-DNM was degraded by the cells as was associated with the cells during the steady state. In the presence of an inhibitor of lysosomal functions, monensin, the degradation of the drug conjugate was inhibited. Monensin did not significantly affect the uptake of ^{125}I -MBSA-DNM.

Competition of degradation of ^{125}I -MBSA-DNM by ligands recognized by the scavenger receptors

In order to determine whether the recognition of MBSA-DNM was mediated through the scavenger receptors, we determined the concentrations of various negatively charged compounds required to cause 50% inhibition of the degradation of ^{125}I -MBSA-DNM ($6\text{ }\mu\text{g}/\text{ml}$) by J774A.1 cells. The data in Table 1 show that the various negatively charged compounds tested can be classed into two distinct groups. Relatively low concentrations ($1\text{--}6\text{ }\mu\text{g}/\text{ml}$) of the effective competitors, namely polyinosinic acid, polyguanylic acid, fucoidin, dextran sulphate and MBSA, brought about 50% inhibition of the degradation of ^{125}I -MBSA-DNM. By contrast, much higher concentrations ($60\text{--}600\text{ }\mu\text{g}/\text{ml}$) of the ineffective competitors, namely polyadenylic acid, polycytidylic acid, heparin, chondroitin sulphate type A and fetuin were needed to bring about the same extent of inhibition of the degradation of ^{125}I -MBSA-DNM by J774A.1 cells.

Specificity of the drug conjugate

The data in Table 2 show that established transformed cell lines of macrophage lineage, namely J774A.1, P388D1 and IC21, degraded 3.6 , 2.2 and $1.9\text{ }\mu\text{g}$ of ^{125}I -MBSA-DNM/mg of cellular protein respectively in 5 h. By contrast, transformed cells of non-macrophage origin, namely L929, EL4, Bowes melanoma and CHO, degraded 0.5 , 0.4 , 0.1 and $0.05\text{ }\mu\text{g}$ of ^{125}I -MBSA-DNM/mg of cellular protein respectively under identical conditions.

Cytotoxic activity of DNM in its free and conjugated form

The effect of free and conjugated DNM on the uptake of ^3H thymidine by the scavenger-receptor-bearing J774A.1 and the scavenger-receptor-deficient Bowes-melanoma cells is shown in Fig. 3. For both the cell lines, free DNM ($0.3\text{ }\mu\text{M}$) inhibited only about 20% of the ^3H thymidine uptake compared with the untreated controls. In contrast, for the receptor-bearing cell line J774A.1, MBSA-DNM ($0.3\text{ }\mu\text{M}$ -DNM) reduced ^3H thymidine uptake by over 75%, whereas for the Bowes-melanoma cell line ^3H thymidine uptake was inhibited by only about 20%. The superior cytotoxic activity of MBSA-DNM was more pronounced at lower concentrations. Thus, for J774A.1 cells, $0.1\text{ }\mu\text{M}$ -DNM in the conjugated form reduced ^3H thymidine uptake by 50% compared with untreated control, whereas inhibition of ^3H thymidine uptake by free DNM ($0.1\text{ }\mu\text{M}$) was less than 0.5%.

To quantify the cytotoxic effect of the drug conjugate more precisely, we incubated J774A.1 cells with DNM, either in free or conjugated form, for 30 min, washed and reincubated the monolayers in drug-free medium. After the indicated time intervals, each monolayer received a 3 h pulse of ^3H thymidine (Fig. 4). At zero time the control cells incorporated about 87000 c.p.m. of ^3H thymidine during the pulse period. As the cells proliferated, the uptake of ^3H thymidine continued to increase linearly over the next 32 h. When the cells were treated with free DNM ($0.2\text{ }\mu\text{M}$), the rate of ^3H thymidine uptake was comparable with that of control cells after an initial lag period. By contrast, exposure of the cells to an equivalent concentration

Fig. 4. [^3H]thymidine incorporation by J774A.1 cells at various periods of time after drug exposure

On day 2 the monolayers received either no additions (▲) or $0.2 \mu\text{M}$ free (○) or conjugated DNM (●) and were incubated for 30 min at 37°C , washed, and reincubated in drug-free medium A. At the indicated times the cells were pulse-labelled with [^3H]thymidine as described in the Materials and methods section. Each point represents the average of three independent determinations ($\pm$ S.D.).

Table 3. MBSA–DNM-mediated inhibition of [^3H]thymidine incorporation by J774A.1 cells: reversal by polyguanylic acid

The monolayers were exposed to MBSA–DNM ($0.1 \mu\text{M}$ -DNM) and/or polyguanylic acid ($200 \mu\text{g/ml}$) for 30 min, washed and processed for [^3H]thymidine incorporation as described in the Materials and methods section. The results are means $\pm$ S.D. (to the nearest integer) of three independent determinations.

Addition	[^3H]thymidine incorporation (%)
1. No addition	100 ± 3
2. MBSA–DNM	60 ± 3
3. MBSA–DNM + polyguanylic acid	106 ± 2
4. Polyguanylic acid	112 ± 3

Fig. 5. Survival of BALB/C mice after transplantation of J774A.1 tumour cells

▲, Untreated; ○, treated with free DNM; ●, treated with conjugated DNM.

of DNM in the conjugated form resulted in total inhibition of the uptake of [^3H]thymidine throughout the experimental period. We have also observed (results not shown) that treatment of J774A.1 cells with $0.1 \mu\text{M}$ -DNM for 30 min in the conjugated form eliminated the ability of these cells to form monolayers

under established culture conditions. However, when the cells were treated with as much as $0.3 \mu\text{M}$ -DNM in the free form, the cells were able to form monolayers as efficiently as the untreated control cells. The presence of polyguanylic acid ($200 \mu\text{g/ml}$) in the medium completely inhibited the cytotoxic activity of the drug conjugate, as shown in Table 3.

Ability of the drug-treated cells to form tumours in mice

The data in Fig. 5 show that transplantation of MBSA–DNM-treated tumour cells into BALB/C mice did not result in formation of tumours, and the mice survived throughout the experimental period. By contrast, injection of untreated or free DNM-treated tumour cells into the mice led to the formation of tumours and all the animals died within 25–35 days.

DISCUSSION

In the present investigation we have shown that MBSA–DNM is taken up by tumour cells of macrophage lineage (J774A.1, P388D1 and IC21) in a saturable fashion, suggesting that the conjugate is recognized by a limited number of binding sites on the cell surface (Fig. 1; Table 2). This high-affinity binding is followed by rapid internalization and degradation of the drug conjugate. The lysosomal inhibitor monensin inhibits the degradation of the drug conjugate (Fig. 2). The results presented in Table 1 show that the degradation of the radiolabelled drug conjugate is inhibited by unlabelled MBSA, fucoidin, dextran sulphate, polyinosinic acid and polyguanylic acid at relatively low concentrations. In contrast, other negatively charged compounds, namely fetuin, chondroitin sulphate A, polyadenylic acid, polycytidylic acid and heparin, which are known to be ineffective as competitors for this receptor system [6], require much higher concentrations to bring about inhibition of the degradation of ^{125}I -MBSA–DNM to the same extent. Taken together, these data indicate that the drug conjugate is taken up by the cells through the scavenger receptors which recognize these ligands. The scavenger-receptor pathway has been reported to consist of at least three distinct molecular entities with varying affinities for different ligands [19]. From the cross-competition experiments reported here it is not clear at present which of these receptors is involved in the high-affinity uptake of MBSA–DNM drug conjugate. The inability of non-macrophage tumour cells such as EL4 (T lymphoma), L929 (fibroblast), and Bowes melanoma (epithelial cells) to take up and degrade MBSA–DNM suggests that these cell lines are deficient in scavenger receptors (Table 2). Conjugation of DNM with MBSA significantly increased the cytotoxic activity of DNM for the scavenger-receptor-bearing cells, especially at low concentrations. Thus the data in Fig. 3 show that, for 50% killing (as measured by [^3H]thymidine uptake) of the scavenger receptor-bearing cells, $0.1 \mu\text{M}$ -DNM was required in the conjugated form, whereas free DNM at the same concentration killed less than 0.5% of these cells. Therefore the drug conjugate was at least 100-fold as effective as the free drug in killing the receptor-bearing cells. The substantially enhanced cytotoxicity at low concentrations of MBSA–DNM compared with the free drug presumably results from the high efficiency of the receptor-mediated uptake and lysosomal degradation of the drug conjugate, permitting rapid build up of pharmacologically effective intracellular concentration of DNM (or a bioactive derivative thereof). We have, however, not excluded the possibility that the intracellular product released after receptor-mediated uptake of MBSA–DNM could have higher cytotoxic activity than the free drug. The data in Fig. 4 show that MBSA–DNM brings about a rapid and total inhibition in the ability of J774A.1 cells to synthesize DNA, as measured by [^3H]thymidine incorporation. Free DNM

under similar conditions causes a transient inhibition of DNA synthesis from which the cells can recover and resume DNA synthesis at rates comparable with those shown by the untreated control. The cytotoxic activity of the drug conjugate results from its efficient uptake through the scavenger receptors. This is indicated by the fact that, in the presence of polyguanylic acid, a known high-affinity ligand for the scavenger receptor which competes with MBSA-DNM effectively for uptake by the J774A.1 cells, the drug conjugate does not exert its cytotoxic effect (Table 3). We have also shown that *ex vivo* treatment of cells derived from J774A.1 intraperitoneal tumours with MBSA-DNM abolishes their ability to form tumours in BALB/C mice, whereas such cells treated with free DNM under identical conditions retain the ability to form tumours in BALB/C mice as the untreated cells (Fig. 5).

The approach of receptor-mediated drug delivery to cancer cells using various receptor systems for lipoproteins, growth factors, etc. has been reported previously (reviewed in [4]). However, since these receptors are found on most normal cells as well, it is likely that drugs delivered through such receptors would not be exclusively targeted to the neoplastic cells. Nevertheless, low-density-lipoprotein-receptor-mediated drug delivery has received most attention so far (reviewed in [20]). However, low-density-lipoprotein-mediated delivery is limited to lipophilic drugs and involves derivatization of polar compounds with lipophilic moieties [21]. The scavenger-receptor system has been used to enhance the tumoricidal activity of macrophages *in vivo* by the administration of acetylated low-density lipoprotein containing a lipophilic derivative of the adjuvant, muramyl tripeptide [22]. Being inherently complex molecules with limited stability, the lipoproteins are likely to prove difficult to formulate into stable pharmacological preparations.

By contrast, we have shown that it is possible to chemically couple an anticancer drug, DNM, to a simple and stable carrier, namely MBSA, to generate a drug conjugate which can distinguish between proliferating cells of diverse origins and cause preferential killing of neoplastic cells only of macrophage lineage *in vitro*. Since normal macrophages are terminally differentiated and do not divide, conjugates such as MBSA-DNM (which affect only the proliferating cells) are not likely to affect the functional roles of the mature normal macrophages *in vivo*. Inasmuch as the scavenger receptors are known to be present on monocytes and endothelial cells, albeit in much lower numbers, the possibility of adverse effects arising as a result of toxicity to these cells by MBSA-DNM cannot be ruled out at present. However, our previous studies with a conjugate of MBSA with the potent anticancer drug methotrexate did not elicit any adverse reactions in hamsters [10]. Further studies in animal models of macrophage tumours, are expected to establish the potentials and risks associated with scavenger-receptor-mediated delivery of antineoplastic drugs in cancer chemotherapy. If successful, this modality would merit serious consideration for combating

histiocytic malignancies which are usually fatal and difficult to control by conventional chemotherapeutic regimens. Use of charge-modified BSA as a general drug carrier for macrophage-associated disorders is also attractive because such preparations could be made sterile and apyrogenic relatively easily and should have longer shelf life than immunotoxins, immunoliposomes etc.

This is communication no. 001/91 from the Institute of Microbial Technology and the work was supported by grants from the Council of Scientific and Industrial Research (C.S.I.R.), New Delhi, India. B.M. was supported by a fellowship from the C.S.I.R. We are grateful to G. C. Mishra and S. Srimal for helpful discussions.

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Received 25 June 1991/18 October 1991; accepted 4 November 1991