Cancer Immunol Immunother (1990) 32: 173–178

Cancer Immunology Immunotherapy

Postoperative active specific immunization in curatively resected colorectal cancer patients with a virus-modified autologous tumor cell vaccine

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Received 6 July 1990/Accepted 17 July 1990

Summary. Active specific immunotherapy was performed in a phase I study in 20 colorectal cancer patients after surgical resection of the tumor. An autologous tumor cell vaccine surface modified by Newcastle disease virus (NDV) was used, which showed the following characteristics. After mechanical and enzymatic dissociation of the tumor tissue an average of 5×10^7 cells/g tissue was obtained. According to trypan blue dye exclusion assay the average viability was 72%. Following irradiation (200 Gy) the inactivation of proliferative activity of the cells could be demonstrated by the absence of incorporation of ^{3}H-labelled thymidine. The cells were, however, still metabolically active as shown by the incorporation of [^{3}H]-uridine and a mixture of ^{3}H-labelled amino acids. Epithelium-specific antigens (detected by mAb HEA125) were expressed on more than 75% cells of the cell suspension indicating a high amount of (epithelium-derived) tumor cells. In order to increase the immunogenicity of the tumor cells the suspended cells were infected by the nonlytic, apathogenic Ulster strain of NDV. The successful modification of tumor cells with NDV could be shown by electron microscopy. Three weeks postoperatively cells were thawed, virus-modified, and inoculated intradermally in the upper thigh. Several cell and virus concentrations were tested in each patient. As control, tumor cells without NDV, NDV alone and normal colon mucosa were used. The number of tumor cells ranged from 2×10^6 up to 2×10^7 cells and NDV concentrations from 4 to 64 hemagglutination units (HU) were tested. Sixteen patients responded with a delayed-type hypersensitivity (DTH) skin reaction to the vaccine. The best DTH reaction, measured 24 h following vaccination, was obtained using a vaccine consisting of 1×10^7 tumor cells and 32 HU NDV (median induration of 8 mm). Response to NDV alone was seen in 2 patients only (median induration of 3 mm); 12 patients responded to tumor cells (1×10^7) alone (median induration of 4 mm). Of 10 patients tested with normal colorectal mucosa, 4 responded with a median induration of 3.5 mm. DTH responses to the vaccine of 1×10^7 tumor cells and 32 HU NDV increased throughout the repeated vaccinations to a median induration of 9.5 mm at the end of the therapy. No severe side-effects in the course of the immunotherapy, except for mild fever in 4/20 patients, were observed. The results of our phase I study show that this type of autologous colorectal tumor cell vaccine is ready for a large clinical trial to prove its efficacy.

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Introduction

Recently immunotherapy has gained increasing interest as a treatment of cancer [5, 6, 9, 11–13]. It could be demonstrated in several animal models that active specific immunotherapy (ASI) leads to the induction of a specific antitumor response [9, 10, 23]. Schirrmacher and his group developed an effective immunotherapy approach in the highly metastatic ESb mouse lymphoma model, where postoperative immunotherapy with virally modified autologous tumor cells cured about 50% of the animals from visceral micrometastases [23]. To prepare the tumor cell vaccine a non-lytic strain Ulster of the Newcastle disease virus (NDV) was used [10, 28]. Such NDV-modified tumor cells were found to have increased tumor immunogenicity and to be effective for antimetastatic therapy in combination with surgical resection of the primary tumor. Postoperative immunization with ESb/NDV led to the establishment of long-lasting systemic antitumor immunity mediated by tumor-associated-antigen-specific cytotoxic T cell clones [27, 28]. The concept of ASI also seems to be promising in treatment of microscopic tumor foci in humans. To find out to what extent this therapy could be transferred to human tumors a phase I study with ASI was performed in patients suffering from colorectal cancer with a high risk of local or systemic recurrence following surgical resection of the primary tumor. It was examined

whether NDV-modified colon tumor cells would induce an immune response and, if so, which dose of tumor cells and virus would lead to a maximal local delayed-type hypersensitivity (DTH) skin reaction.

Materials and methods

Patients. A group of 20 colorectal cancer patients (9 rectal tumors, 11 colon tumors; patients had an average age of 64 years) entered the clinical phase I study. All of them underwent colon or rectal tumor resection. Staging revealed Dukes B in 12 and Dukes C in 8 patients. All patients signed an informed consent for this study according to institutional and federal guidelines.

Preparation of viable single-cell suspension and storage. Following surgical resection, specimens were immediately examined by a pathologist and staged according to the Astler and Coller [1] classification. Tumor tissue from 20 patients and in 10 cases also tissue from uninvolved mucosa was transported to the clinical laboratory at 4° C in RPMI medium supplemented with 300 µg/ml penicillin, 300 µg/ml streptomycin and 500 µg/ml gentamicin. All tissue samples were washed with Hanks' balanced salt solution (HBSS) and minced into 3-to-5-mm^3 pieces under sterile conditions. Necrotic debris and adipose tissue were removed. Following transfer into trypsination flasks, the tissue samples were dissociated using 0.14% type I collagenase and 0.1% type I DNase (Sigma, St. Louis, USA) in HBSS. The tissue was incubated at 37° C for 30 min under gentle agitation to achieve better dissociation. Supernatant containing dissociated cells and fragments was filtered through a 40-µm mesh and thereafter centrifuged at 1200 rpm for 10 min. The cells were resuspended in 40 µl HBSS, counted, and the viability was determined using the trypan blue dye exclusion test. After pelleting the cells again, a freezing medium (HBSS supplemented with 7.5% dimethylsulfoxide and 5% human serum albumin) was added and 1×10^7 cells were frozen in a vial using a programmable freezer (BV 9, Cryoson, Schöllkrippen, FRG) down to –80° C. The vials were stored in liquid nitrogen until the preparation of the vaccine [15].

Preparation of virus-modified tumor cell vaccines. Routinely the first vaccination was performed 3 weeks following surgery. To prepare the vaccine the cells were thawed rapidly in a 37° C water bath and transferred out of the cryo-vial into a 50-ml tube. HBSS and 0.1% DNase were added dropwise to the cells. Following several washings using HBSS the number and viability of the cells were checked. Cells, thereafter, were incubated with NDV at 37° C for 1 hour. After a final centrifugation the cell pellet was resuspended in 200 µl NaCl and inactivated by irradiation with 200 Gy (^{137}Cs source; Gammacell 1000, Atomic Energy of Canada, Ottawa).

Immunization of patients and recording of response. Patients were vaccinated intradermally in the upper thigh with vaccination sites 6 cm apart. In each patient several cell and virus concentrations were tested separately or in combination using a checkerboard protocol to find out optimal parameters for the vaccine. The number of tumor cells ranged from 2×10^6 up to 2×10^7 and the amount of NDV from 2 to 64 hemagglutination units (HU). For the control, tumor cells without NDV, NDV alone and cells from normal mucosa were used for vaccination. In 5 patients we also used purified carcinoembryonic antigen (a generous gift from J.-P. Mach, Lausanne, Switzerland), at a concentration of 30 µg and 60 µg, as a vaccine in the same manner. For control of the immune response, local delayed-type hypersensitivity skin reactions at the vaccination site were recorded 24 h and 48 h following injection, and induration was measured as the diameter (mm). Indurations measuring more than 3 mm in diameter were considered positive. For statistical analysis the Wilcoxon matched-pairs signed rank tests was performed. All vaccinations and controls of local reaction were performed by one person to minimize individual variability. Vaccinations were repeated every 10 days up to four times. For documentation of side-effects differential white and red blood cell counts, aminopeptidase, glutamate-oxaloacetate transaminase, glutamate-pyruvate transaminase, γ-glutamyl-transpeptidase, electrolytes and creatinine were monitored during therapy.

Test for microbial contamination. Prior to vaccination, 10 µl cell suspension was collected and plated at the surface of blood agar, and incubated for 24 h at 37° C. Thereafter the colonies were counted and the species of germs subsequently determined by a standard biochemical differentiation.

Immunoperoxidase staining. Cytospins were performed with 2×10^5 tumor cells (7 min, 55 *g*). Immunoperoxidase staining was performed as described elsewhere [19]. Briefly, the air-dried samples were fixed for 10 min in 100% acetone at room temperature and incubated for 60 min with the mouse monoclonal antibody HEA125 (directed against the epithelium-specific antigen Egp34 [18]. After rinsing, the specimens were incubated fo 60 min at room temperature with a biotinylated sheep anti-(mouse IgG) and thereafter for 60 min at room temperature with a biotinylated steptavidin-peroxidase complex (both Amersham, Budec, UK). Following an additional rinsing, the preparations were incubated for 10 min at room temperature in the substrate (3-amino-9-ethylcarbazole, Sigma). Finally the cells were stained embedded in Euparal (Chroma).

Transmission electron microscopy. The virus-infected cells were fixed first with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) and, after washing with this buffer, postfixed with 1% osmium tetroxide in cacodylate buffer (for 30 min each at room temperature). Following stepwise dehydration in acetone (70%, 90%, 100%) the samples were embedded in epoxy resin according to Spurr [25]. Thin sections were cut with an ultramicrotome (OM U3, Reichart), stained with uranyl acetate (1% in 70% acetone) following lead citrate (1%, aqueous) and observed using an electron microscope.

Incorporation of tritium-labelled thymidine, uridine and a mixture of amino acids. Each batch of 5×10^5 cells isolated from the surgically removed tumor tissue was incubated as described in detail elsewhere [24] with 1 µCi/ml [^{3}H]thymidine (sp. act. 777 MBq/mg; Amersham), [^{3}H]-uridine (spec. act. 759 MBq/mg; Amersham) or an ^{3}H-labelled amino acid mixture (leucine, sp. act. 35.7 GBq/mg; lysine monohydrochloride, sp. act. 15.5 GBq/mg; phenylalanine, sp. act. 27.6 GBq/mg; proline, sp. act. 31.23 GBq/mg; tyrosine, sp. act. 20.7 GBq/mg; Amersham) in HBSS at 37° C for 30 min. The samples were then rinsed twice with HBSS and precipitated in 5% ice-cold trichloroacetic acid. Thereafter they were applied to a Whatman filter, rinsed with 96% methylated ethanol and air-dried. After addition of 5 ml scintillation solution the activity of the probes was determined using a beta counter (LS 7000, Beckmann).

Results

Characterization of the vaccine

Cytological data. From all 20 patients undergoing operation, sufficient tumor cells could be obtained to perform the active specific immunotherapy protocol. The yield of cells from tumors of colon or rectal origin was $5 \pm 3 \times 10^7$/g tumor tissue on average. Cell yield per gram of tissue was slightly greater for tumor samples than for normal mucosa. The viability of cells was about 72% (Table 1). Since colorectal carcinomas are derived from epithelial cells, we used the epithelium-specific monoclonal antibody HEA125 to test for the presence of tumor cells within the preparations. In cytospin preparations of dissociated cells more than 75% of the cells were HEA125-positive (Fig. 1). The vaccine was characterized microscopically and shown to consist of a mixture of cell types. Besides tumor cells, erythrocytes, leukocytes, other cells, and debris were de-

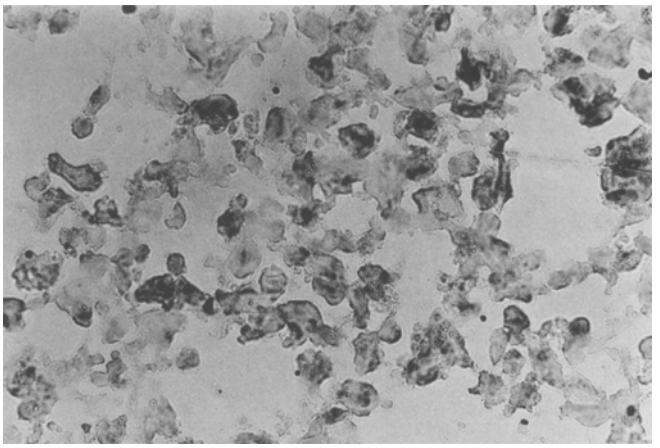

Fig. 1. Sample of dissociated cells stained with the monoclonal antibody HEA125. Phase-contrast optics, ×700

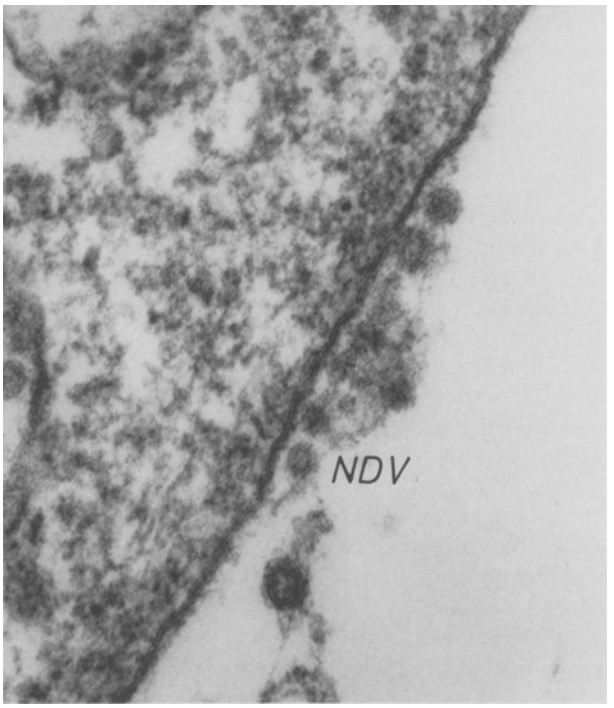

Fig. 2. Transmission electron microscopy of tumor cell vaccine. Newcastle disease virus (NDV) particles are attached to tumor cell surface

tected. Electron-microscopic investigations of cells incubated for 1 h with NDV showed that in one of seven to ten cell sections virus particles were identified at the cell surface (Fig. 2). NDV was also associated with fragments of cell membranes or unbound between the cells.

X-irradiation effect. The effect of irradiation on the biological activity, in particular on replication, transcription, and translation of the cell preparations, was investigated for individual dissociated samples of the 20 primary tumors. No thymidine incorporation was seen after irradiation. In 5/13 samples [^{3}H]-uridine and in 3/10 samples ^{3}H-labelled amino acid incorporation exceeded 500 cpm/5×10^5 cells after irradiation, which indicates metabolic activity.

Table 1. Viability and yield of cells dissociated from colorectal cancer tissue

Investigated parameters		Results
Viability		
Trypan blue dye exclusion test, $n = 10$	After dissociation	$72 \pm 12\%$
	After storage in liquid N_2	$75 \pm 22\%$
Propidium iodide, $n = 8$	Before irradiation	$82 \pm 15\%$
	After irradiation	$84 \pm 9\%$
Yield of cells, $n = 20$	Per gram tissue	$5 \pm 3 \times 10^7$
	Total	$9 \pm 6 \times 10^7$

Table 2. Local delayed-type hypersensitivity (DTH) reaction to the control (24 h p.i.)

Preparation	DTH reaction positive	Median induration (mm)	
		First application	Last application
NDV 4–64 HU[a]	2/20	3.0 (3.0–3.5)	3.5 (3.0–4.0)
Tumor cells 1×10^7	12/20	4.0 (3.0–9.5)	–
	5/12	–	5.0 (4.0–12.0)
Uninvolved mucosa 2×10^6–2×10^7	4/10	3.5 (3.0–6.5)	3.5 (3.0–7.0)
Carcinoembryonic antigen 30–60 µg	0/5	–	–

[a] HU, hemagglutinating units

Table 3. Local DTH reaction to the vaccine (24 h p.i.)

Vaccine	DTH reaction positive	Median induration (mm)	
		First vaccination	Last vaccination
2×10^6 tumor cells			
+ 4 HU NDV	3/6	3.0 (3.0–4.0)	–
+ 32 HU NDV	7/12	4.0 (3.5–7.5)	5.0 (3.0–8.5)
+ 64 HU NDV	3/6	4.5 (3.0–7.0)	5.0 (3.0–7.5)
1×10^7 tumor cells			
+ 4 HU NDV	6/9	4.0 (3.0– 8.0)	4.5 (3.0– 9.0)
+ 32 HU NDV	16/20	8.0 (3.5–14.0)	9.5 (5.0–15.5)
+ 64 HU NDV	6/10	6.5 (3.0–13.0)	7.2 (3.0–14.5)
2×10^7 tumor cells			
+ 32 HU NDV	2/4	6.5 (3.0–14.0)	–

Bacterial contamination. Preparations of dissociated colorectal primary tumors were contaminated mainly by commensals of the intestinal tract, and was found impossible to eliminate them by irradiation. Surviving micro-organisms were seen even after irradiating the samples with 600 Gy. After supplementing the processing medium with antibiotics, however, micro-organisms were no longer detected in the preparations.

Table 4. Local DTH reaction to the vaccine (24 h p.i.) of 16 patients

Induration (mm)				
Preparations consisting of 1×10^7 tumor cells + 32 HU NDV			Preparations consisting of 1×10^7 tumor cells alone	
	First vaccination	Last vaccination	First application	Last application
	9.0	14.0		
	10.0	11.0	3.0	4.5
	5.0	7.0	5.0	6.0
	10.0	8.0		
	10.0	15.5		
	7.0	9.0		
	5.0	6.0		
	3.5	7.0		
	5.0	5.0	3.0	4.0
	4.0	13.0		
	14.0	10.0		
	8.0	10.0		
	9.0	9.5		
	8.0	9.5		
	10.0	12.0	8.0	12.0
	4.0	5.0	3.0	5.0
Median	8	9.5	3	5

Clinical results

Side-effects. Of the 20 patients, 4 developed a mild fever (<38.5° C) during the first vaccination, and 10 patients recorded a mild itching at the vaccination site. Liver enzyme values and hematological indices did not show any significant changes during vaccination. No other side-effects could be observed. Following intradermal application neither local ulceration nor local necrosis could be observed.

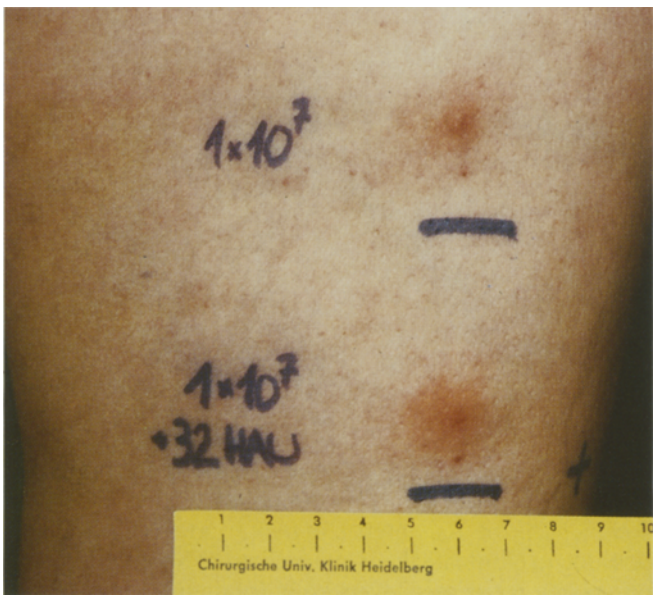

Fig. 3. Local delayed-type hypersensitivity skin responses 24 h p.i. of the vaccine: skin reaction to a vaccine consisting of 1×10^7 cells incubated with 32 hemagglutinating units NDV and without NDV preparation

Skin reaction to autologous tumor cells. Peak reactions of local inflammation and induration were observed 24–48 h post injection and induration usually lasted up to 7 days. Of the 20 patients, 12 reacted at the first vaccination with a local DTH reaction to tumor cells given as a sample of 1×10^7 cells. The median induration was 4 mm (Table 2). Vaccination could be performed only once in 15 patients because there was an insufficient number of tumor cells. In 5 patients vaccination with tumor cells alone could be carried out up to four times along with the virus-modified tumor cell vaccine. The DTH response in these patients increased from a median of 3 mm at the beginning of the vaccination up to 5 mm at the end of the vaccination. Statistical evaluation of the increase of the skin reaction using the Wilcoxon matched-pairs signet rank test revealed a significance of $P = 0.03$. Data are shown in Table 4.

Skin reaction to NDV. Of the 20 patients, 2 responded with a median induration of 3.0 mm to NDV, and the number of patients reacting, as well as the local reaction, did not significantly increase throughout vaccination (Table 2).

Skin reaction to mucosa. In 10 patients the DTH responses to cells of normal, uninvolved autologous colon mucosa without NDV were tested, 4 of them revealing a median induration of 3.5 mm, which was constant throughout four vaccinations (Table 2).

Skin reaction to carcinoembryonic antigen. None of the 5 patients inoculated with purified carcinoembryonic antigen at a dose of 30 μg or 60 μg showed any skin reaction, neither at the first nor at the fourth and the last vaccination (Table 2).

Skin reaction to autologous tumor cells incubated with NDV. The best DTH reaction was seen when a vaccine

consisting of 1×10^7 irradiated tumor cells and 32 HU NDV (Fig. 3) was applied. Out of 20 patients 16 responded with a median induration of 8.0 mm, ranging from 3.5 mm to 14.0 mm as measured 24 h p.i. Local skin induration following intradermal inoculation of this vaccine increased up to 9.5 mm, ranging from 5.0 mm to 15.5 mm at the last vaccination (Tables 3, 4). Statistical evaluation of this increase reveals a significance of $P = 0.0231$ in a Wilcoxon matched-pairs signed rank test. When a vaccine consisting of 1×10^7 tumor cells and 4 HU NDV was tested in 9 patients, 6 of them reacted with a median induration of 4.0 mm, and 6 of 10 patients reacted with a median induration of 6.5 mm to a vaccine consisting of 1×10^7 tumor cells incubated with 64 HU NDV. Skin reactions to a vaccine consisting of fewer tumor cells were less marked: 7 of 12 patients reacted with a median induration of 4.0 mm to a vaccine of 2×10^6 cells and 32 HU NDV. Increasing the number of tumor cells did not improve the DTH reaction: 2 of 4 patients showed an induration of 6.5 mm following vaccination of 2×10^7 tumor cells and 32 HU NDV.

Discussion

The biological efficacy of ASI has already been demonstrated in several animal models [9, 22, 23], and clinical trials of ASI in colorectal cancer patients have been performed using a vaccine of autologous tumor cells mixed with bacillus calmette-Guérin (BCG). A prolongation of disease-free interval and increased survival in patients with Dukes B_2 and C colorectal cancer could be shown by Hoover et al. [12] and Jessup et al. [13]. Because of our own encouraging results in the Esb animal tumor model with tumor cell vaccines modified by infection with NDV [22, 23], we have performed a phase I ASI study with an analogous protocol of tumor vaccines consisting of NDV-infected intact autologous tumor cells. NDV is well known to be non-toxic for humans [5]. Several ways of immune stimulation by NDV are suggested and described in the literature: induction of interferon α and β as well as of tumor necrosis factor α [4, 16, 17]. A stimulation by NDV of cellular accumulation of mRNAs for stress proteins has also been described [8]. In the animal model an increased CD4-positive T-cell-mediated helper response and an increased number of tumor-specific CD8-positive cytotoxic T lymphocyte precursors could be observed following vaccination [21, 27].

The efficacy of the postoperative therapeutic effect of vaccination in the animal tumor model depended on the viability of the inoculated cells and on a successful NDV adsorption [10, 22, 23, 28]. The adsorption of NDV to cells dissociated from tissue of colorectal tumors was shown by electron microscopy. According to our trypan blue dye exclusion test and incorporation data, a sufficiently high percentage of viable cells was obtained after tissue dissociation. The average yield of isolated cells from primary tumors was higher than previously described [12, 13]. Because of the staining characteristics of the epithelium-specific monoclonal antibody HEA125, the proportion of tumor cells in our dissociated cell preparations was at least 75%.

Even after low-dose irradiation most cells degenerate after further incubation in tissue culture as a consequence of chromosome and DNA damage [26, 29]. From our incorporation data following 200 Gy irradiation we could conclude that the dissociated irradiated cells no longer replicated and, therefore, could be used as a safe live vaccine.

During dissociation of colorectal tumors, contamination with intestinal bacteria has to be taken into account. According to Hoover et al. [12] and Jessup et al. [13] bacterial contamination does not affect the delayed-type hypersensitivity (DTH) skin reaction. Nevertheless, we eliminated live microbial contaminations from cell suspensions dissociated from colorectal carcinomas by supplementing the preparation medium with antibiotics.

To obtain optimal effects the correct dose of NDV and cells is important. In the animal tumor model it was shown that vaccines combined with a low dose of NDV induced the best antitumor immunity [10]. To achieve an optimal DTH reaction it seems to be necessary to vaccinate with at least $(0.5-1.0) \times 10^7$ tumor cells. The optimal concentration of NDV virus was 32 HU/1×10^7 cells, this being a rather small value and similar to that used in the ESb animal tumor model [10, 22, 23]. Since we already could observe a reaction to the first vaccination 24 h p.i., this might represent a preexisting immunity. It has been shown that humoral and cell-mediated immunity directed to autologous tumor-associated antigens are detectable in most patients suffering colorectal cancer already in the preoperative period [14]. ASI, therefore, probably boosts a preexisting immunity instead of inducing a new one. This might be the reason for the positive reaction to tumor cells alone in 12 of the 20 patients at the time of the first vaccination. Although the DTH reaction is a very important means for evaluating cell-mediated immunity, the question still remains whether it really corresponds to the systemic immune response with antitumor protection [2]. Whether a different time interval of vaccination would lead to a better DTH reaction also has to be investigated. The finding of the weak reaction to uninvolved mucosa as well as the lack of any reaction to vaccination with carcinoembryonic antigen and NDV supports the hypothesis of a specific immune response to tumor cells. We could show a significant increase of this immune response to a modified tumor cell vaccine throughout vaccination. An increase of local reactivity to tumor cells alone at the end of the vaccination as compared to the first vaccination could also be observed, although this could be tested in 5 patients only because of limitations in cellular material.

No severe concomitant side-effects were observed. Efforts have to be made to find parameters correlating to the degree of immune response and to define clearly which patient population might profit from such a vaccination protocol. Further clinical investigations in a phase II/III trial therefore have to be carried out for a better understanding of this kind of therapy and to prove the clinical value of ASI.

Acknowledgements. The study was supported by grants of the *Tumorzentrum Heidelberg-Mannheim.* The skilful technical assistance of Mrs. P. Kuprion is appreciated. The tumors were dissociated by Mrs. S. Daffinger and E. Senfi.

References

1. Astler VB, Coller FA (1954) The prognostic significance of direct extension of carcinoma of the colon and rectum. Ann Surg 139: 846–852
2. Ballow M (1985) Cutaneous delayed hypersensitivity to measure in vivo cell mediated immunity. In: Yoshida T (ed.) Investigation of cell mediated immunity. Livingstone, Edinburgh, pp 179–190
3. Barnstable CJ, Bodmer WF, Brown G, Galfre G, Milstein C, Williams AF, Ziegler A (1978) Production of monoclonal antibodies to group A erythrocytes. HLA and other cell surface antigens – new tools for genetic analysis. Cell 14: 9–20
4. Brown GE, Simon EH, Chung C (1980) Interferon production by individual L cells. J Gen Virol 47: 171–182
5. Cassel WA, Garret RE (1965) Newcastle disease virus as an antineoplastic agent. Cancer 18: 863–868
6. Cassel WA, Murray DR, Phillips H (1983) A phase II study on the postsurgical management of stage II malignant melanoma with a Newcastle disease virus oncolysate. Cancer 52: 856–860
7. Charon DJ, McDevitt HO (1980) Characterization of HLA-D region antigens by two-dimensional gel electrophoresis. J Exp Med 152: 18s–36s
8. Collins PL, Hightower LE (1982) Newcastle disease virus stimulates the cellular accumulation of stress (heat shock) m-RNA's and proteins. J Virol 44: 703–707
9. Hanna Jr MG, Peters LC (1978) Specific immunotherapy of established visceral micrometastases by BCG-tumor cell vaccine alone or as an adjunct to surgery. Cancer 42: 2613–2625
10. Heicappell R, Schirrmacher V, von Hoegen P, Ahlert T, Appelhans B (1986). Prevention of metastatic spread by postoperative immunotherapy with virally modified autologous tumor cells: I. Parameters of optimal therapeutic effects. Int J Cancer 37: 569–577
11. Hollinshead A (1986) Immunotherapy trials: current status and future directions with special emphasis on biologic drugs. Springer Semin Immunopathol 9: 85–103
12. Hoover HC, Surdyke MG, Dangel RB, Peters LC, Hanna Jr MG (1985) Prospectively randomized trial of adjuvant active-specific immunotherapy for human colorectal cancer. Cancer 55: 1236–1243
13. Jessup MJ, McBride CM, Ames FC, Guarda L, Ota DM, Romsdal MM, Martin RC (1986) Active specific immunotherapy of Dukes B_2 and C colorectal carcinoma: comparison of two doses of the vaccine. Cancer Immunol Immunother 21: 233–239
14. Jessup MJ, Kefeng O, Kanellopoulus K, Campbell DE, Cleary KR, Hickey CM, Reading CL, Savage HE (1988) Expression of autoantigens in human colorectal carcinomas. Arch Surg 122: 1435–1439
15. Liebrich W, Lehner B, Möller P, Schirrmacher V, Schlag P (1988) Preparation and characterization of a new type of tumor cell vaccine: Surface modified human colon cells using Newcastle disease virus (NDV). Clin Exp Metastasis 6 [Suppl 1]: 80–81
16. Lorence RM, Rood PA, Kelly KW (1988) Newcastle disease virus as an antineoplastic agent. Induction of tumor-necrosis-factor alpha and augmentation of TNF alpha cytotoxicity. J Natl Cancer Inst 80: 1305–1312
17. Marcus PI, Svithlik C, Selellick MJ (1983) Interferon induction by viruses: X. A model for interferon induction by Newcastle disease virus. J Gen Virol 64: 2419–2431
18. Momburg F, Moldenhauer G, Hämmerling GJ, Möller P (1987) Immunohistochemical study of the expression of a Mr 34,000 human epithelium-specific surface glycoprotein in normal and malignant tissues. Cancer Res 47: 2883–2891
19. Möller P, Matthaei-Maurer DU, Moldenhauer G (1989) CD30 (Ki-1) antigen expression in a subset of gastric mucosal plasma cells and in a parimary gastric plasmacytoma. Am J Clin Pathol 91: 18–23
20. Murray DR, Cassel WA, Torbin A, Olkowski Z, Moore MC (1977) Viral oncolysate in the management of malignant melanoma: II. Clinical studies. Cancer 40: 680–686
21. Schild HJ, von Hoegen P, Schirrmacher V (1988) Modification of tumor cells by a low dose of Newcastle disease virus: II. Augmented tumor-specific T cell response as a result of CD4+ and CD8+ immune T cell cooperation. Cancer Immunol Immunother 28: 22
22. Schirrmacher V (1986) Postoperative activation of tumor-specific T cells as a means to achieve immune control of minimal residual disease. In: Fortner JG, Rhoads JE (eds) Accomplishments in cancer research 1986. General Motors Cancer Res Found, Lippincott, Philadelphia, pp 218–232
23. Schirrmacher V, Heicappel R (1987) Prevention of metastatic spread by postoperative immunotherapy with virally modified autologous tumor cells. II. Establishment of specific systemic anti tumor immunity. Clin Exp Metastasis 5: 147–156
24. Schlag P, Verser J, Geier G, Breitig D, Merkle P (1979) Incorporation studies of nucleic acid precursors in gastric cancer. J Cancer Res Clin Oncol 95: 273–280
25. Spurr AR (1969) A low viscosity epoxy resin embedding medium for electron microscopy. J Ultrastruct Res 26: 3143–43
26. Thompson T, Suit D (1969) Proliferation kinetics of X-irradiated mouse L cells studied with time-lapse photography. Int J Radiat Biol 15: 247–362
27. Von Hoegen P, Heicappell R, Griesbach A, Altevogt P, Schirrmacher V (1989) Prevention of metastatic spread by postoperative immunotherapy with virally modified autologous tumor cells: III. Postoperative activation of tumor-specific CTLP from mice with metastases requires stimulation with the specific antigen plus additional signals. Invasion Metastasis 9: 117–133
28. Von Hoegen P, Weber E, Schirrmacher V (1988) Modification of tumor cells by a low dose Newcastle disease virus: I. Augmentation of the tumor specific T cell response in the absence of an anti-viral response. Eur J Immunol 18: 1159–1166
29. Watanabe I, Okada S (1966) Study of mechanisms of radiation-induced reproductive death of mammalian cells in culture: estimation of stage at cell death and biological description of processes leading to cell death. Radiat Res 27: 290–306