Colonization of the rat liver by syngeneic tumor cells
An experimental approach by in vivo and in situ studies

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Summary. Syngeneic colon carcinoma cells and glioma cells were injected into the portal vein of BD IX rats. After various time periods the animals were sacrificed and the livers and lungs were fixed and prepared for histology. Atypical cells were observed in the liver 4 and 7 days after the injection of tumor cells, whereas distinct colonies of both colon carcinoma and glioma cells were demonstrated after 14 days. Lung metastases of both tumor cell types were seen after 14 and 30 days. Furthermore, injection of glioma and carcinoma cells into the tail vein gave detectable lung metastases after 7 and 4 days respectively. Intraperitoneal injection of tumor cells resulted in the accumulation of large tumor masses, particularly in the mesentery. By in situ perfusions of the liver with tumor cells included in the perfusion medium it was possible to establish that all the tumor cells were arrested in the course of 4 min. In contrast, normal rat leukocytes were not trapped in the liver, whereas trypsin-treated leukocytes were, suggesting the importance of trypsin-sensitive structures for binding to hepatic tissue. The binding of both glioma and carcinoma cells to the liver and the ensuing growth of tumor nodules in this organ indicate a lack of specificity on part of the malignant cell types for metastasis to the liver. Both tumor cell types colonized the first organ encountered after injection.

Key words: Tumor cells, syngeneic - Rat liver - Portal vein - In vivo - In situ

Introduction

A major problem facing current cancer therapy is metastasis of malignant cells from a primary tumor to adjacent or distant sites of the body. This process is characterized by the escape of tumor cells from their primary lodging, and invasion of and transportation by blood or lymph vessels to distant organs, where the cells adhere, penetrate and eventually multiply. Tumor cells successful in this final achievement represent a small percentage of the cells invading the vascular or lymphatic systems (Liotta et al. 1974). The arrest of tumor cells in a particular organ may involve a high degree of specificity; certain lymphomas do not infiltrate the lungs but metastasize extensively to the liver (Nicolson 1982).

The liver is a major target organ for a large number of metastatic tumors. Metastatic spread to this particular organ has been studied by various experimental approaches. Intrasplicinc injections of rat colon carcinoma cells in nude mice resulted in liver colonization (Giavazzi et al. 1986). Furthermore, aggregates of the same carcinoma cells were injected in the portal vein of syngeneic rats and were found to produce large hepatic metastases (van der Elst et al. 1986). Murine colon carcinoma cells were also found to metastasize to the liver when injected into the caecal wall of mice (Bresalier et al. 1987). In the study presented here single cell suspensions of syngeneic colon carcinoma cells and glioma cells were injected into the portal vein of BD IX rats. The primary aim was to establish whether any of the two cell lines could colonize the liver and secondly, whether there were any differences in the extravasation of two syngeneic tumor cell lines of such different sites of origin, once they were introduced into the circulatory system. Intravenous injections of tumor cells have been shown to result in the development of metastases correlating with the spontaneous metastases of the same tumor cells from primary tumors (Kripke et al. 1978). This particular in vivo approach to the study of interactions between rat
tumor cells and hepatic tissue was further extended by in situ liver perfusion. Tumor cells were introduced into the perfusate and the kinetics of disappearance was monitored and compared with that of isolated normal rat leukocytes. The data presented demonstrate that both colon carcinoma and glioma cells have the capacity to colonize the rat liver under the chosen in vivo and in situ conditions.

Materials and methods

Cells. BD IX rats and syngeneic rat glioma cell lines BT 3 and 4 were obtained from Dr. O.D. Lærum, The Gade Institute, Department of Pathology, University of Bergen, Bergen, Norway. The cell lines were kept in vitro in Dulbecco's modified Eagle's medium (DME) containing 10% fetal calf serum, and 100 IU of streptomycin and penicillin per ml. The colon carcinoma cell line, DHD/K2/Trb (DHD), was kindly provided by Dr. M. Martin, Laboratoire d'Immunologie, Faculté de Médecine, Dijon, France (Martin et al. 1983). The cells were cultured in vitro in DME with 10% fetal calf serum and 50 μg/ml of gentamycin. The cells were passaged by trypsinization (0.25% in Ca²⁺ and Mg²⁺-free phosphate-buffered saline (PBS), pH 7.4) and split in a ratio of 1:5.

In vivo experiments. BD IX rats were given 1 mg/100 g body weight of Hypnorm (Janssen Pharmaceutica, Beerse, Belgium) and 0.5 mg/100 g body weight of Dormicin (F. Hofmann-LaRoche, Basel, Switzerland) intramuscularly, and thereafter access was gained to the peritoneum. Colon carcinoma or glioma cells obtained by trypsinization of cultured cells were reconstituted in 0.9% sterile saline and injected into the portal vein at a concentration of 2 × 10⁶ cells in a volume of 300 μl. Prior to the injections, the cells were gently shaken at intervals in order to avoid clumping. Control animals received 300 μl of PBS. To avoid bleeding complications the injection site was gently compressed with a sterile cloth. After suturing the abdominal incision, the animals were kept for various time periods before sacrifice. The same number of tumor cells was injected into other animals either in the tail vein or directly into the peritoneal cavity.

In order to detect possible liver metastases, the animals were anesthetized as described above after 4 days, 1 and 2 weeks or 1 month after the portal vein injection of colon carcinoma or glioma cells. Then 50 IU heparin/100 g body weight (Nycomed, Oslo, Norway) was injected into the inferior vena cava followed by exposure of the peritoneum. The animals were then given a second (lethal) dose of Hypnorm and Dormicin, following which the thorax was opened and 60–80 ml sterile 0.9% saline was injected into the left ventricle. At the same time, peritoneal arteries and veins were cut. After the liver had been washed free of blood, 20 ml of McDowell's buffer (PBS with 1% glutaraldehyde, 4% formaldehyde and 80 mM sucrose) was injected for fixation of the whole liver. Four thin slices from each liver were obtained at identical anatomical sites; they were processed, sectioned and stained with hematoxylin and eosin. Thin tissue slices were also obtained from the lung tissue and prepared in the same fashion. The specimens were examined by light microscopy and photographed in a Leitz Wetzlar inverted phase microscope using a Nikon FG 20 camera.

In situ experiments. BD IX rats were given phenobarbital (5 mg/100 g body weight) and the liver was perfused with RPMI 1640 medium to wash it free of blood. The liver was removed and connected to a recirculation perfusion system with a flow rate of 30 ml/min. The RPMI 1640 medium was substituted with RPMI 1640 containing either glioma or colon carcinoma cells and 1% albumin. In some experiments albumin was replaced with 10% fetal calf serum or 10% BD IX rat serum. The total recirculation volume was 20 ml and the cell density was 10⁶ cells per ml. In order to avoid fluctuations in the pH or O₂ tension of the perfusion medium, the solution was continuously bubbled with 5% CO₂/95% O₂. After the tumor cells had been connected to the perfusion system, samples of the eluate from the liver was taken every 2 min and the experiments continued for 15 min. Control perfusions were performed with syngeneic leukocytes obtained by Lymphoprep (Nycomed, Oslo, Norway) centrifugation of rat blood. Normally, 10⁶ leukocytes per ml were used in the same experimental procedure as described above. The pH of the perfusion medium was not found to change during a 15 min perfusion period.

Results

Metastases in vivo

BD IX rats exposed to DHD colon carcinoma cells or BT 3 and 4 glioma cells for 4 weeks were typically found to have enlarged livers with small nodules under the surface. Rats kept for 14 days after injection of carcinoma and glioma cells had enlarged livers in both cases, but only the latter tumor cell type gave visible nodules on the surface. Examination of thin slices from the livers revealed that DHD colon carcinoma cells had formed a large number of colonies in the hepatic tissue both after 14 and 30 days (Table 1). In one animal atypical cells were observed after only 4 days exposure to DHD cells. In this particular animal lung metastases were also observed. This was also the case when colon carcinoma cells were injected into the tail vein. Four days after injection lung metastases were observed, and lung and liver metastases, and in one case peritoneal cavity-associated tumor cells, were detected 30 days after the injection (Table 1).

Both BT 3 and BT 4 glioma cells were found to give large tumor colonies in the liver 14 days after intraportal injection (Tables 2 and 3). Injection of BT 3 cells into the peritoneal cavity gave large tumor masses in the peritoneum itself, mainly associated with the mesentery, but also to a certain extent with the peritoneal wall (Table 2). BT 4 cells were found in the mesentery 7 days after intraportal injection. Metastases to the liver were also observed with both glioma cell types after they had first been found to colonize the peritoneum. Small cell colonies (not seen in the normal liver, but not forming distinct tumor masses) were also observed in the liver 4 and 7 days after intraportal injection (Tables 2 and 3), as was also the case for DHD.