Eur J Cancer Clin Oncol, Vol. 21, No. 8, pp. 907-912, 1985.
Printed in Great Britain.

0277-5379/85$3.00+0.00

Inflammatory Tumor Response to Monoclonal Antibody Infusion*

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Abstract—*Two patients with melanoma and one with apudoma, all three with metastatic disease, received monoclonal antibody infusions with mAb R-24, specific for the disialoganglioside G_{D3}. This marker was shown to be restricted to melanoma cells and a few other tumors of neural crest origin. Following treatment with mAb R-24 both melanoma patients showed inflammatory cutaneous responses around tumor nodules, i.e. blister formation or inflammatory perinodular halos. Local pain in bulky intestinal tumor sites occurred in all three patients about* 3 hr *after onset of antibody infusion. Adverse side-effects of antibody application were not observed with antibody doses up to* 200 mg *(single) and* 440 mg *total dose. The presented data indicate that mAb R-24 is active* in vivo.

INTRODUCTION

In vivo application of murine monoclonal antibodies to patients with solid tumors represents a novel approach to diagnostics and cancer therapy [1-3]. Whereas frequently the lack of antibody specificity limits this approach, mAb R-24 recognizing disialoganglioside G_{D3} on malignant melanoma and tumors of neural crest origin meets the criteria for tumor restriction best, defining G_{D3} as one of the most important markers for these tumor systems to date [4]. Moreover, functional studies indicate that mAb R-24 inhibits melanoma cell growth through induction of morphological changes [5], activates human complement [6] and mediates high-level ADCC [7]. Therefore, mAb R-24 fulfils several criteria for its utilization as an antitumor agent very well. The purpose of this pilot study was to obtain data on the *in vivo* toxicity of this mAb. Here we report our initial experiences with systemic and local intra-arterial mAb infusion in three patients with G_{D3}-positive metastatic tumors.

Accepted 22 February 1985.
*Supported by grants from the Deutsche Forschungsgemeinschaft Di 245/3-2, Kn 180/3-2. Part of this paper has been reported at the Behring Symposium, Marburg, F.R.G., September 1983, and at the AACR/ASCO Cancer meeting in Toronto, Canada, May 1984.
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MATERIALS AND METHODS

Patients

Patient AA initially presented at the age of 42 yr with an invasive rectal polyp, which was diagnosed as apudoma and removed. One and a half years later multiple metastases of the liver were operated. After surgery the patient failed on extensive chemotherapy (methotrexate/5-FU, streptozotocin/5-FU and mitomycin C) and radiotherapy for progressive disease in both lungs and liver. Chemotherapy was given until 5 weeks before the start of R-24 antibody application. Values of blood chemistry were normal except for hemoglobin (10.9 g%). The patient had been on no other medication at this time. Karnofsky performance status was approximately 70%.

The melanoma of patient AB (age 27 yr) was disseminated over the whole body, showing small subcutaneous pigmented tumor nodes. The tumor progressed under chemotherapy with cisplatin and vindesine and multiple metastases were demonstrable in liver, spleen and adrenals. Monoclonal antibody R-24 application was given 3 months after the patient's last chemotherapy. Laboratory studies showed no abnormal values at this time. Karnofsky performance status 80%.

AC was a 36-yr-old patient with extensive subcutaneous malignant melanoma at the left thorax (6 × 8 cm) and multiple 3-4 cm large exulcerative metastases consuming the whole left

arm with massive edema. Further sites of lymph node metastases were the supraclavicular regions, the left groin, the mediastinum, the left axilla and bones. Because of progressive tumor, the patient was treated with various regimens of chemotherapy: DTIC, cisplatin, ifosfamide and vindesine, of which the combination of cisplatin and vindesine gave a partial response of short duration only. Because of the massive tumor burden in the area of the left arm and thorax, a femoral catheter was placed into the left a. brachialis for local antibody application. The patient's last course of chemotherapy had been given 4 weeks previously. Laboratory studies showed an Hb of 8.3 g%, but no other abnormal values. Karnofsky performance status was 60%.

Materials and methods

Production and specificity of mAb R-24 has been described [4]. Antibody, purified by ammonium sulfate precipitation, was diluted in 250 ml normal saline + 5% human albumin for infusion. Pyrogenicity and sterility were checked by standard procedures. Consent was obtained from each patient after full explanation of the purpose, nature and risks, which may be a consequence of monoclonal antibody application.

The infusion time was 6 hr. Serum levels of murine mAb were determined in protein A-MHA on SK-MEL-28 melanoma cells as described [4]. Formation of anti-mouse antibody in patients' sera as a consequence of R-24 infusion were tested in an enzyme-linked immunoassay.* Immunoperoxidase staining procedures of frozen tissue sections were performed as described by Stein *et al.* [8]. Instead of diaminobenzidine, 3-amino-9-ethylcarbazol (Sigma, Taufkirche, F.R.G.) was used as substrate. Patients were eligible for this study when <95% of tumor cells in frozen stained sections showed an homogeneous strong staining with mAb R-24.

RESULTS

Patient 1 (42 yr) was unresponsive to cytostatic chemotherapy for apudoma of the rectum, metastatic primarily to lung and liver. He received mAb R-24 infusions of 6 hr duration over 2 weeks with increasing doses of antibody (day 1: 1 mg; day 4: 6 mg; day 8: 30 mg; day 11: 100 mg; day 15: 140 mg). Infusion of 6 mg antibody or more induced severe intra-abdominal pain localized to the tumor-infiltrated liver approximately 3 hr after start of infusion. mAb R-24 was detectable in serum only after doses higher than 30 mg. A time course of antibody detected is shown in Fig. 1. Specimens of urine and spinal fluid were negative for mAb R-24. Human anti-mouse antibody formation started after the fifth mAb-infusion.

Tumor growth continued in patient 2 (26 yr) despite cytostatic chemotherapy for metastatic malignant melanoma. Disease primarily manifested in skin, liver, spleen and adrenals. Following infusion of 200 mg mAb R-24, bright inflammatory halos around cutaneous tumor nodules (Fig. 2) as well as local pain at bulky visceral tumor sites develped 3 hr after start of antibody infusion. Free serum antibody R-24 was detectable by protein A-MHA up to 2 days (day 1: 1/124; day 2: 1/32; day 3: 0) following antibody application.

Patient 3 (36 yr) had bulky metastatic malignant melanoma unresponsive to cytostatic chemotherapy. Tumor manifested around the left upper chest ('cancer en cuirasse') and the left arm, consuming nearly all functional structures including bone, with widespread bulky lymph node metastases. Local antibody perfusion of the left arm was facilitated through an intra-arterial catheter placed in the a. brachialis. Intensive pain at perfused tumor sites developed 3 hr after the start of the first, second and third antibody infusions (day 1: 105 mg; day 3: 135 mg; day 6: 200 mg). Free R-24 serum antibody was seen for 2 days after the first and for 3 days after the following two Ab-applications. Two cutaneous tumor nodules in the perfused arm showed blister formation. Although no measurable reduction in tumor size was detected, subjectively arm movements in the left shoulder became easier due to less pain after 5 days of antibody perfusion.

Effects of mAb application were sought in all three patients. Blood pressure, pulse and temper-

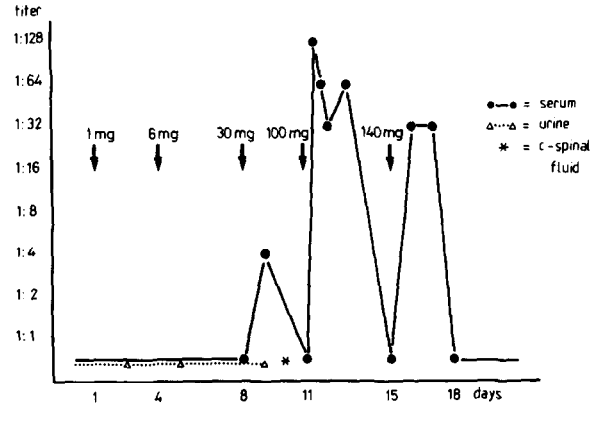

Fig. 1. Titers of mAb R-24 in serum, urine and cerebrospinal fluid after application of increasing doses of mAb in patient AA.

*Details obtainable from authors upon request.

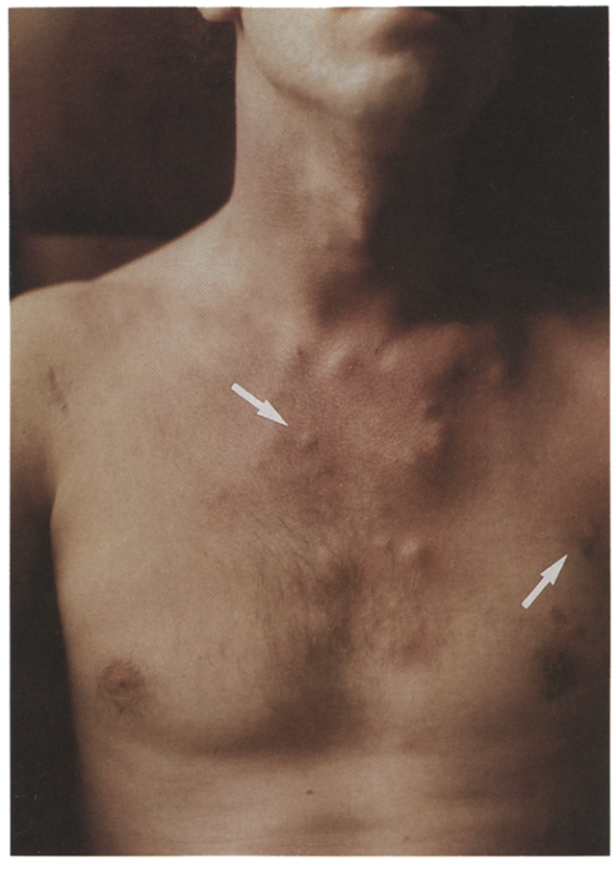

Fig. 2. Inflammatory response at the tumor site after application of mAb R-24 in patient AB.

ature were assessed in all three patients and remained unchanged during and after antibody application. Blood counts, platelets included, liver enzymes (glutamic oxalacetic transaminase, glutamic pyruvic transaminase, glutamic acid dehydroxygenase, alkaline phosphatase, γ-glutamyl transpeptidase) renal function (urea, uric acid, creatinine) and electrolytes ($Na+/K+$, CA^{2+}, were determined before and during treatment with mAb R-24. We could not detect any variations of these parameters in all three patients. Thyroid values (T3, T4, TSH) and cortisol levels, which were controlled before and after antibody application in patient 1, also remained unchanged. We only saw a rise in glutamic pyruvic transaminase (8 → 51 U/l) and glutamic acid dehydroxygenase (4.0 → 44 U/l) after the application of 30, 100 and 140 mg mAb R-24, together with a reduction in size of one big liver metastasis, documented by computed tomography in patient 1, which returned to normal 10 days afterwards. Mild skin rashes were observed about 5 hr after starting mAb infusion, particularly at higher mAb doses in all three patients. They continued for up to 5 hr and subsided spontaneously. In two instances an antihistaminicum (clemastin hydrogen fumarat 4 mg) was given; however, specific medical treatment was never necessary.

DISCUSSION

Infusion of ganglioside G_{D3}-specific monoclonal antibody is shown to induce a local inflammatory response in cutaneous tumor sites. Pain in bulky tumor masses (visceral or muscular) regularly occurred 3 hr after antibody infusions had started. This local response at the tumor site confirms our initial studies, demonstrating the extraordinary specificity of this antibody for malignant melanoma [4], and more recent immunohistological studies, on the tissue distribution of ganglioside G_{D3}, detected in primary and metastatic malignant melanoma, other human tumors, nevi and 50 different normal human tissues [Dippold *et al.*, in press]. The histomorphological correlate and immune histology of the observed inflammatory effect at the tumor site was not established, for ethical reasons or because patients refused biopsies. Meanwhile we were able to demonstrate mAb at the tumor site 2 weeks after low-dose mAb application (5 mg/m^2) for 3 weeks and tumor necrosis in another patient (unpublished data).

Three mechanisms which may be involved in this *in vivo* antibody effect can be demonstrated *in vitro*. Thus, G_{D3}-specific mAb R-24 effectively activates human complement [6]. In addition, R-24 mediates unusually strong antibody-mediated cellular cytotoxicity [7], as well as cytomorphological and cytotoxic changes on G_{D3}-positive tumor cells exposed to mAb R-24 [5]. Some or all of these known phenomena may contribute to the effects observed *in vivo*.

The purpose of this study has been to determine whether the application of mAb R-24 causes toxicity in patients with G_{D3}-positive tumors and advanced disease. Particularly, since we know that a minor population of normal cells (<0.1%) in the human body, especially in the brain stem [9], the zona reticularis of the adrenals and the juxtaglomerum of the kidney, stained R-24 positive by immunohistological procedures [Dippold *et al.*, in press], we were concerned that vital functions may be affected by the application of this specific antibody. Neurologic and ophthalmic examinations were performed prior to and after infusions and did not show any changes.

This concern also determined our strategy. Thus we started with small doses of mAb (1, 6, 30, 100 and up to 140 mg) in the first patient. Since this patient did not develop any signs of antibody toxicity but responded to R-24 by anti-mouse Ab after the last dose, we gave an even higher single dose to patient 2 (200 mg). This high antibody dose resulted in a local inflammatory response at the area of subcutaneous tumor nodules; pain at internal tumor sites, however, did not lead to adverse side-effects. We therefore continued to apply higher antibody doses locally in patient 3 to see if an immediate, more dramatic effect could be achieved by this antibody than in the case of patient 2. Blister formation and pain at the tumor site were an acute effect, and better mobility of the arm about 5 days after infusion of the last antibody dose a more long-term effect.

This first experience using mAb R-24 *in vivo* demonstrates that no major side-effects are to be expected when treating patients with antibody doses up to 200 mg single dose and 440 mg total dose. The inflammatory response at the tumor site indicates that mAb R-24 is also active *in vivo*.

Shrinkage of one liver metastasis was shown in patient 1, 1 day after the third Ab application (30 mg). No further change occurred during or after the following treatments. The patient received chemotherapy 5 days after the last antibody application. Patient 2 remained stable for 3 months after a single dose of mAb R-24 and then progressed. Tumor lesions of patient 3 had shown blister formation for 4 days after antibody treatment, which resumed spontaneously. The edema of his left hand disappeared completely and the pain and movability in his left shoulder improved 5 days after his last antibody treatment, lasting for almost 3 weeks. The tumor did not

progress for 5 weeks following antibody application. Thereafter, because of the massive tumor burden, another trial of cytostatic chemotherapy was undertaken.

Most recently we observed an immediate inflammatory response and perhaps more importantly tumor regression following one cycle of R-24 treatment. This finding will be the subject of a further report.

Acknowledgements—We acknowledge the constant support and discussion of Drs A. Houghton, H. Oettgen and L. J. Old. We thank E. Boosfeld and B. Kinkel for their technical help.

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