ANTITUMOR ACTIVITY OF BACTERIOPHAGES IN MURINE EXPERIMENTAL CANCER MODELS CAUSED POSSIBLY BY INHIBITION OF β3 INTEGRIN SIGNALING PATHWAY

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Summary. – Bacteriophages (phages) as bacterial viruses are generally believed to have no intrinsic tropism for mammalian cells. In this study the interactions between phages and various eukaryotic cells were investigated. Binding of phages to the membranes of cancer and normal blood cells was observed. Moreover, it was shown that the wild-type phage T4 (wtT4) and its substrain HAP1 with enhanced affinity for melanoma cells inhibit markedly and significantly experimental lung metastasis of murine B16 melanoma cells by 47% and 80%, respectively. A possible molecular mechanism of these effects, namely a specific interaction between the Lys-Gly-Asp motif of the phage protein 24 and β3-integrin receptors on target cells is proposed. It was also shown that anti-β3 antibodies and synthetic peptides mimicking natural β3 ligands inhibit the phage binding to cancer cells. This is in line with the well-described β3 integrin-dependent mechanism of tumor metastasis. It is concluded that the blocking of β3 integrins by phage preparations results in a significant decrease in tumor invasiveness.

Key words: phage T4; melanoma; antimetastatic activity; β3-integrins

Introduction

There have been many attempts to apply phages in treating bacterial infections. The history of phage therapy can be divided into several characteristic stages: early enthusiasm, critical scepticism, abandonment, and then a renewed interest in its application as a means to eradicate antibiotic-resistant pathogens (Stone, 2002; Clarke, 2003). While the ability of phages to destroy bacteria has been known and extensively investigated since their discovery, our knowledge about the phage interactions with mammalian cells is very limited. Phages have been generally believed to have no intrinsic tropism for those cells.

Probably, the only suggestion that phages may have an anticancer activity originated from observations made by Bloch (1940). He has shown that phages can accumulate in cancer tissue and eventually inhibit tumor growth. These results corresponded to the data of Kańtoch *et al*., who have demonstrated that cancer cells bind phages both *in vitro* and *in vivo* (Kańtoch and Mordarski, 1958). Wenger *et al.* (1978) have suggested that phages may attach to the plasma membrane of lymphocytes. Nevertheless, none of these

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Abbreviations: CB = experimental constant of binding; ECM = extracellular matrix; FACS = fluorescence-activated cell sorter; HAP1 = HAP1 substrain of T4 phage; IIET = Institute of Immunology and Experimental Therapy, Wroclaw; LPS = lipopolysacharide; MAb = monoclonal antibody; PBS = phosphate-buffered saline; p.i. = post infection or inoculation; wtT4 = wild-type T4 phage

observations has resulted in more extensive investigation in this area and the studies have been finally abandoned.

Given the recent renewed interest in phages as antibacterial agents, we turned our attention to their interactions with eukaryotic cells. Recently, we proposed a hypothesis that was formulated on the basis of preliminary experimental data and a theoretical analysis of sequences of phage proteins. The hypothesis concerns the prospective interactions of phages and eukaryotic cells and their possible molecular basis. At least some phages (e.g. T4) possess proteins in their structures containing a Lys-Gly-Asp motif, which is a ligand for the αIIbβ3 integrin and has a low affinity for αvβ3. This probably allows the binding to and eventual interactions of phages with cells that express β3 integrins (Gorski *et al*., 2003).

Integrins comprise a large family of cell-surface receptors expressed on a wide variety of cells (Arnaout *et al*., 2002; Giancotti and Ruoslahti, 1999; Mousa, 2002). They mediate cell-cell and cell-extracellular matrix (ECM) interactions that are important for the maintenance of tissue integrity and promotion of cellular migration, regulation of gene expression, cell survival, adhesion, and differentiation. They also carry out functions in development, angiogenesis, wound healing, and thrombosis (Adair and Yeager, 2002; Schwartz *et al*., 1995). Integrins mediate adhesive events during various cancer stages. In particular, the role of overexpression of the αvβ3 integrin has been demonstrated in various tumors, e.g. in melanomas (Hosotani *et al*., 2002; Li *et al*., 2001; Miziejewski, 1999). Importantly, the αIIβ3 integrin is characteristic of platelets. The ligand binding to αIIbβ3 is required for platelet aggregation (Basani *et al*., 2000; Philips *et al*., 1991), and a defect or qualitative abnormality in this integrin expression results in the Glanzmann thrombasthenia, an exceptional, genetically heterogeneous syndrome associated with a bleeding tendency (D'Andrea *et al*., 2002; Belluci and Caen, 2002).

In this study we describe the results of studies on the interactions between phages and various eukaryotic cells with the aim to elucidate their possible molecular basis and potential significance.

Materials and Methods

Phages. wtT4 was purchased from ATCC, Rockville, MD, USA. HAP1, a T4 phage substrain with a high affinity for melanoma cells was selected as follows. The material applied was either a lysate of *Escherichia coli* B filtered through Millipore 0.22 μm filters or a highly purified phage preparation. T4 phage lysates contained usually approx. 1,500 EU of endotoxin per ml. wtT4 and HAP1 were purified by filtration through polysulfone membranes, gel filtration on Sepharose 4B and cellulofine sulphate (both Millipore) chromatography (Boratyński *et al*., 2004). In this way phage preparations containing 3–7 EU of endotoxin per ml, as determined by chromogenic Limulus Amebocyte Lysate (QLC-1000 Chromogenic Endpoint LAL, Bio Whittaker, USA) were obtained. They were dialyzed against PBS and applied to *in vivo* experiments. Phage concentration in preparations was measured by the two-layer method of Adams (Adams, 1959). To prepare a control for phage lysates *E. coli* B cultures were disrupted by ultrasound and filtered through Millipore 0.22 μm filters. LPS concentration in these preparations was estimated by the Limulus Amebocyte Lysate QLC-1000 Chromogenic Endpoint LAL Kit (Bio Whittaker, USA), gas chromatography and mass spectrometry.

In order to label phages, 1 part of the purified phage preparation in PBS was diluted with 9 parts of 0.01 mol/l phosphate buffer pH 8.8–9.0 containing 0.25 μl/ml SYBR® Green I in DMSO (Molecular Probes, USA), incubated at 37°C for 3 hrs, and dialyzed against PBS for 2 days.

Eukaryotic cells. The melanoma cell lines Hs294T (human) and B16 (murine) as well as human lung cancer cell line A549 were obtained from ACTT (Rockville, MD USA). The LLC cell line was obtained from Dr. I. Wodinsky, National Cancer Institute, Bethesda, MD, USA. Platelets were freshly isolated from the blood of either human volunteers or a Glanzmann's patient (Dick and Crichton, 1972).

Peptides and antibodies. For functional blocking, the following antibodies and peptides were used: an anti-mouse integrin β3 antibody (β3 antibody), anti-mouse integrin αIIbβ3 antibody (αIIbβ3 antibody) (both from BD Biosciences PharMingen, USA), anti-human integrin αvβ3 antibody (αIIβ3 antibody, Chemicon, USA), and anti-human integrin αIIbβ3 antibody (αIIβ3 antibody, Abciximab-ReoPro©, a gift from Prof. C. Cierniewski, Medical University of Lodz, Lodz, Poland), Eptifibatidum, a cyclic KGD motif-containing heptapeptide (Integrilin™, Schering-Plough Corporation, a gift from Schering-Plough Central East AG, Poland), and RGDFV, a cyclic RGD motif-containing pentapeptide (Calbiochem).

For FACS analysis, the αIIβ antibody (FITC-labeled antibody) and anti-β3 antibody (FITC-labeled antibody, BD Biosciences PharMingen, USA) were employed.

Direct evaluation of binding of phages to mammalian cells in vitro A phage preparation (10^5 PFU/100 μl) was added to 96-well plates precoated with cancer cells in culture medium (10^4 cells/100 μl), incubated at 37°C for 1–2 hrs, and the phage concentration was estimated. Wells without cells (PBS only) with identically applied phages served for estimation of the phage "start" concentration. Antibodies (10 μg/ml), oligopeptides (375 μg/ml), or PBS (control) were added to the wells and incubated for 30–45 mins before addition of phages. The phage concentration after incubation was subtracted from the start concentration. The difference represented the number of phage particles bound to the cells. This allowed the calculation of the percentage of bound phages. The phage preparation (10^8 PFU) was incubated with a platelet suspension (10^8 platelets) in PBS at 37°C for 1 hr, centrifuged at 4,500 rpm for 30 mins at 10°C with a minimum deceleration. The phage concentration in the supernatant was estimated and the percentage of bound phages was calculated.

Confocal microscopy as detection method for phages bound to cells. SYBR green-labeled phages (10^9 PFU) were incubated with B16 cells or platelets (10^6 cells) at 37°C for 2 hrs, the sample was centrifuged at 1,000 rpm for 10 mins at 20°C with minimum

deceleration and the supernatant was discarded. The cells were observed by a confocal microscope (λ_{ex} = 488 nm, BioRad MRC 1024, IITD). To exclude any participation of potential SYBR background in the observed fluorescence of SYBR-labeled phage samples a control sample was prepared. The control cells were incubated with a SYBR-treated PBS sample.

Selection of HAP1. The bacterial lysate was incubated with B16 cells (2 x 10^9 PFU per 2 x 10^6 cells, 3 hrs, 37°C), the cells were thoroughly washed with saline and cultured by the two-layer method (Adams, 1959) until lysis was complete. Then the soft agar was scratched, shaken for 10 mins with 5 ml of the culture medium, centrifuged, the supernatant was filtered (Millipore 0.22 µm) and the phage concentration was estimated. This preparation was repeatedly (altogether 7 times) incubated with B16 cells. The last supernatant was cultured to obtain single phage plaques. Ten plaques were subcultured and the constant of binding (CB) for each culture was calculated. The culture with the highest CB (HAP1) was chosen for further investigation.

Estimation of CB. CB was used to compare in simple manner the ability of phages to bind eukaryotic cells. CB enables rating of binding potency of particular phage substrains. It represents the number of cells per 1,000 that, after incubation with phage and intensive washing, remain bound with phage particles. The purpose of CB calculation was to indicate the T4 phage substrain with the highest binding potency. Phage preparations were incubated with the cell suspension (10^9 PFU per 10^6 cells, 1 hr, 37°C). The cells were thoroughly washed with PBS, centrifuged (1,000 rpm, 10 mins, 20°C), and the cells were suspended in PBS and counted. CB was calculated according to the formula

$$CB = 1000\ a/c$$

in which a = phage concentration in the final cell suspension, b = phage concentration in the last discarded supernatant, c = cell number in the final cell suspension. This calculation was considered valid only for a ≥10b.

FACS. To determine CD61 (β3) and CD41 (αIIβ) expression by flow cytometry, 2 x 10^5 B16 or LLC cells were mixed with MAb pre-chilled to 4°C. The cells were incubated in ice bath for 30 mins and washed twice with PBS supplemented with 2% fetal bovine serum. The cell surface fluorescence was measured using a FACS Calibur flow cytometer (Becton Dickinson, USA). Damaged cells were labeled by adding propidium iodide each test tube to final concentration 5 µg/ml just before data acquisition. Data analysis was performed using the Becton Dickinson Cell Quest software.

In vivo antimetastatic assay. Six- to twelve-week-old female C57Bl/6/IiW mice bred and kept in IIET under standard minimal-disease conditions. Athymic NCr nu/nu female mice were received as a gift from the National Cancer Institute, Frederic Cancer Research and Development Center, Frederick, MD, USA, and kept under pathogen-free conditions. All experiments were performed according to *Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Marketing and Education* issued by the New York Academy of Sciences' ad hoc Committee on Animal Research and were approved by the 1st Local Committee for Experiments with the Use of Laboratory Animals, Wroclaw, Poland. Mice were inoculated intravenously with 3 x 10^5 B16 cells collected from *in vitro* culture in 0.2 ml of saline into the lateral tail vein. Phages, 2–4 x 10^8 PFU in 0.2 ml per mouse in the case of lysates and 4–8 x 10^7 PFU in 0.2 ml per mouse in the case of purified phages, were injected intraperitoneally. The mice were sacrificed by cervical dislocation on day 21 p.i. and the metastatic lung colonies were counted.

Statistical methods. The Student's *t*-test and STATISTICA 5.0 software package were employed. The differences with $P<0.05$ were considered significant.

Results

Binding of phages to mammalian cells in vitro

In preliminary experiments binding of wtT4 to murine cancer cells was observed. This finding represented the stimulus for attempting selection of a phage with a higher affinity for cancer cells. A serial *in vitro* passage method developed by us allowed selection of such a phage, namely HAP1.

Table 1. *In vitro* binding of wtT4 and HAP1 to mouse B16 and human melanoma Hs294T cells

Experimental group	B16 cells		Hs294T cells	
	Phage concentration Mean PFU x 10^6/ml ± SD (N)	% of binding	Phage concentration Mean PFU x 10^6/ml ± SD (N)	% of binding
wtT4 start concentration (control)	2.59 ± 0.11 (16)	–	2.31 ± 0.16 (14)	–
Cells + wtT4	1.98 ± 0.20* (16)	23.6	1.81 ± 0.16* (13)	21.7
Cells + KGD + wtT4	2.51 ± 0.34** (10)	3.1	2.16 ± 0.17** (6)	6.5
Cells + RGD + wtT4	2.59 ± 0.11** (6)	0.0	NT	NT
Cells + anti-αIIbβ3 MAb + wtT4	2.03 ± 0.13 (6)	21.6	2.06 ± 0.15* (6)	10.8
Cells+ anti-β3 MAb + wtT4	2.53 ± 0.21** (6)	2.3	NT	NT
Cells+ anti-αvβ3 MAb + wtT4	NT	NT	2.27 ± 0.15** (6)	1.7
HAP1 start concentration (control)	2.53 ± 0.26 (10)	–	2.41 ± 0.18 (7)	–
Cells + HAP1	1.74 ± 0.12* (10)	31.2	1.63 ± 0.17* (7)	32.4

N = number of tests; *Statistically significant difference from an adequate control; **Statistically significant inhibition of binding; wtT4 = wtT4 lysate; HAP1 = HAP1 lysate; KGD = a peptide with KGD-like motif (Integrilin™); RGD = a peptide with RGD motif; NT = not tested. For the experimental procedure see *Materials and Methods.*

Table 2. *In vitro* binding of wtT4 and HAP1 to mouse lung cancer cells LLC and human lung cancer cells A549

Experimental group	LLC cells		A549 cells	
	Phage concentration Mean PFU x 10^6/ml ± SD (N)	% of binding	Phage concentration Mean PFU x 10^6/ml	% of binding ± SD (N)
WtT4 start concentration (control)	2.26 ± 0.04 (13)	–	2.58 ± 0.15 (6)	–
Cells + wtT4	1.78 ± 0.11* (13)	21.2	2.32 ± 0.06* (6)	10.7
Cells + KGD + wtT4	2.06 ± 0.16** (12)	8.8	2.51 ± 0.08** (6)	2.7
Cells + RGD + wtT4	2.22 ± 0.21** (6)	1.8	NT	NT
HAP1 start concentration (control)	2,00 ± 0,24 (12)	–	2.53 ± 0.14 (6)	–
Cells + HAP1	1.51 ± 0.28* (12)	24.5	2.25 ± 0.10* (7)	11.1
Cells + KGD + HAP1	1.71 ± 0.22 (6)	14.5	NT	NT
Cells + RGD + HAP1	1.75 ± 0.12 (6)	12.5	NT	NT

N = number of tests; *Statistically significant difference from an adequate control; **Statistically significant inhibition of binding; wtT4 = wtT4 lysate; HAP1 = HAP1 lysate; KGD = a peptide with KGD-like motif (Integrilin™); RGD = a peptide with RGD motif; NT = not tested. For the experimental procedure see *Materials and Methods.*

Table 3. Expression of CD61 (ß3) and CD41 (αIIb) cell surface integrins on mouse melanoma B16 cells and mouse lung cancer LLC cells

Group	Mouse melanoma cells (B16)		Mouse lung cancer cells (LLC)	
	N	Mean percentage of positive cell population ± SD	N	Mean percentage of positive cell population ± SD
Control	3	1.1 ± 0.8	7	1.6 ± 0.9
CD61	3	25.3 ± 10.8*	7	81.8 ± 14.9*
CD41	3	1.4 ± 1.4	3	0.6 ± 0.6

The results are calculated by subtraction of the common area under the graph representing the negative control and the graph representing the expression of the antigen from the area under the graph representing the expression of the antigen. The results marked with asterisks differ significantly from the control. For the experimental procedure see *Materials and Methods.*

Table 4. Antimetastatic effect of T4 and HAP1 in the C57BL/6 mouse B16 melanoma model

Experimental group of mice	Number of lung colonies ± SD (N)
Saline (control)[a]	55 ± 19 (24)
E. coli B [b]	60 ± 19 (10)
wt T4 (1+21)[c]	29 ± 20 (24)*
wt T4 (1)[d]	28 ± 16 (6)*
wt T4 (21)[e]	35 ± 24 (6)*
HAP1[f]	11 ± 13 (12)**

N = number of mice inoculated with B16 melanoma cells. *Statistically significant difference from saline. **Statistically significant difference from saline and wtT4 (21).
[a]Mice treated with 0.9% NaCl.
[b]Mice treated with *E. coli B* disrupted by ultrasound (control of LPS).
[c]Mice treated with wtT4; the first dose given 1 hr before inoculation with B16, then 21 doses given daily.
[d]Mice treated with wtT4; the first dose given 1 hr before inoculation with B16.
[e]Mice treated with wtT4 in 21 daily doses.
[f]Mice treated with HAP1; the first dose given 1 hr before inoculation with B16, then 21 doses given daily.

The ability of wtT4 and HAP1 to bind to mouse (B16) and human (HS294T) melanoma cells quantified by CB was evaluated. The results showed that HAP1 was bound to the cells more strongly than wtT4. The CB values for HAP1/B16 and wtT4/B16 were 29 and 16, and for HAP1/HS294T and wtT4/HS294T were 4 and 2, respectively. This binding could be also observed by direct biological assay. Again, the binding of HAP1 was significantly stronger than that of wtT4 (Table 1). This result was confirmed by confocal microscopy, using wtT4 and HAP1 labeled with a green fluorescent dye (SYBR I) (Fig. 1). Both the phages were able to bind also to mouse (LLC) and human (A549) lung cancer cells (Table 2).

Insight into the molecular mechanisms of phage-cell binding

According to the structure of T4 phage capsid proteins, namely the presence of the KGD ligand sequence for the β3 integrin receptors and our hypothesis about the molecular basis of phage-mammalian cell interactions (Gorski *et al.*, 2003), we decided to highlight the role of αIIbβ3 and αvβ3 integrins in this process. Particularly, the influence on cell-phage binding of both anti-β3 integrin antibodies and synthetic peptides, which mimick the ligands for β3 integrins due to the presence of RGD-like or KGD-like motifs, was investigated.

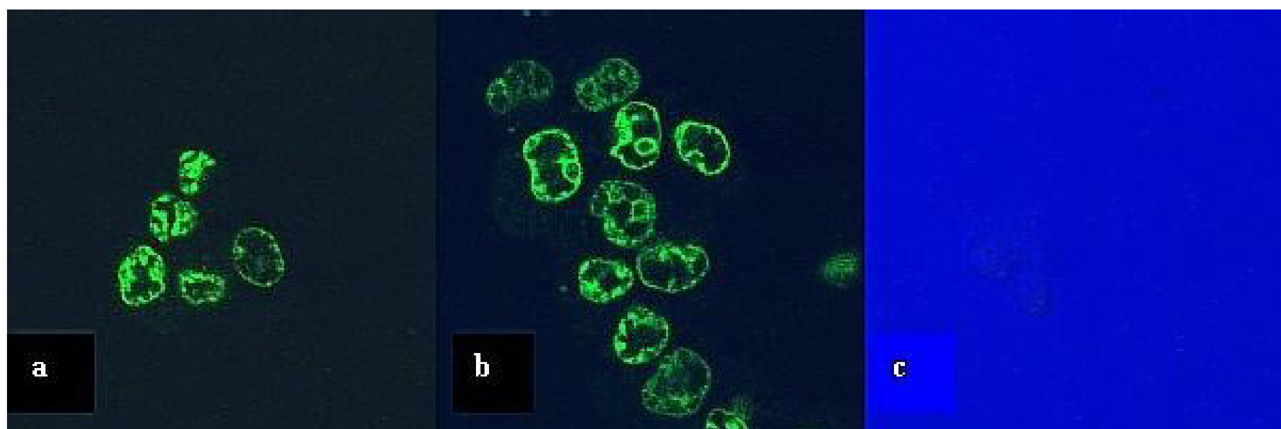

Fig. 1

Confocal microscopy of labeled phages bound to melanoma B16 cell membranes

B16 melanoma cells after incubation with the labeled wtT4 (a). B16 melanoma cells after incubation with the labeled HAP1 (b). B16 melanoma cells not incubated with a labeled phage, negative control (c). For the experimental procedure see Materials and Methods.

With all human or murine melanoma and lung cancer cells the T4 phage binding was almost completely blocked by the anti-β3 antibodies and/or KGD- and RGD-containing peptides (Tables 1 and 2). An irrelevant antibody or peptide (without RGD and KGD motifs) had no effect on the binding (data not shown). Importantly, an antibody directed against a murine αIIbβ3 heterodimeric complex did not affect the binding of phages to B16 cells (Table 1). In accordance with this, FACS analysis showed no expression of αIIbβ3 but a high expression of αvβ3 on B16 or LLC cells (Table 3).

In human melanoma Hs294T cells, the binding of T4 phage was significantly blocked by the KGD-containing peptide and both the αvβ3 and αIIbβ3m antibodies, although the effect of the αIIbβ3 antibody was weaker than that of the αvβ3 antibody (Table 1). The irrelevant antibody or irrelevant non-RGD non-KGD peptide caused no effect on the cell-phage binding (data not shown).

It is known that the αIIbβ3 integrin is the most abundant platelet cell-surface protein. Therefore the ability of the phages to interact with human platelets was investigated. As this activation is known to result from activation of the receptor function of the αIIbβ3 integrin, resting and collagen-activated platelets were used (Phillips *et al.*, 2001). A binding of 22.5% (resting platelets) and 44.5% (collagen-activated platelets) was observed. Moreover, platelets from a Glanzmann's thrombasthenia patient lacking functional αIIbβ3 integrin were examined for the binding. In this case no binding was observed.

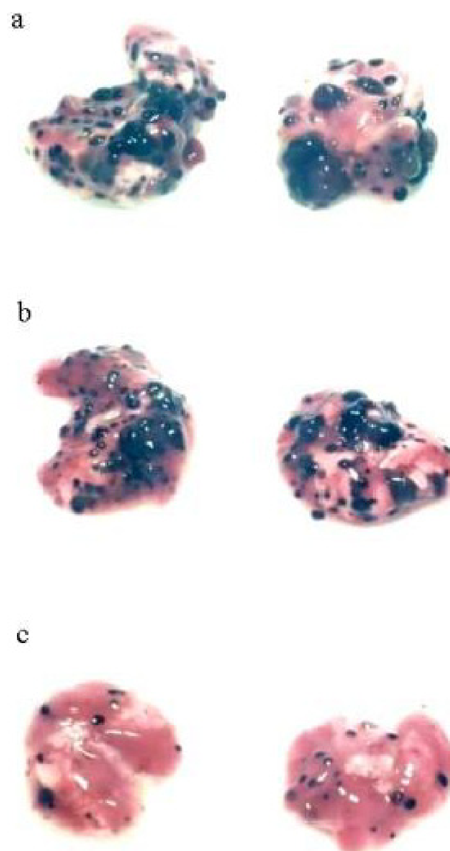

Fig. 2

Colonies of murine melanoma B16 cells in mice treated with HAP1

Lungs of mice inoculated with B16 melanoma cells. Mice treated with 0.9% NaCl, negative control (a). Mice treated with *E. coli* B disrupted by ultrasound, negative control (b). Mice treated with HAP1 (c). For the experimental procedure see *Materials and Methods.*

Antimetastatic effect of phages

Both wtT4 and HAP1 were examined for their antimetastatic potential *in vivo* in transplantable mouse melanoma B16 cell model. A statistically significant inhibition of tumor lung-colony formation was observed both in mice treated with wtT4 (47%) and HAP1 (80%) (Table 4). Moreover, the difference between the inhibition caused by HAP1 and wtT4 was statistically significant. No tumor inhibition was observed in mice treated with the *E. coli* B culture disrupted by ultrasound, which served as a control for a potential activity of LPS and other bacterial residues (Fig. 2). These results stimulated us to purify both phage lysates and test them in nude (NCr nu/nu) mice in order to exclude possible effect of bacterial components. A significant antimetastatic effect of these purified preparations was observed. The inhibition of metastases formation reached statistically significant values of 51% for T4 and 70% for HAP1. This suggests that the antimetastatic effect may be due rather to direct phage-cell (cancer or endothelial) interactions than to the influence of phages on the host immune, at least T cell-dependent response.

The antimetastatic activity of the phages was not dependent on the schedule of treatment. Single phage administration 1 hr before tumor cell inoculation led to the same therapeutic effect as that obtained with daily injections during the whole experiment (Table 4). It should be stressed that no toxic side-effects of the applied treatment were observed.

Discussion

Our results show the unexpected ability of phages to interact with cancer cells and human platelets *in vitro*. We observed binding of phages to cancer cells and platelets in direct biological tests and by use of confocal microscopy. The binding was also visualized by electron microscopy; both phages were located at the cell surface, attached or situated very closely to the cell membrane (data not shown). HAP1, which is able to bind to melanoma cells significantly more strongly than the parental wtT4, is also able to inhibit melanoma metastasis much more effectively. This indicates that the specific binding of phages to cancer cells is a main mechanism of the antimetastatic activity of phages.

In this paper we mentioned the presence of the protein 24 containing the KGD amino acid motif at the T4 phage head. The whole protein is highly exposed: it is located in 5 copies on each corner of the geometrical phage head, which makes altogether 55 copies for each phage particle. Potential interactions may be significantly increased by this exposure as well as by a potential cooperative action of the 5 copies (connected at each head corner) of the protein. The 3D structure of this protein is so far unknown and its knowledge seems to be necessary for evaluation of the exposition of KGD in protein 24 and the role of KGD in interactions with eukaryotic cells. At present we cannot exclude other possible molecular mechanisms that may be involved in phage-eukaryotic cell interactions, and we still admit the possibility of a more complex molecular basis of this effect. Nevertheless, the binding of phages to cancer cells can be inhibited by substances able to block β3 integrins. This strongly suggests that the KGD motif and thus β3 integrins play an important role in the observed interactions. Recent data demonstrate the importance of β3 integrins for tumor expansion and metastasis. The blocking of these integrins function results in anticancer and antimetastatic effects on many types of tumors. Importantly, this refers not only to cancer cells but also to vessels: blocking the vascular (endothelial) β3 integrins significantly inhibits metastasis (Hosotani *et al*., 2002; Miziejewski, 1999). In preliminary experiments we also observed binding of phages to mouse and human endothelial cells (unpublished data). Therefore we consider phage-cell interactions in general and the binding of phages to cancer or endothelial cell membranes (blocking β3 integrins) in particular a major mechanism of the antimetastatic effects of phages. No effect of wtT4 on cell cycle and apoptosis was detected by FACS analysis. It should also be stressed that no antiproliferative effect of the phages in the *in vitro* treatment of B16 cells could be detected (data not shown). The results of the experiment performed on athymic (NCr nu/nu) mice confirmed our hypothesis that the antimetastatic effect of phages (phage treatment) may be due rather to direct phage-cell (cancer or endothelial) interactions than to the efffect of phages on host immune, at least T cell-dependent response.

Studies on the role of LPS in cancer growth have resulted in quite controversial findings. Some reports indicate an antitumor activity of LPS (Pitari *et al*., 2003; Satoh *et al*., 2002), but others describe a stimulating effect of LPS on cancer growth and metastasis (Harmey *et al*., 2002; Wang *et al*., 2003). With these data in mind, we decided to include in our experiments a control culture of *E. coli* B disrupted by ultrasound. Such a preparation contained all the bacterial impurities, which could be present in the phage lysates tested, but it did not contain phages. The treatment of the melanoma cells with this preparation resulted in a small and insignificant increase in the number of melanoma lung colonies. An antimetastatic effect was definitely not observed.

Moreover, our results obtained with highly purified phages confirmed that the antimetastatic effect of the treatment of melanoma cells with phage lysates resulted from the activity of phages but not bacteria.

Sequencing of gene 24 of HAP1 revealed no differences from that of wtT4 (unpublished data). Thus it seems probable

that an additional binding motif(s) was generated in other capsid proteins of the phage. On the other hand, an alternation in the genes encoding proteins active in phage capsid folding could result in an altered exposition of existing motifs leading to a more effective binding. A comparison of gene structure of HAP1 and wtT4 will be the aim of our further studies.

Phages are the most abundant forms of life on the Earth, being virtually omnipresent. The idea to use phages as tools of therapy of bacterial infections arose soon after discovery of phages. The possibility of treatment of bacterial infections in animals as well as humans with phages is still being investigated and discussed (Sulkavelidze *et al*., 2001; Weber-Dabrowska *et al*., 2000). Also a potential use of phages in fighting against non-bacterial diseases should be considered. Another fact is that new anticancer drugs available recently offer little or no advantage over existing medications, though costing much more (Garattini, 2002). Therefore, novel strategies of anticancer treatment, based not only on the destruction of cancer cells but also on the interference with the host and tumor microenvironment are urgently needed. The possible role of phages in cancer prevention should also be regarded.

The data presented herein open, in our opinion, a revolution in general understanding of the role of phages in the environment and in the human body. We believe that these observations call for more extensive studies on the interaction of phages with cancer as well as other eukaryotic cells and on potential application of phages to the treatment and/or prevention of cancer and, perhaps, other diseases dependent on the β3-integrin activity.

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