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Involvement of T Lymphocytes in Curative Effect of a New Immunomodulator OM 163 on Rat Colon Cancer Metastases

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In a model of colon cancer in syngeneic rats, a new immunomodulator, OM 163, induced the complete disappearance of peritoneal carcinomatosis (nodules measuring 1–5 mm) in 41 out of 82 rats. Those results were confirmed in a survival experiment in which 3 out of 10 treated rats died free of tumour 10, 18 and 28 months after the tumour cell injection while all the untreated control rats died of their tumours within 3 months. OM 163 had a systemic effect, since injected intraperitoneally it completely inhibited the growth of lung metastases in 13 out of 20 rats. The antitumour effect of OM 163 was also observed in two rat strains on original tumours. Lymphocyte infiltration was observed in the tumours mainly constituted of $CD4^+$ and $CD8^+$ cells. The treatment had no effect in nude rats, confirming the involvement of T lymphocytes. Furthermore, rats cured by OM 163 were protected against a second challenge of tumour cells and in a Winn's assay, splenocytes from cured rats protected normal rats against tumour cells.
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INTRODUCTION

COLORECTAL CANCER is the most frequent cause of cancer death in industrialised countries. Despite a general improvement in surgical techniques, patients die of primary tumours (25% of patients cannot be operated), of local recurrences and/or of metastases. Immunotherapy of gastrointestinal cancers has met with little success. The results of trials with recombinant cytokines: interferons (IFN), tumour necrosis factor (TNF)α, interleukin 2 (IL-2), have been disappointing [1–3]. However, immunotherapy has recently been shown to be effective as an adjuvant to surgery. The association of 5-fluorouracil plus levamisole reduced the risk of recurrence by 41% and overall mortality by 33%, the median follow-up being 3 years [4]. Immunotherapy with an autologous tumour cell-BCG vaccine showed 24% improvement of the disease-free period, the median follow-up being 28 months [5]. These results are sufficiently encouraging to revive the interest in immunotherapy.

The new immunomodulator OM 163 is a fractionated alkaline extract of *Escherichia coli*. Fractionation is carried out by ultrafiltration and diethylaminoethyl (DEAE) ion-exchange chromatography. Any remaining lipopolysaccharide is minimised by extracting it into Triton X-114. The lipopolysaccharide content, as measured by the Limulus amoebocyte assay, is lower than 10 ng/mg of OM 163. We investigated the antitumour effect of OM 163 in a model of colon cancer in the rat. In this model, as in the human, colon cancer metastases are chemoresistant [6] and natural killer (NK) resistant [7]; BCG is without effect [8] as are recombinant TNF (rTNF) and recombinant IL-2 (rIL-2); crude lentinan is effective [9] but not the soluble preparation for human use. Therefore, this model is close to human disease and is suitable for evaluating antitumour immunotherapy. Here we report the curative effect of OM 163 on rats bearing colon cancer metastases and the involvement of T lymphocytes in this effect.

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MATERIALS AND METHODS

Animals and tumour cells

BD IX, Fischer 344 and nude rats, 3–5-month-old males and females, were purchased from IFFA credo (l'Arbresle, France) or from CNRS (Orléans, France).

The tumour cell clone DHD/K12-PRO b (named PRO b), the cell culture DHD/K12 and the grafted tumour DHD were obtained from a colon adenocarcinoma chemically induced in inbred BD IX rats [10], hence they are syngeneic to BD IX rats. Those cells, PRO b and DHD/K12, were kept in culture as previously described [11]. The line of grafted tumour Fischer dimethylhydrazine tumour C (FHC) was obtained from a colon adenocarcinoma chemically induced in inbred Fisher 344 rats, hence they are syngeneic to Fisher 344 rats. The absence of contamination by bacteria, mycoplasma or fungi was checked monthly and before each *in vivo* injection by fluorescent staining of extranuclear DNA with bisbenzimide.

Immunomodulator OM 163

OM 163 (OM Laboratories, Meyrin, Switzerland) is an alkaline extract of *Escherichia coli* produced under conditions which leave the bacteria essentially intact as observed by light microscopy. The extract is purified by ion-exchange chromatography (DEAE) and extracted with Triton X-114 to minimise endotoxin content. The activity of the final product is tested *in vitro* for nitric oxide production (NO_2^-) in a murine macrophage assay. The final product contains principally bacterial proteins with acidic isoelectric points, due to deamidation under the alkaline extraction conditions. The lipopolysaccharide content,

as measured by the Limulus amoebocyte assay, is lower than 10 ng/mg of OM 163. Injected intraperitoneally (i.p.) in mice up to 300 mg/kg OM 163 does not produce toxicity.

Peritoneal carcinomatoses and artificial lung metastases

All the untreated BD IX rats injected i.p. with 10^6 viable PRO b cells or in the femoral vein with 7×10^6 viable PRO b cells exhibited either peritoneal carcinomatoses and haemorrhagic ascites or lung metastases [12, 13]. They died of their tumours between the 8th and the 12th week.

Immunotherapy

The treatment of the peritoneal carcinomatoses was always initiated on day 14 and those of lung metastases on day 3 after the tumour cell injection (day 0). At that time, all rats had macroscopic peritoneal nodules or microscopic lung metastases. Treatment consisted of five i.p. injections administered twice a week. Experiments took 6 weeks and ended before the death of control rats. An autopsy was carried out on all rats at the end of each experiment.

The differences in tumour growth between rats were large. Peritoneal carcinomatoses was scored on a scale from 0 to 4. Scoring was performed in a blind fashion. Rats were taken without reference to their ear tags and scored after inspection of their abdominal cavities. Rats of similar scores were placed in defined classes of peritoneal carcinomatoses. Class 0 was defined as no visible nodules; class 1: few nodules <2 mm diameter; class 2: nodules <5 mm diameter and too numerous to count; class 3: peritoneal cavity invaded with nodules up to 1 cm diameter; class 4: peritoneal cavity fully invaded with tumour masses, some of which measured several cm in diameter. After scoring, the ear tags were read. For lung metastases rats were scored after investigation of lungs under the microscope and placed in two classes, with or without metastases. The procedure was the same as above.

The number of rats per treatment group was 10. The significance of the effect of OM 163 on the peritoneal carcinomatoses, the lung metastases and on the production of ascites was analysed by a Kruskal–Wallis test, and the effect on the survival by a log rank test.

Immunisation experiment and Winn's assay

In the immunisation experiment, rats were i.p. injected with 10^6 PRO b cells and treated with OM 163. Four months after the i.p. tumour cell injection, cured rats and normal rats were subcutaneously (s.c.) injected with 10^6 PRO b cells. The tumours were measured weekly and mean volume calculated according to the formula $L \times I^2/2$ where L is the length and I the width.

The Winn's assay consisted of an i.p. injection of 100×10^6 splenocytes mixed with 10^6 PRO b cells in normal rats. Splenocytes were taken from tumour-bearing rats treated or not with OM 163 and from normal rats. Control rats received PRO b cells alone or splenocytes alone (splenocytes from untreated tumour-bearing rats).

Histological and immunoenzymatic study

Tumour nodules were embedded in tissue freezing medium and frozen in isopentane in liquid nitrogen for cryosection or fixed in Bouin's solution and embedded in paraffin. Conventional 3 μm sections were stained with modified trichrome Masson and haematoxylin–eosin. Cryosections (4–5 μm) were fixed in acetone and incubated with monoclonal antibodies from mouse ascitic fluid specific for rat leucocytes then with goat anti-mouse IgG biotinylated antibodies as second reagent and stained with the streptavidin-biotin-peroxidase complex. Monoclonal antibodies used were W3/25 which stained CD4 cells and macrophages, OX 8 which stained CD8 cells (both were obtained from Seralab, Crawley Down, Sussex, U.K.) and KiM2R (a gift from Dr H.H. Wacker, Kiel, Germany) which stained macrophages.

Tumour nodules were taken from omentum or mesentery at each time from three untreated control rats and three treated rats. Nodules were taken after the first, third and the fifth injections of OM 163, i.e. on days 17, 24 and 30, then 2 weeks after the last injection on day 41. The density of stained cells was independently evaluated by two observers on a scale from 0 to 5.

RESULTS

OM 163 induced disappearance or slowed down the growth of peritoneal carcinomatoses and artificial lung metastases

OM 163 induced the regression, i.e. induced the disappearance of tumour nodules, or slowed down the growth of peritoneal carcinomatoses which consisted of numerous nodules measuring 1–5 mm. Furthermore, OM 163 significantly inhibited the production of ascites (Table 1). Dose–response experiments showed an antitumour effect from 10 to 40 mg/kg when OM 163 was injected i.p., the best effect to treat peritoneal carcinomatoses being with 10 mg/kg. Among the treated rats, 50% showed a complete disappearance of their tumours and 95% did not exhibit ascites after the treatment at the dose of 10 mg/kg whereas 100% of untreated rats had tumours and haemorrhagic ascites 6 weeks after the tumour cell injection. OM 163 antitumour efficacy was highly reproducible, cumulative data of eight experiments show 41 complete regressions out of 82 treated rats with a distribution of four to six complete regressions per group of 10 rats and six in a group of 12 rats (Table 1).

In a survival experiment three out of the 10 rats treated with OM 163 died free of tumour 10, 18 and 27 months after the tumour cell injection at 15, 23 and 30 months (the median of life of BD IX rats is 27 months). A tooth problem was responsible for the death of the first two rats. All the untreated control rats died with tumour masses of several centimeters and haemorrhagic ascites of 15–60 ml within 3 months after the tumour cell injection (Fig. 1). The lifespan of rats from the treated group was significantly enhanced ($P < 0.01$) in comparison with those from the untreated control group (Fig. 2). These results are confirmed in a second survival experiment in progress in which

Table 1. Effect of OM 163 on the growth of macroscopic peritoneal carcinomatoses induced by colon cancer cells in syngeneic rats

OM 163 treatment	Number of rats with carcinomatoses of class					Volume of the ascites (ml/rat)	
	0	1	2	3	4	Range	Mean
None	0	6	18	21	36	0–98	24
10 mg/kg	41	14	18	5	4	0–40	2

Statistical significance of the treatment effect on the tumour growth and on the ascites production: $P < 0.01$ (Kruskal–Wallis test). The treatment started 14 days after the tumour cell injection, all the rats were killed 6 weeks later and an autopsy was carried out on each of them. Definition of different classes of carcinomatoses is given in Materials and Methods. Cumulative data from eight experiments (82 treated and 81 untreated rats).

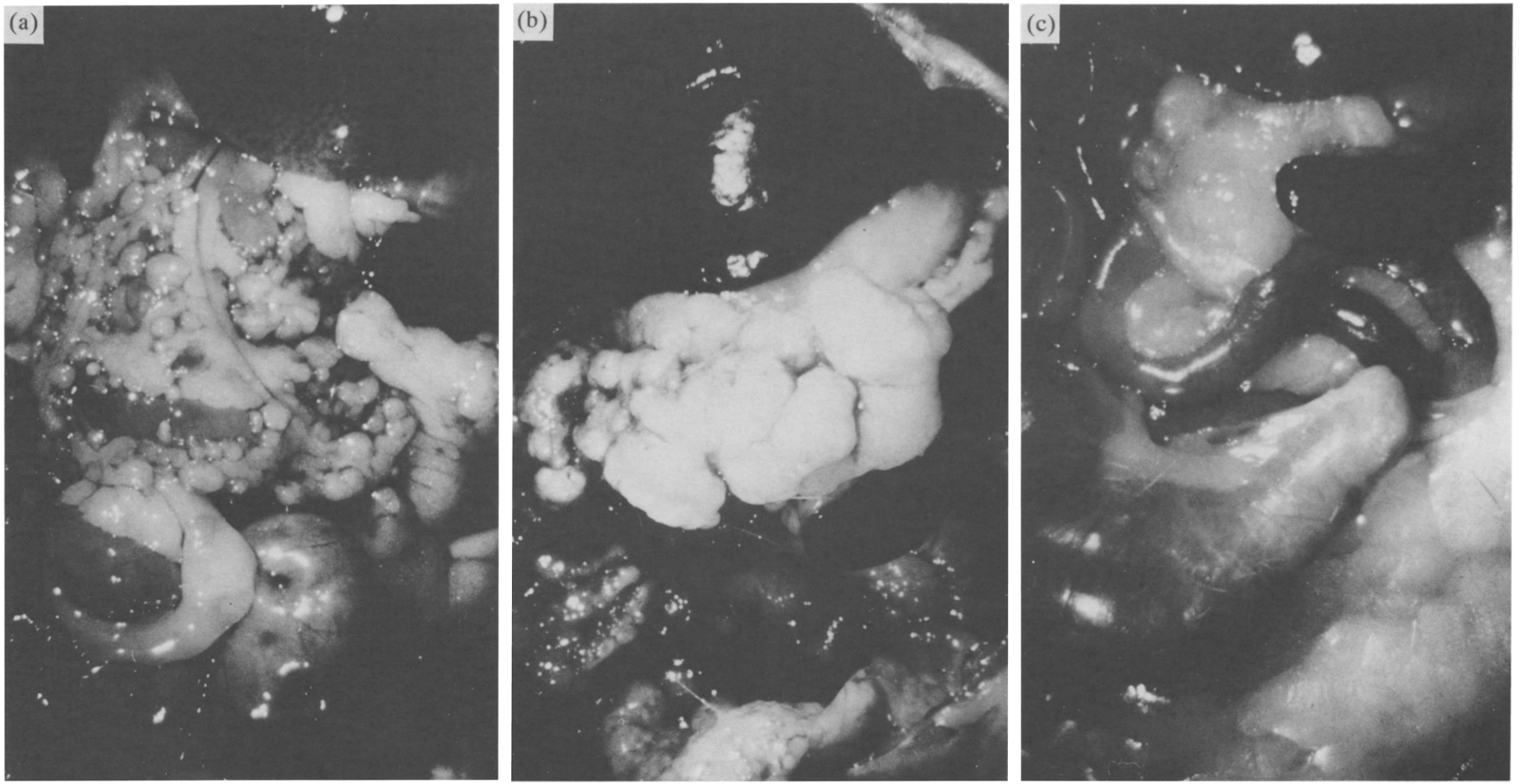

Fig. 1. Autopsy of mesentery and omentum of BD IX rats which were injected with 10^6 PRO b tumour cells into the peritoneal cavity. (a) Autopsy was performed on day 14 after the tumour cell injection when treatment with OM 163 was started; numerous nodules up to 5 *mm* are visible. (b) Autopsy was performed on the death of untreated control rat, tumour masses of several cm invading the whole peritoneal cavity are visible. (c) Autopsy was performed on the death of treated rat, there are no visible nodules.

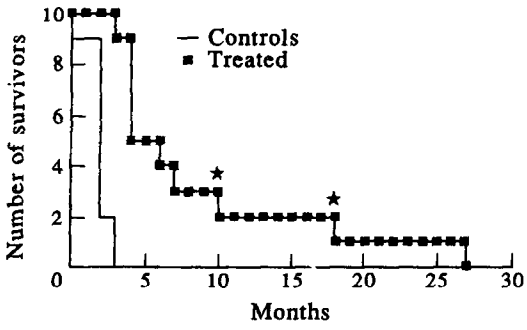

Fig. 2. Survival of rats bearing macroscopic peritoneal carcinomatoses and treated with OM 163. BD IX rats were given the injection of 10^6 syngeneic PRO b tumour cells into the peritoneal cavity on day 0. The treatment was initiated 14 days later; at that time all rats had numerous peritoneal tumour nodules up to 5 mm. Treatment consisted of five i.p. injections of 10 mg OM 163/kg administered twice a week. An autopsy was carried out on all rats. All the control untreated rats died of their tumours. The lifespan of treated rats was enhanced ($P < 0.01$) and three rats died free of tumour; a tooth problem was responsible for the death of two rats★.

5 months after the tumour cell injection four out of the nine treated rats are still alive whereas all the untreated control rats died within 3 months.

OM 163 efficacy was not restricted to the DHD/K12-PRO b cell clone; the same effect was observed on DHD-K12 cells as well as on dissociated cells from DHD tumours. DHD/K12-PRO b cells were cloned from DHD/K12 cells which were established in culture from DHD tumour. Results are shown in Table 2 and they were confirmed in two other independent experiments. OM 163 efficacy was not restricted to the BD IX/DHD model; the same effect was observed in Fisher 344 rats on peritoneal carcinomatoses induced by dissociated cells from FHC tumours (Table 2).

OM 163 efficacy was not restricted to peritoneal carcinomatoses. When injected i.p. OM 163 had a systemic effect shown by its action on artificial lung metastases. Six weeks after the tumour cell injection no metastases were observed under the microscope in lungs of 65% of treated rats whereas 95% of control untreated rats had metastases of several millimeters

Table 2. Effect of OM 163 on the growth of macroscopic peritoneal carcinomatoses induced by colon cancer cells coming either from a culture or from a tumour

Origin of cancer cells	Treatment*	Number of rats without tumours	Number of rats with tumours	Total number of rats
Culture DHD/K12†	Without	0	12	12
	With	7	4	11
Graft tumour DHD†	Without	1	9	10
	With	5	5	10
Graft tumour FHC‡	Without	1	9	10
	With	5	5	10

*Statistical significance of the treatment effect: $P < 0.01$ (Kruskal–Wallis test). The dose of OM 163 was 10 mg/kg, the treatment started 14 days after the tumour cell injection when the nodules measured 1 to 5 mm. Six weeks later, rats were killed and an autopsy was carried out on each of them. †Injected in syngeneic BD IX rats. ‡Injected in syngeneic Fisher 344 rats.

(Table 3). Dose–effect experiments showed an antitumour effect from 25 to 50 mg/kg when OM 163 was injected i.p., the best effect to treat lung metastases with was the dose of 25 mg/kg.

OM 163 was without side-effects in rats and without cytotoxic effects on PRO b cells

OM 163 administered i.p. up to five times with doses as high as 50 mg/kg was without overt toxicity, i.e. loss of weight, digestive bleeding, peritoneal fibrosis.

In a photometric microassay, OM 163 up to 1 mg/ml (i.e. 0.2 mg/10^4 cells) was not directly toxic to PRO b cells. The mean optical density of PRO b cells cultivated 72 h in culture medium was 0.543 ± 0.018 without OM 163 and 0.541 ± 0.021 with OM 163.

T lymphocytes were involved in the antitumour effect of OM 163

In untreated control tumours, staining with trichrome Masson showed a weak peripheral inflammatory reaction (from 1 to 1.5 on the arbitrary scale) but did not show infiltrating inflammatory cells. In treated rats a strong inflammatory reaction from 1.5 to 5 was observed. This reaction was seen from day 17 to 41 with a maximum on day 24. From day 34 in the rats treated by OM 163 some tumour nodules were replaced by fibrous granuloma constituted by epithelioid cells, multinucleated giant cells and fibroblasts.

In untreated control tumours the number of cells stained by W3/25 monoclonal antibody or OX 8 monoclonal antibody was low (from one to two) and did not change from day 17 to 41. On the contrary, in treated tumours the number of stained cells increased from one to five with a maximum on day 24 (Fig. 3). This increase was observed in three out of three independent experiments for W3/25 and in two out of three independent experiments for OX 8-stained cells. The number of KiM2R-stained cells was low and did not change in either untreated or treated tumours.

OM 163 had no significant effect on the growth of peritoneal carcinomatoses induced by PRO b cells in nude rats (Table 4).

Rats cured by OM 163 were immunised against PRO b cells

Rats cured of PRO b tumours by OM 163 were protected against a second challenge of PRO b cells (Fig. 4). Four months after the first tumour cell injection, PRO b cells were s.c. injected in nine rats cured of their peritoneal carcinomatosis. Eight rats did not develop tumours or developed a tumour which was completely rejected. However, one rat exhibited a tumour of 0.21 cm^3 120 days after the tumour cell injection. All the control rats exhibited tumours with a mean volume of 7.7 ± 2.5 cm^3 70 days after the tumour cell injection. At that time the control rats were sacrificed for ethical reasons.

Table 3. Effect of OM 163 on the growth of lung metastases induced by colon cancer cells in syngeneic rats

OM 163 treatment*	Number of rats Without tumours	With tumours	% without tumours
None	1	19	5
25 mg/kg	13	7	65

*Statistical significance of the treatment effect: $P < 0.01$ (Kruskal–Wallis test), results of two independent experiments with PRO b cells. The treatment administered i.p. started 3 days after the tumour cell injection, 6 weeks later the rats were killed and an autopsy was carried out on each of them.

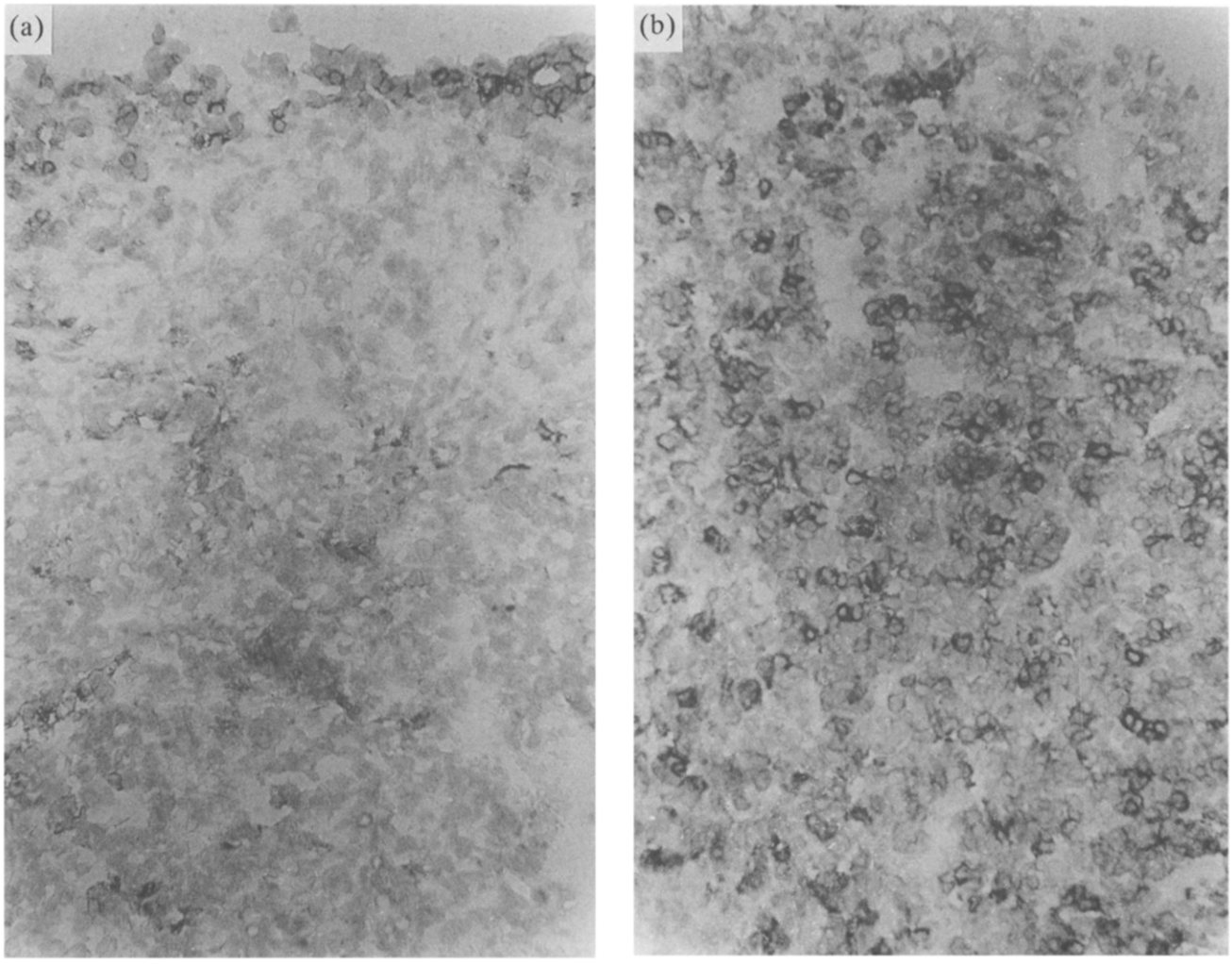

Fig. 3. CD8 cells in untreated (a) and treated (b) tumour nodules. Histological sections of tumour nodules were taken on day 24 after tumour cell injection from mesentery of untreated control rat or during the treatment with OM 163 (×300).

In a Winn's assay, all the rats injected with PRO b cells mixed with splenocytes either from normal rats or from untreated tumour-bearing rats died of peritoneal carcinomatosis within 3 months (Fig. 5). On the contrary, the lifespan of the rats injected with PRO b cells mixed with splenocytes from treated tumour-bearing rats was significantly enhanced ($P < 0.01$), four out of these eight rats were still alive 12 months after the injection. In this group two rats died free of tumours, a tooth problem being responsible for their death. All the rats injected with PRO b cells alone died of carcinomatosis, as did all the rats injected only with the splenocytes from tumour-bearing rats, which shows the

Table 4. Effect of OM 163 on the growth of macroscopic peritoneal carcinomatoses induced by colon cancer cells in nude rats

OM 163 treatment	Number of rats with carcinomatoses of class 0	1	2	3	4
None	0	2	4	4	0
10 mg/kg	2	2	3	3	0

Statistical significance of the treatment effect on the tumour growth: non-significant (Kruskal–Wallis test). The treatment started 14 days after the tumour cell injection, all the rats were killed 7 weeks later and an autopsy was carried out on each of them. Definition of different classes of carcinomatoses is given in Materials and Methods.

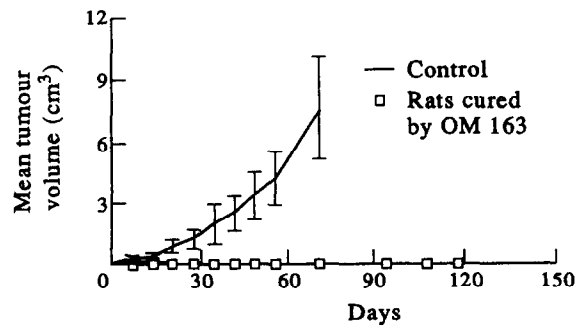

Fig. 4. Growth of subcutaneous tumours in normal rats or in tumour-bearing rats cured by OM 163. Tumour-bearing rats cured by OM 163 and normal rats (control) were s.c. injected with PRO b cells 4 months after the first tumour cell injection. Tumour volumes were measured weekly. The control rats were sacrificed 70 days after the tumour cell injection for ethical reasons.

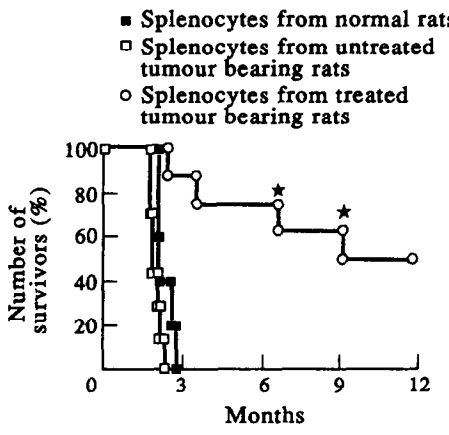

Fig. 5. Survival of rats injected i.p. with PRO b cells mixed with splenocytes from normal rats or from untreated or treated tumour-bearing rats. All the rats died of their tumours except two★ which died free of tumours after tooth problems. The rats injected with PRO b cells alone or with splenocytes alone (from untreated tumour-bearing rats) died of their tumours in the same time as the rats of the two first groups (■, □).

presence of tumour cells in the spleen of the untreated tumour-bearing rats. These results were confirmed in a second experiment.

DISCUSSION

Immunotherapy in humans is limited by many factors including the location and the stage of the tumour, the immune status of the host and the level of antigenicity of tumour cells, the tumour heterogeneity and the toxicity of the immunomodulator. Our results show that OM 163 overcame these barriers in our animal models.

—In the BD IX rat model, OM 163 was effective both on peritoneal carcinomatoses and on artificial lung metastases, showing a systemic antitumour effect. OM 163 induced the disappearance of macroscopic tumours since when the treatment started the carcinomatoses consisted of numerous nodules measuring up to 5 mm.

—The tumorigenicity is dependent on the immunosuppressive effect of tumour cells. In fact, when the treatment started the rats were already immunosuppressed and their splenocytes failed to proliferate in mixed lymphocyte tumour cell culture. We showed here that OM 163, although not directly toxic for PRO b cells was effective in immunosuppressed rats.

—The antitumour effect of OM 163 was not specific to the cellular clone PRO b since it was effective on tumours induced with DHD/K12 cells (from which PRO b cells were cloned) and on tumours induced with cell dissociations from grafted DHD tumours (from which K12 cells were established in culture). These results are important because of tumour heterogeneity and interactions between tumour cell subpopulations [14]. The DHD/K12 cell line is composed at least of two tumour cell variants [11] and DHD tumour dissociation is composed of tumour cell variants, macrophages, lymphocytes, fibroblasts, etc.

—The antitumour effect of OM 163 was not specific to the BD IX/DHD model since it was effective on tumours induced with dissociated cells from FHC tumours in Fisher rats.

—OM 163 was not toxic for rats at the effective doses, furthermore, five i.p. injections administered 3 days apart at the dose of 50 mg/kg were without overt toxicity. OM 163 is prepared from gram negative bacteria and, therefore, every effort is made during the preparation to eliminate any contamination with lipopolysaccharide. Detectable levels are extremely low. Work is in progress to further reduce these minute levels to avoid any risk of toxicity in man.

In another model of colon cancer in rat (CC531 colon adenocarcinoma in syngeneic WAG rats), Marquet [15] and Ijzermans [16] reported antitumour activity of recombinant mouse TNF and recombinant rat IFN-γ. Tumour cubes were implanted under the renal capsule and 1 week later the tumours were enucleated and weighed. Recombinant mouse TNF was injected intravenously (i.v.) at a dose of 2 μg/rat. The treatment resulted in inhibition of the tumour growth: the mean tumour weight was of 48 ± 13 mg in controls and 24 ± 7 mg in treated rats ($P < 0.001$) [15]. Recombinant rat IFN-γ therapy was given i.v. at a dose of 5×10^5 units per day for 5 days starting on the day of implantation. The treatment led to a significant inhibition of tumour growth: the mean tumour weight was of 31 ± 7 mg in controls and 19 ± 7 mg in treated rats ($P < 0.05$) [16]. In our model rMTNF and rRIFN-γ are effective against microscopic but not against macroscopic tumours. None of these treatments was able to cure the rats or to induce the tumour regression observed with OM 163. To our knowledge, the other treatments in the other models of colon cancer in the rat are not effective.

A possible synergy between lipopolysaccharide and OM 163 has been considered. In our model, LPS had an antitumour activity at the optimum dosage of 100 μg/kg [17] which is 1000–2000 times higher than the possible traces of LPS administered with OM 163. At the dose of 1 μg/kg LPS alone was not active and added to OM 163 LPS did not modify its antitumour activity. Thus it is highly improbable that LPS interferes with the OM 163 antitumour activity, which suggests that this immunotherapy could be applied to humans.

OM 163 was not directly toxic for tumour cells so OM 163 modified the relationship between the host and its tumour. The specific host's immune responses and likely T lymphocytes are involved in this therapy because: (a) OM 163 was not efficient in nude rats, (b) $CD4^+$ and $CD8^+$ cells increase in tumours during the treatment, (c) tumour-bearing rats cured by OM 163 reject subsequent injection of PRO b cells and (d) splenocytes taken from tumour-bearing rats cured by OM 163 protect normal rats against PRO b cells.

Ongoing research on the mechanism of this immunotherapy tends to confirm the involvement of T cells. However, macrophages are directly activated by OM 163. They become cytotoxic for PRO b cells and they produce TNF, IL-1 and IL-6 *in vivo* and *in vitro*. Therefore, macrophages are probably the first cells involved in a cascade of reactions including tumour cell lysis, tumour antigen processing and T cell activation leading to the rejection of tumours.

1. Kemeny N, Childs B, Larchian W, Rosado K, Kelsen D. A phase II trial of recombinant tumor necrosis factor in patients with advanced colorectal carcinoma. *Cancer* 1990, **66**, 659–663.
2. Thompson JA, Lee DJ, Lindgren CG, *et al.* Influence of schedule of interleukin 2 administration on therapy with interleukin 2 and lymphokine activated killer cells. *Cancer Res* 1989, **49**, 235–240.
3. Walder S, Wiernik PH. Clinical update on the role of fluorouracil and recombinant interferon alfa-2a in the treatment of colorectal carcinoma. *Semin Oncol* 1990, **17**, 16–21.
4. Moertel CG, Fleming TR, McDonald JS, *et al.* Levamisole and fluorouracil for adjuvant therapy of resected colon carcinoma. *New Engl J Med* 1990, **322**, 352–358.

5. Hoover HC, Surdyke MG, Brandhorst JS, Peters LC, Hanna MG. Five-year follow-up of a controlled trial of active specific immunotherapy in colorectal cancer. *Proc ASCO* 1990, **9**, 106–106.
6. Chauffert B, Martin F, Caignard A, Jeannin JF, Leclerc A. Cytofluorescence localization of adriamycine in resistant colon cancer cells. *Cancer Chemother Pharmacol* 1984, **13**, 14–18.
7. Shimizu T, Pelletier H, Hamman A, Olsson NO, Martin MS, Martin F. Effects of a single injection of anti-asialo GM1 serum on natural cytotoxicity and the growth of a regressive colonic tumor in syngeneic rats. *Int J Cancer* 1987, **40**, 676–680.
8. Martin MS, Martin F, Justrabo E, Michel MF, Lagneau A. BCG therapy as an adjuvant of surgery in the treatment of 5 transplantable lines of rat colon cancer. *Eur J Cancer* 1978, suppl. 1, 123–125.
9. Jeannin JF, Lagadec P, Pelletier H, *et al.* Regression induced by lentinan of peritoneal carcinomatoses in a model of colon cancer in rat. *Int J Immunopharmacol* 1988, **10**, 855–861.
10. Martin MS, Bastien H, Martin F, Michiels R, Martin MR, Justrabo E. Transplantation of intestinal carcinoma in inbred rats. *Biomedicine* 1973, **12**, 555–558.
11. Martin F, Caignard A, Jeannin JF, Leclerc A, Martin M. Selection by trypsin of two sublines of rat colon cancer cells forming progressive or regressive tumors. *Int J Cancer* 1983, **32**, 623–627.
12. Lagadec P, Jeannin JF, Reisser D, Pelletier H, Olsson O. Treatment with endotoxins of peritoneal carcinomatoses induced by colon tumor cells in the rat. *Invasion and Metastasis* 1987, **7**, 83–95.
13. Chauffert B, Shimizu T, Caignard A, *et al.* Use of specific monoclonal antibody for studying the liver metastatic invasion of rat colon cancer. *In vivo* 1988, **2**, 301–306.
14. Caignard A, Martin MS, Michel MF, Martin F. Interaction between two cellular subpopulations of a rat colonic carcinoma when inoculated to the syngeneic host. *Int J Cancer* 1985, **6**, 273–279.
15. Marquet RL, Ijzermans JNM, De Bruin RWF, Fiers W, Jeekel J. Anti-tumor activity of recombinant mouse tumor necrosis factor (TNF) on colon cancer in rats is promoted by recombinant rat interferon gamma; toxicity is reduced by indomethacin. *Int J Cancer* 1987, **40**, 550–553.
16. Ijzermans JNM, Marquet RL, Bouwman RWF, van der Meide PH, Jeekel J. Successful treatment of colon cancer in rats with recombinant interferon-gamma. *Br J Cancer* 1987, **56**, 795–796.
17. Jeannin JF, Onier N, Lagadec P, Von Jeney N, Stutz P, Liehl E. Antitumor effect of synthetic derivatives of Lipid A in an experimental model of colon cancer in the rat. *Gastroenterology* 1991, **101**, 726–734.

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Preclinical Activity of Taxotere (RP 56976, NSC 628503) Against Freshly Explanted Clonogenic Human Tumour Cells: Comparison with Taxol and Conventional Antineoplastic Agents

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Taxotere (TER) and taxol (TA) are new antitumour agents currently undergoing clinical evaluation. We studied the antineoplastic effects of these agents (final concentrations: 4.0, 0.4, 0.04 μmol/l) on the *in vitro* proliferation of clonogenic cells from freshly explanted human tumours using a capillary soft agar cloning system. We also compared the activity of these new compounds to conventional antineoplastic agents (bleomycin, cisplatin, dacarbazine, doxorubicin, etoposide, 5-fluorouracil, vinblastine, interferon-α_2). Using a 21–28-day continuous drug exposure, 54/81 specimens (67%) were evaluable for comparisons, and using a 1-h drug exposure followed by 21–28 days incubation, 50/80 specimens (63%) were similarly evaluable. With both schedules, TA and TER showed concentration-related antitumour activity. At 0.4 μmol/l, median colony survival was 0.61 × control (range 0.09–0.96) for TA and 0.51 × control (0.15–0.81) for TER in the 1-h incubation ($P = 0.0002$). Median colony formation was also reduced significantly more by TER as compared to TA in the long-term incubation schedule. Statistical analysis indicated that TER but not TA was significantly more active than cisplatin ($P = 0.02$), doxorubicin ($P = 0.01$), 5-fluorouracil ($P = 0.01$) and interferon-α_2 ($P = 0.01$). We conclude that TER and TA are more active against *in vitro* tumour colony formation from freshly explanted human tumours. TER appears to be slightly more active than taxol and promises to be active against tumours resistant to conventional antineoplastics.
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INTRODUCTION

TAXOL AND taxotere are new antitumour agents obtained either by extraction (taxol) from the bark of *Taxus brevifolia* or by semisynthesis (taxotere) from 10-deacetyl baccatin III, a non-cytotoxic precursor extracted from the needles of the European yew, *Taxus baccata* L. [1–3]. Both agents induce the formation of stable microtubule polymers and thus disturb the architecture of the cytoskeleton as well as the orderly progression through mitosis [4, 5]. Taxol has shown activity against cisplatin-refractory ovarian cancer, breast cancer, lung cancer and possibly