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New cell lines of gastric and pancreatic cancer: distinct morphology, growth characteristics, expression of epithelial and immunoregulatory antigens

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Abstract Two new cell lines from stomach cancers and one from a pancreatic carcinoma are presented. MZ-GC-1 was established from a hepatic metastasis of a well differentiated gastric adenocarcinoma. MZ-GC-2 was derived from ascites induced by a poorly differentiated gastric adenocarcinoma. MZ-PC-1 originated from the pleural effusion of a moderately well differentiated pancreatic ductal adenocarcinoma. MZ-GC-1 cells were adherent and partially polarized, connected tightly via desmosomes. In contrast MZ-GC-2 cells consisted of slightly adherent or floating subpopulations and displayed no desmosomes. MZ-PC-1 cells were adherent and showed polarized growth, connected by apical junctional complexes. Cell doubling times were 7 days for MZ-GC-1 and 45 h for MZ-GC-2 and MZ-PC-1 cells. MZ-GC-2 and MZ-PC-1 gave rise to nude mouse tumours, resembling the original lesions. Chromosome analysis of the cell lines revealed a high range of numerical abnormalities. Each cell line had cytokeratin patterns fitting well to typical in vivo patterns. Furthermore the cell lines expressed a panel of antigens typical for gastrointestinal epithelia. Unique for MZ-PC-1 were high amounts of secreted Ca19-9. γ-Interferon enhanced HLA-class I antigens up to twofold and induced ICAM-1 expression on each cell line. HLA-class II antigens were differentially enhanced by γ-interferon. Due to their distinct characteristics the three tumour cell lines may be useful models in the investigation of the cell biology and immunogenicity of gastrointestinal tumours.

Key words Stomach neoplasms · Pancreatic neoplasms · Cell differentiation · Histocompatibility antigens · Interferon gamma

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Introduction

The spectrum of gastric and pancreatic adenocarcinoma cell lines is limited. Most established cell lines have not been characterized extensively with regard to variables reflecting tumour differentiation. For example, expression of gastrointestinal epithelial antigens, cytokeratin polypeptides, tumour markers and immunoregulatory molecules have not been investigated with few exceptions, in newly established cell lines of gastric [27, 36, 41] or pancreatic carcinomas [2, 3, 8, 10, 13, 16, 17, 18]. The investigation of these characteristics makes it possible to determine the variability of a gastric or pancreatic carcinoma cell line. To develop new treatment strategies for these cancers, it is important that a panel of different tumour cell lines is available which might reflect the variability of the clinicopathological features of these carcinomas. We are particularly interested in the expression of HLA-antigens and adhesion molecules by tumour cell lines, because one focus of our research is the cellular immunity against gastrointestinal tumours. Here we report the establishment of cell lines from a moderately well differentiated pancreatic adenocarcinoma (MZ-PC-1), from a well differentiated gastric carcinoma (MZ-GC-1) and from a poorly differentiated gastric carcinoma (MZ-GC-2). The characterisation of the cell lines includes the morphology in tissue culture, growth kinetics, cloning efficiency, heterotransplantation into nude mice, histology of transplanted tumours, expression pattern of cytokeratin polypeptides, gastrointestinal epithelial anti-

gens, tumour antigens and immunoregulatory antigens and modulation of the latter by interferons.

Materials and methods

MZ-GC-1 was derived from a liver metastasis of a male patient, 56-years-old, who suffered from recurrent and metastatic gastric cancer 1 year after diagnosis of gastric cancer and subtotal gastrectomy. Histological examination of the recurrent tumour and liver metastases revealed a well differentiated, partially papillary adenocarcinoma. MZ-GC-2 was established from malignant ascites of a female patient, 51-years-old, suffering from gastric cancer with peritoneal carcinomatosis. Histological examination of the primary tumour after total gastrectomy revealed a poorly differentiated adenocarcinoma with parts composed of signet ring cells. MZ-PC-1 was established from a male patient, 46-year-old, suffering from advanced and metastatic pancreatic cancer. Diagnosis was obtained by exploratory laparotomy. Histological examination of a tumour sample revealed a moderately well-differentiated ductal adenocarcinoma. Chemotherapy was initiated. After 7 months of tumour growth arrest it progressed slowly and a maglinant pleural effusion developed, from which the tumour cell line MZ-PC-1 was established.

All tumor cell lines were established with the patients informed consent and with the approval of the local ethics committee. The hepatic metastasis of a gastric carcinoma (MZ-GC-1) was finely minced with scissors. Larger tissue clumps were removed by sedimentation. The cell suspension was washed twice and resuspended in tissue culture medium at a cell concentration of approximately 5×10^5 viable cells per ml. Cells from ascitic fluid (MZ-GC-2) or pleural effusion (MZ-PC-1) were pelleted and resuspended in tissue culture medium at the same cell concentration. The tissue culture medium consisted of CMRL (Gibco, New York, USA), supplemented with 15% fetal bovine serum (FBS, Biochrom, Berlin, Germany), 2 mM glutamine, 1% non essential amino acids, 100 U/ml penicillin and 100 µg/ml streptomycin. A 5 ml cell suspension was transferred into a 25 cm^2 tissue culture flask (Greiner, Nürtingen, Germany) and kept at 37° C and 5% carbon dioxide atmosphere. Adherent tumour cells were detached by 0.05% EDTA in PBS after reaching confluence and were passed to another flask. Fibroblasts and mesenchymal cells were removed by repeated short EDTA treatment. After 5–10 passages the tumour cell lines were adapted to DMEM tissue culture medium, (Gibco), 10% FBS, supplemented as described above. All cultures were regularly tested for fungal, bacterial and mycoplasma infections. Infected cultures were discarded.

Phase contrast micrographs of the cultured cell lines were taken by an inverted microscope (Zeiss, Oberkochen, Germany). Transmission and scanning electron microscopy was performed as described [12].

To determine the growth kinetics of the cell lines, fixed cell numbers were seeded into parallel tissue culture flasks on day 0. In 24 h intervals cells of one flask were detached and counted. A growth curve was calculated by logarithmic regression analysis and the doubling time was estimated from this curve. Cloning of the cell lines was performed in 96 flat bottom well plates (Greiner, Nürtingen, Germany). Cells were seeded at concentrations of 30,

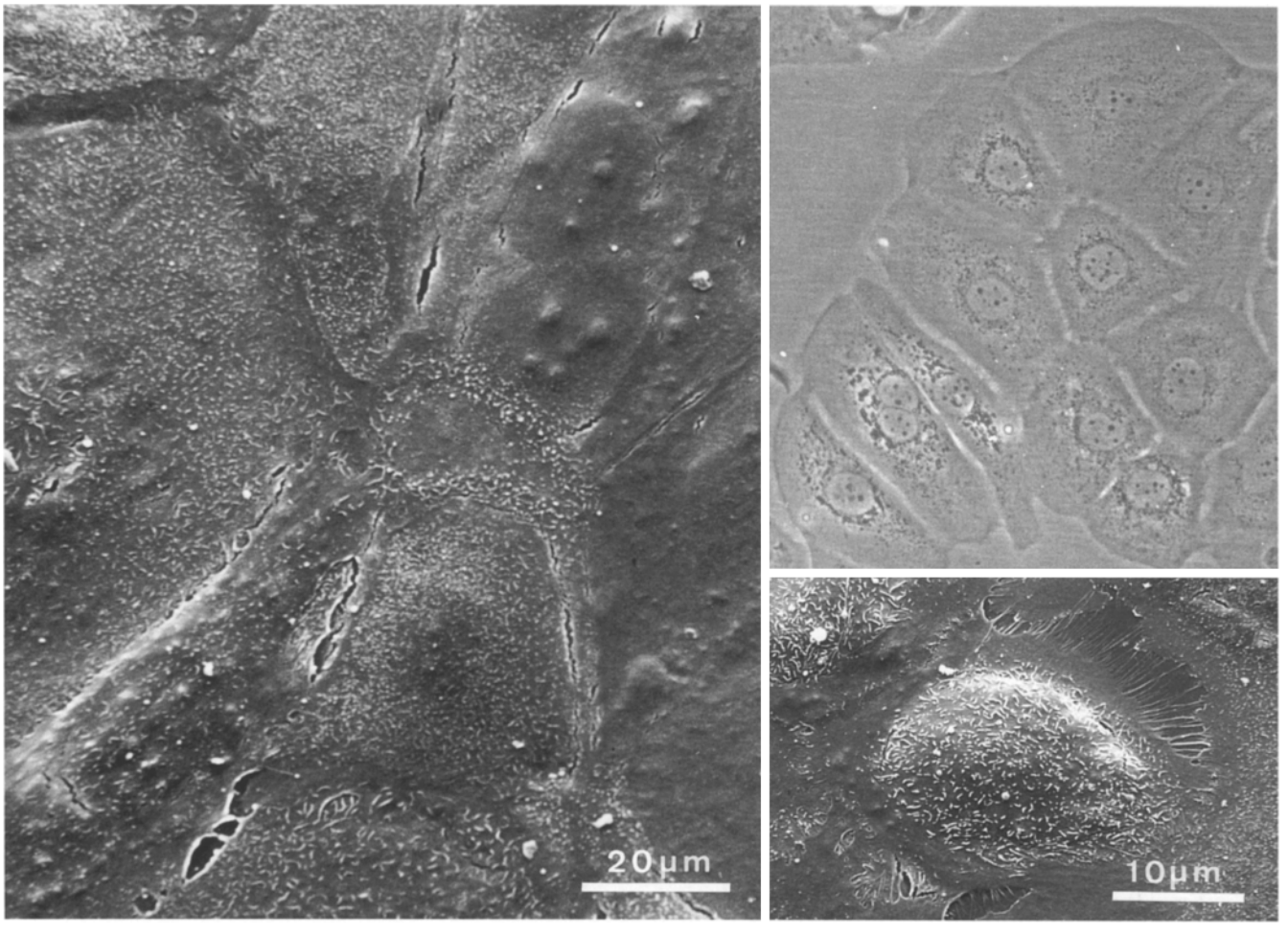

Fig. 1 MZ-GC-1, scanning electron micrograph (left side and lower inset) and phase contrast micrograph (×200, upper inset) showing polygonal cells with microvilli at the surface, growing gland-like around a lumen

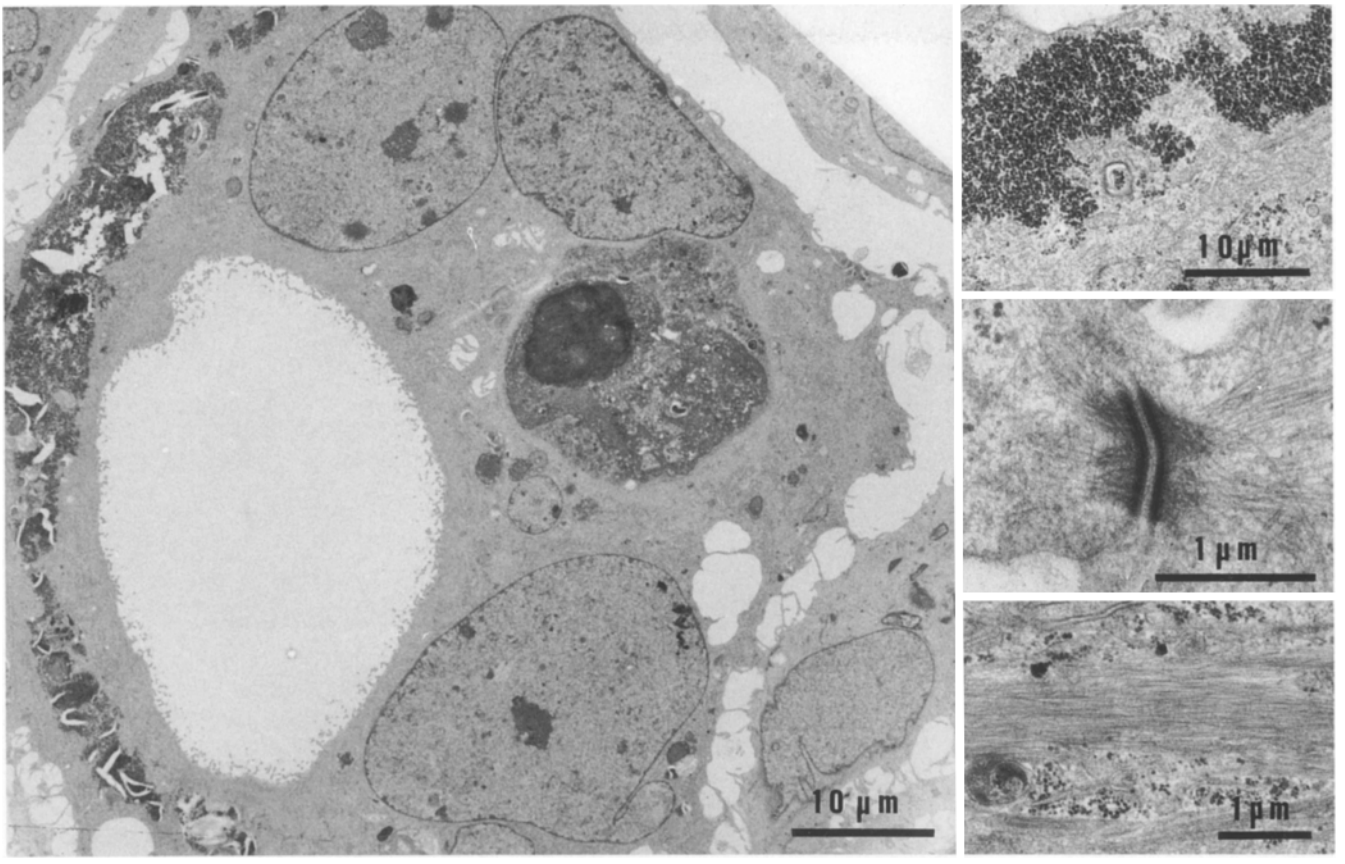

Fig. 2 MZ-GC-1, transmission electron micrographs showing a cell with intracellular vacuoles lined by microvilli (left side), glycogen deposits (right upper inset), tight intercellular contacts via desmosomes (right middle inset), intermediate filaments (right lower inset)

10, 3 and 1 cell per well in CMRL tissue culture medium, 15% FCS, supplemented as described above. In some experiments irradiated (10,000 rad) NIH-3T3 fibroblasts served as feeder cells in concentrations of 1.5×10^5 per well. The percentage of clonogenic cells was estimated by analysing the Poisson distribution of the relationship between tumour cell dose per well and the fraction of wells without tumour growth [37]. Tumour cell clones were expanded for further analysis.

For heterotransplantation 2×10^7 cells were injected subcutaneously into the back of 4–6 weeks old nude mice (NMRI, nu/nu, w.). The mice were observed for tumour growth up to 2 months thereafter.

For chromosomal studies subconfluent cells were fed 1 day before harvesting in order to obtain a high mitotic rate. Colcemide (0.5 µg/ml) was applied 4 h beforehand. After a hypotonic shock of 30 min in 0.075 M potassium chloride, cells were fixed with methanol/acetic acid 3:1, washed several times and spread by an air drying procedure for chromosome analysis.

The cytokeratin pattern of the cell lines was investigated by an indirect immunoperoxydase method on cells grown on tissue culture slides and by 2-dimensional gel electrophoresis. The antibodies, which were used in this analysis and detailed methods were described previously [22]. In addition, monoclonal antibody 2D7 agaginst cytokeratin 13 was purchased from Eurodiagnostics (Apeldoorn, The Netherlands).

The expression of gastrointestinal epithelial antigens by cultured cells was tested by an ELISA-test using rabbit-anti mouse Ig-peroxidase (Dakopatts, Copenhagen, Denmark). Between 300–1000 cells per well in 10 µl culture medium were seeded into microtest plates (Falcon No. 3034) incubated overnight at 37° C 5% carbondioxide atmosphere to allow attachment of cells. For the detection of intracellular antigens, cells were fixed with acetone/methanol (1:2) for 10 min at −20° C and dried under an air stream. Otherwise cells were washed three times with PBS. The staining procedure was performed as described [6]. The percentage of positive cells was estimated visually by phase contrast microscopy. To test antigen expression by nude mouse tumours, frozen tissue sections were prepared and tested by an indirect immunoperoxidase method as described [5]. The carbohydrate antigen 19-9 (Ca19-9) and carcinoembryonic antigen (CEA) concentrations in tissue culture supernatants were determined by a commercial Ca19-9 radioimmunoassay (Abbott) and a commercial CEA Enzymimmunoassay (Tandem-E-CEA, Hybritech, Hürth, Germany).

For the detection and modulation of HLA antigens and ICAM-1, tumour cells were grown with culture medium, 5000 U/ml interferon-α2a (Hoffmann-La Roche, Grenznach-Wyhlen, Germany) or 100 U/ml γ-interferon (Ernst-Böhringer Institut für Arzneimittelforschung, Vienna, Austria) for 1 days at 37° C, 5% carbondioxide. The antigen expression of the cells was tested with the respective antibodies by a β-galactosidase-enzyme linked immunosorbent assay as described [4, 5] with the modification that 2×10^4 tumour cells served as antigens. Test values were derived from a Dynatech microfluor reader and expressed as fluorescence units. The means of repeated experiments with triplicate values and their 95% confidence intervals were calculated. If the 95% confidence intervals of two means did not overlap, samples were assumed to be different.

The following monoclonal antibodies were used in form of diluted nude mouse ascites or tissue culture supernatants at antibody concentrations of approximately 5 µg/ml: W6/32 to HLA-class I [1] and L-243 to HLA-DR [19], Mü-A80 to HLA-A [32], Ma 2.1 to HLA-A2/HLA-B17 [21], Th4 to HLA-B (unpublished, a generous gift of C. Müller, Sektion für Transplantationsimmunologie und Immunhämatologie, Abteilung Innere Medizin II, Universität Tübingen Germany), B7/21 to HLA-DP [40], Tü22 to HLA-DQ [28], p3.58[b] to ICAM-1 [11], Pa-25 and Pa-G14 to epithelial anti-

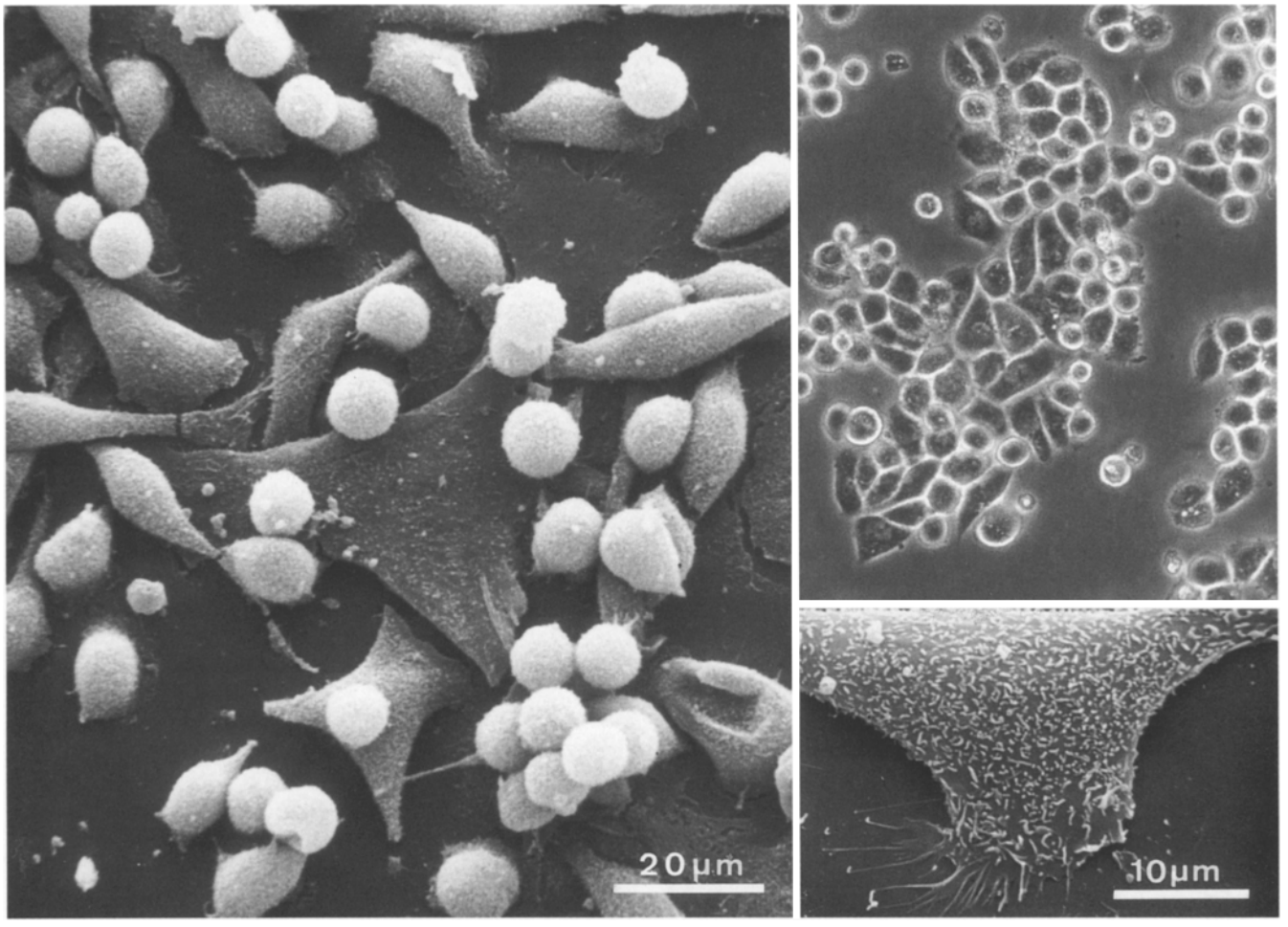

Fig. 3 MZ-GC-2, scanning electron micrograph (left side and lower inset) and phase contrast micrograph (×200, upper inset) showing slightly adherent or floating cell populations with microvilli at the cell surfaces and tumour cells displaying pseudopods

gens [4, 5], mouse monoclonal antibody 19.9 to Ca 19-9 [14]. Monoclonal antibodies against A, B and Lewis[a, b] blood group antigens were purchased from Biotest Diagnostica (Frankfurt, Germany). A peroxidase-conjugated rabbit anti CEA antibody was purchased from Dako (Copenhagen, Denmark).

Results

MZ-GC-1 cells were large, polygonal and adherent, growing in islands until reaching confluence (Fig. 1). Ultrastructurally some of the MZ-GC-1 cells appeared polarized, growing in a gland-like pattern around a lumen with microvilli at the apical surfaces and tight intercellular contacts at the lateral surfaces via desmosomes (Figs. 1, 2). Other differentiation characteristics were bundles of intermediate filaments, glycogen deposits and intracellular vacuoles, sometimes of enormous size and lined by microvilli (Fig. 2). In contrast with MZ-GC-1, MZ-GC-2 cells were small, more rounded and consisted of slightly adherent and floating subpopulations (Fig. 3). MZ-GC-2 cells had microvilli, only occasionally loose intercellular contacts and rarely intracellular vacuoles (Fig. 4). MZ-PC-1 cells were polygonal and adherent in tissue culture. In lower density MZ-PC-1 cells were forming islands. In higher density MZ-PC-1 became a confluent monolayer, showing dome formation (Fig. 5). Ultrastructurally MZ-PC-1 cells had microvilli and their lateral cell surfaces were connected by desmosomes and apical junctional complexes. Occasionally cells were grouped around duct-like lumina (Fig. 6).

Chromosome analysis of MZ-GC-1 revealed a broad range of chromosome numbers from haploid to tetraploid with a peak between 70 and 80 chromosomes. Chromosome numbers of MZ-GC-2 had a more narrow range with 80% of the karyotypes having between 55 and 70 chromosomes. MZ-PC-1 is subtetraploid with 90% of the karyotypes having between 90 and 100 chromosomes.

In tissue culture MZ-PC-1 and MZ-GC-1 had a doubling time of 45 h. The frequency of clonogenic cells was 15%. In contrast to that MZ-GC-1 was growing slowly with a doubling time of 7 days and had a low cloning frequency of 1%. This frequency could be increased to 6% by co-culture with irradiated NIH-3T3 mouse fibroblasts as feeder cells.

Transplantation into nude mice was regularly successful for MZ-PC-1 and MZ-GC-2 but never for MZ-GC-1. MZ-PC-1 nude mouse tumours displayed the pattern of

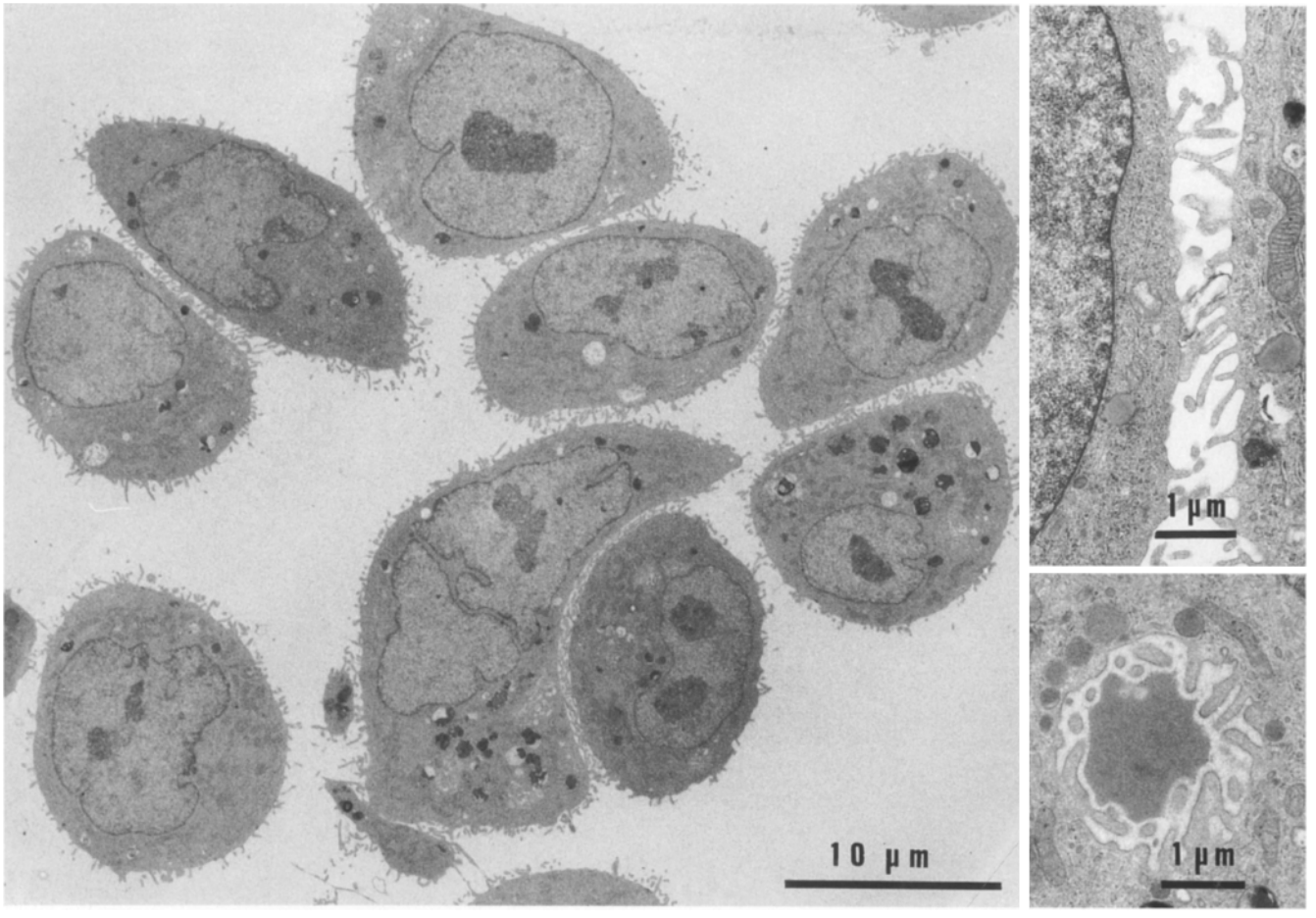

Fig. 4 MZ-GC-2, transmission electron micrograph showing small tumour cells only occasionally in loose contact with each other via surfaces covered with microvilli (right upper inset) but without tight connections via desmosomes. Cells were rarely displaying small intracellular vacuoles (right lower inset)

moderately well differentiated adenocarcinomas seen in the original tumour. The xenografts were composed of glandular structures where lumina were surrounded by one or several layers of columnar cells (Fig. 7). Xenografted MZ-GC-2 tumours were poorly differentiated (Fig. 8 like the original tumour). In both MZ-PC-1 and MZ-GC-2 xenografts periodic acid-Schiff staining showed cytoplasmatic mucin.

Each tumour cell line secreted CEA and Ca 19-9 (Table 1). MZ-PC-1 was secreting very high amounts of Ca19-9, corresponding to a high serum level of the patient at the time of culture initiation. None of the tumours secreted α-fetoprotein. Each cell line expressed HLA-class I, Lewis^a and Lewis^b blood group antigens, Ca19-9, CEA and the epithelial antigens EPM-1 and Exo-1 (Table 2), but no blood group A or B antigens (data not shown). Only MZ-GC-2 expressed HLA-DR. The same antigen patterns as in vitro were displayed by nude mouse tumours of MZ-PC-1 and MZ-GC-2.

The analysis of the intermediate filament protein expression by the cell lines in vitro is summarized in Table 3. Immunocytochemical tests showed that MZ-GC-1 ex-

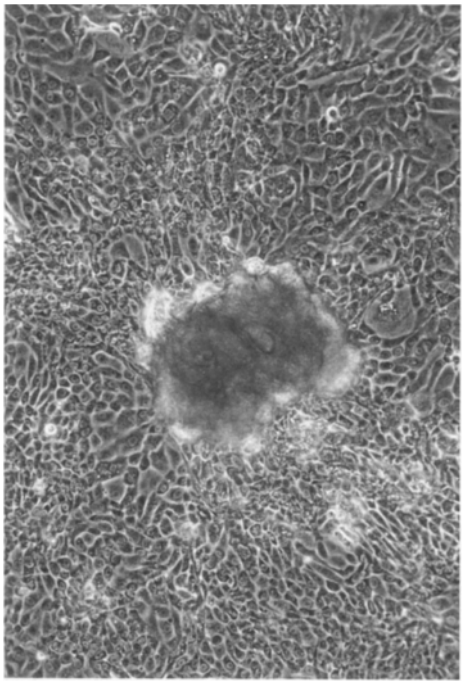

Fig. 5 MZ-PC-1, phase contrast micrograph (×100) showing dome formation of cells as a sign of cellular polarization

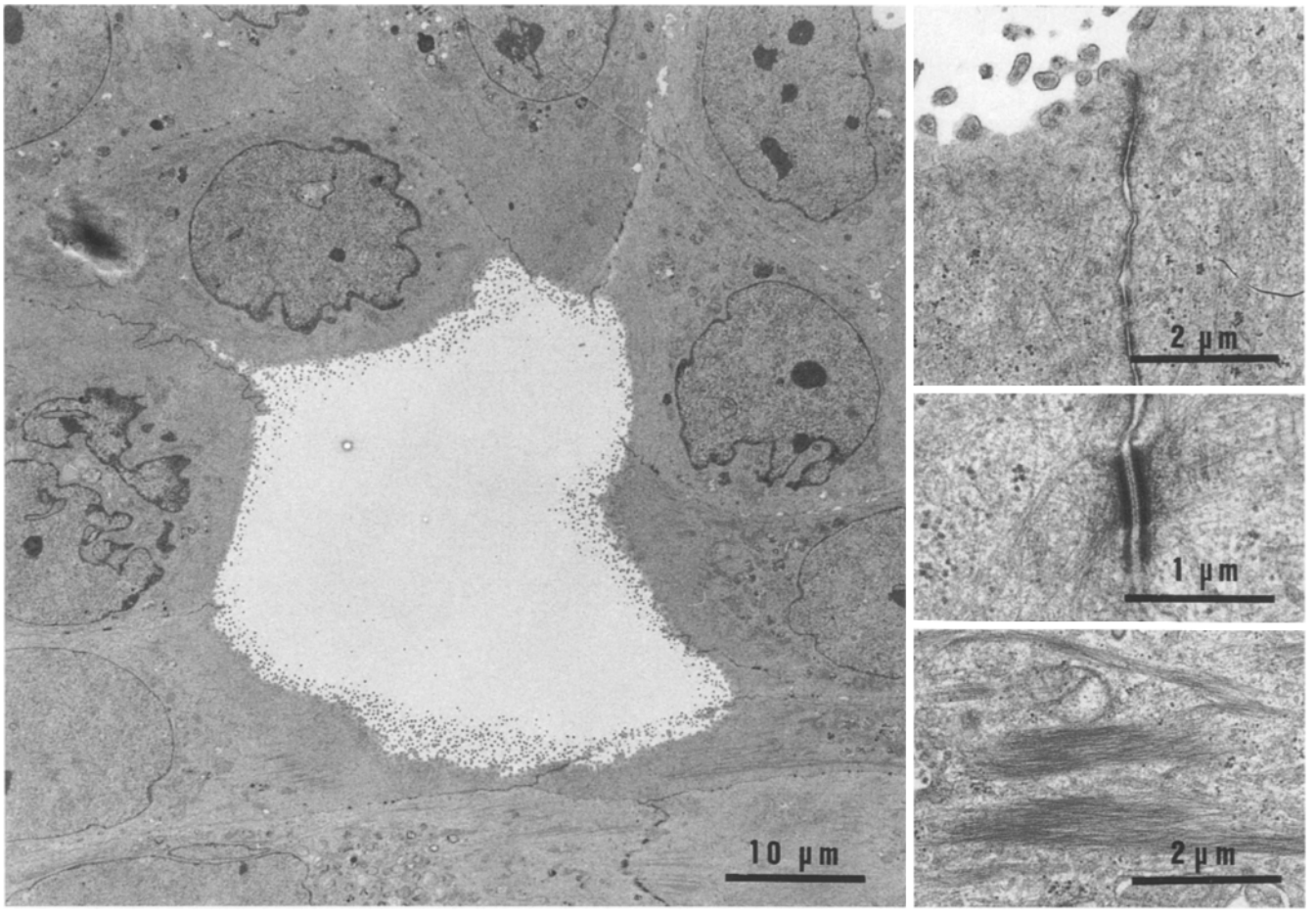

Fig. 6 MZ-PC-1, transmission electron micrograph showing grouping of cells around a duct-like lumen, their apical surfaces covered with microvilli (left side) and their lateral surfaces connected by apical tight junctions (right upper inset) and desmosomes (right middle inset). Cells were also displaying intermediate filaments (right lower inset)

pressed uniformly CK18 and CK19, cytokeratins of the simple epithelial type. Biochemical analysis by cell extraction and 2 dimensional gel electrophoresis revealed that MZ-GC-2 expressed predominantly CK 8, 18 and 19. Few MZ-GC-2 cells expressed CK 7 and CK20 in addition when tested by immunocytochemistry. MZ-PC-1 expressed CK 7, 8, 18 and 19 predominantly. Immunocytochemical tests showed that a few MZ-PC-1 cells expressed CK 4, 5 and 13, while CK 20 was not expressed. Remarkably, about 20% of MZ-PC-1 cells showed expression of vimentin, a mesenchymal intermediate filament protein.

HLA-class I antigens, HLA-A2 and HLA B antigens, which were expressed by each of the three tumour cell lines, could be upregulated by γ-interferon more than by α-interferon (Fig. 9). It should be noted that the expression of HLA-A2 and HLA-B was more than doubled by γ-interferon in each tumour. γ-Interferon but not α-interferon modulated HLA-class II antigen expression by the cell lines. γ-Interferon enhanced or induced HLA-DR, -DP and -DQ on MZ-GC-2, HLA-DR and -DP on MZ-

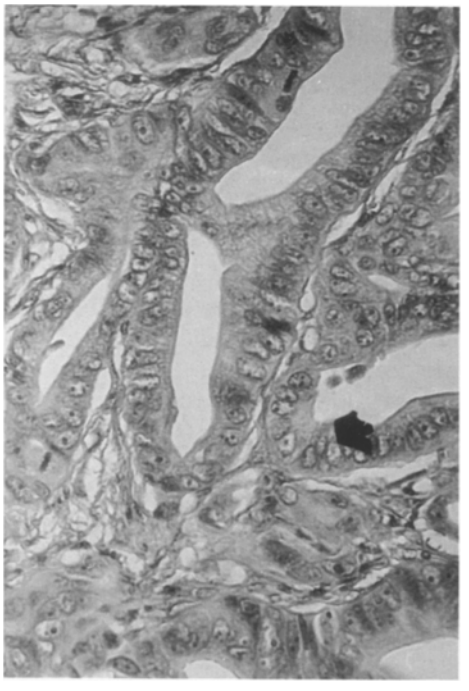

Fig. 7 MZ-PC-1 grown in a nude mouse for 6 weeks subcutaneously (×200) showing a moderately well differentiated adenocarcinoma with duct-like structures lined by a one or several layers of columnar epithelial cells

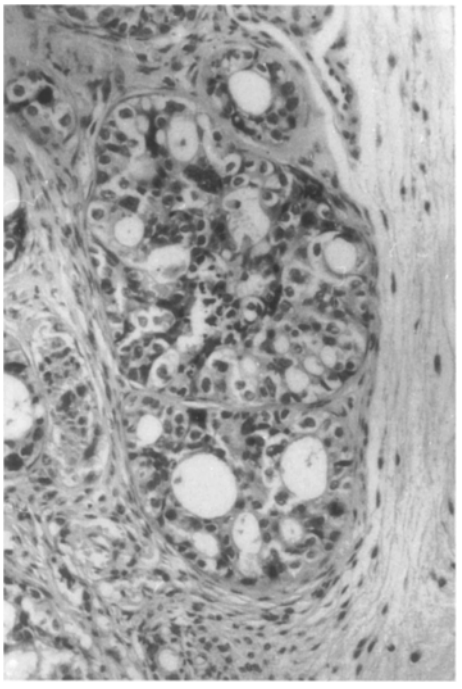

Fig. 8 MZ-GC-2 grown in a nude mouse for 6 weeks subcutaneously (×200) showing a poorly differentiated adenocarcinoma, resembling the original tumour

Table 1 Carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (Ca19-9) secretion by gastric and pancreatic carcinoma (secretion in tissue cultures by 10^7 cells per 24 h)

Cell line	CEA (ng)	Ca19-9 (U)
MZ-GC-1	110	1,440
MZ-GC-2	2	460
MZ-PC-1	30	100,000

PC-1 and only HLA-DR on MZ-GC-1. ICAM-1 was marginally expressed by MZ-GC-1 and MZ-PC-1 and not detectable with our assay on MZ-GC-2. The ICAM-1 expression could be upregulated exclusively by γ-interferon on each of the tumours.

Discussion

The two gastric adenocarcinoma cell lines MZ-GC-1, MZ-GC-2 and MZ-PC-1, the pancreatic cancer cell line, complement existing cell lines of the same tissue origin by their distinct differentiation, growth characteristics and expression of epithelial and immunoregulatory antigens. MZ-PC-1 shares some characteristics with other moderately to well differentiated adenocarcinoma cell lines of the pancreas as AsPC-1, SUIT-2, PK-8, PK-9, PK-12, PK-16, KP-2, RWP2, Capan-1, Capan-2, SW 1990 [2, 3, 8, 10, 13, 16, 17, 18]. These tumour cell lines grow as adherent monolayers in tissue culture. Xenografts into nude mice are forming moderately well differentiated adenocarcinomas. However the ultrastructural differentiation characteristics of MZ-PC-1 have only been described for Capan-1 [20]. Confluent MZ-PC-1 cells showed polarization in tissue culture, forming domes and duct-like structures with microvilli at the apices and apical junctional complexes (tight junctions, intermediate junctions) besides desmosomes joining cells together at the lateral surfaces. Unique for MZ-PC-1 seems to be the amount of secreted Ca19-9. Ca19-9 secretion has been described for two other moderately well differentiated pancreatic carcinoma cell lines, SUIT-2 and KP-2 [8, 10], but not to this extent.

MZ-GC-1 represents a rare cell line of a well differentiated gastric adenocarcinoma. To our knowledge only one other cell line NCI-N87 of a well differentiated gastric adenocarcinoma has been reported [27]. Signs of differentiation displayed by MZ-GC-1 are the growth in islands of adherent, tightly cohesive, polygonal cells, the occasional polarity of MZ-GC-1 cells with microvilli at the apical surfaces and with lateral cell contacts via desmosomes and the decreased growth potential compared to other gastric cancer cell lines. In contrast to MZ-GC-1, MZ-GC-2 cells consisted of either slightly adherent or floating cells like other poorly differentiated or undifferentiated gastric carcinoma cell lines [36, 41]. The in vitro growth potential of MZ-GC-2 was superior to MZ-GC-1 and in contrast to MZ-GC-1 MZ-GC-2 gave rise to undifferentiated tumours in nude mice.

The expression of cytokeratin polypeptides by each cell line confirms their epithelial nature. Each cell line expressed cytokeratins of the simple epithelium type, that is CK 8, 18, 19 (with CK8 not tested on MZ-GC-1). The presence of high levels of CK 7 distinguishes MZ-PC-1 from the gastric cancer cell lines as it is also typical for pancreatic versus gastric carcinomas in vivo [25]. In good agreement with our data, Schüssler et al. [34] detected CK7 in addition to CK8, 18 and 19 in 10/12 pancreatic cancer cell lines tested. The detection of minorities of cells of MZ-PC-1 expressing CK 4, 5 or 13, stratified epithelium type cytokeratins, may indicate a certain tendency to squamous metaplasia. These cytokeratins may also be focally expressed in pancreatic carcinomas in vivo [25]. The absence of CK 20 from the gastric cancer cell lines (with the exception of a few MZ-GC-2 cells) is remarkable, because foveolar stomach epithelium is uniformly CK 20 positive, whereas glandular epithelial cells of the corpus and pylorus do not express CK 20 [24]. However, a proportion of gastric carcinomas in vivo also lack CK 20 or exhibit a very low level of expression (22% or 28% respectively, [26]). The additional expression of vimentin by MZ-PC-1 has also been described in other cultured carcinoma cell lines [23], including some pancreatic lines [34] and does not speak against the epithelial nature of this line. In conclusion, the cytokeratin patterns observed in the cell lines fit well with the typical in vivo patterns and thus reflect the faithful maintenance of differentiation qualities of the respective carcinoma types.

Each of the three cell lines expressed antigens, which characterize gastrointestinal epithelia, like Lewis^a and

Table 2 Expression of HLA and epithelial differentiation antigens by gastric and pancreatic carcinoma cell lines (tested by an indirect immunoperoxidase ELISA on cells grown in microtest plates or frozen tissue sections in the case of nude mouse tumours; –, negative; (+), <5% of cells positive; +, 5–20% of cells positive; ++ 21–80% of cells positive; +++ >80% of cells positive)

Antigens	MZ-GC-1 Tissue culture	MZ-GC-2 Tissue culture	MZ-PC-1 Tissue culture	MZ-GC-2 Nude mouse tumour	MZ-PC-1 Nude mouse tumour
HLA-ABC	+++	+++	+++	+++	+++
HLA-DR	–	+++	–	+	–
Ca19-9	+	+	+++	++	+++
CEA	+++	+	+++	++	+++
Le^a	+	++	+++	++	+++
Le^b	+	+++	+++	+++	+++
EPM-1[a]	++	++	(+)	+	+
Exo-1[a]	++	++	(+)	+	+

[a] staining after fixation with acetone/methanol

Table 3 Expression of intermediate filament proteins in gastric and pancreatic carcinoma cell lines (–, negative; (+), <5% of cells positive; +, 5–20% of cells positive; ++ 21–80% of cells positive; +++ >80% of cells positive)

Cell line	Cytokeratin expression in 2D gel	Cytokeratin expression detected by immunocytochemical staining (Cytokeratin number)							Vimentin
		7	18	19	20	4	5	13	
MZ-GC-1	not tested	–	+++	+++	–	–	–	–	–
MZ-GC-2	CK 8, 18, 19	(+)	+++	+++	(+)	–	–	–	–
MZ-PC-1	CK 7, 8, 18, 19	+++	+++	+++	–	(+)	(+)	(+)	+

$Lewis^b$ blood group antigens, sialyl $Lewis^a$ (Ca19-9) and epithelial antigens EPM-1 and Exo-1. $Lewis^a$ and $Lewis^b$ blood group antigens are co-expressed in most gastric and pancreatic carcinomas and in normal pancreatic tissue. In normal gastric tissue either $Lewis^a$ or $Lewis^b$ antigens are expressed depending on the blood group secretor status of the individual [31]. Ca19-9 represents a differentiation antigen of normal ductal cells of the pancreas [9, 29] and is expressed by the majority of pancreatic carcinomas. About 50% of the gastric carcinomas express Ca19-9, whereas in most cases normal gastric epithelia are Ca19-9 negative [31]. EPM-1, a high molecular weight glycoprotein, is expressed by normal and malignant epithelial cells [4]. EXO-1 is a carbohydrate epitope present on both glycosphingolipids and mucin proteins [5, 33]. Both epithelial antigens are found in normal gastric and pancreatic tissues as well as in pancreatic and gastric cancer, with varying intensity.

As we are interested in the immune response against cancer, we investigated the expression of HLA antigens and ICAM-1 and their modulation by interferon. Total losses of HLA-class I or selective losses of single class I allels have been described for a variety of human tumours [7, 39]. These may contribute to progression by evading the immune response of the host in the form of HLA-class I restricted, tumour reactive, cytotoxic T-cells. The three tumour cell lines described here expressed HLA-ABC, particularly HLA-A2 and HLA-B alleles. We cannot rule out however, that selective losses of a single HLA-A allel, HLA-B allel or HLA-C occurred in the cell lines. The expression of HLA-A2 and HLA-B by the lines was more than doubled by γ-interferon, possibly indicating a low basal expression. Normal gastric epithelia express HLA-class II antigens, however, HLA-DR expression by gastric cancer cell lines seems to be an exception [15]. Remarkably, MZ-GC-2 showed expression of HLA-DR. HLA-DR, -DP, and -DQ expression by the cell lines was upregulated by γ-interferon in a differential way, as described for other gastric cancer cell lines [30]. HLA-class II expression in tumours may play a role in antigen presentation, a suggestion supported by the observations that HLA-DR on gastric cancer can stimulate allogeneic lymphocytes [30] and that autologous, HLA-DR restricted tumour reactive T-cells crossreacting with MZ-GC-2 have been detected against a sarcoma (Heike et al. manuscript in preparation). ICAM-1 represents an adhesion molecule for T-cells on antigen presenting cells and is frequently expressed de novo by gastric and pancreatic carcinomas [15, 35]. The ICAM-1 expression by the cell lines investigated here could be induced or enhanced by γ-interferon, a phenomena observed for many cell types. It is not clear however, whether ICAM-1 expression or shedding by epithelial cancer cell lines is a marker for metastatic potential as observed in malignant melanoma [11]. Membrane bound ICAM-1 may also act as an co-stimulatory molecule for tumour reactive T-cells and NK-cells [38]. We observed that ICAM-1 expression by tumour cells was necessary for non-MHC restricted T-cell cytotoxicity against γ-interferon treated MZ-PC-1 cells and some other tumour cell lines (Heike et al., manuscript in preparation).

From their distinct characteristics these three tumour cell lines are seen to complement existing cell lines of gastric and pancreatic cancer. They may be useful models in which to investigate certain aspects of the cell biology and immune mechanisms involved in gastrointestinal tumour biology.

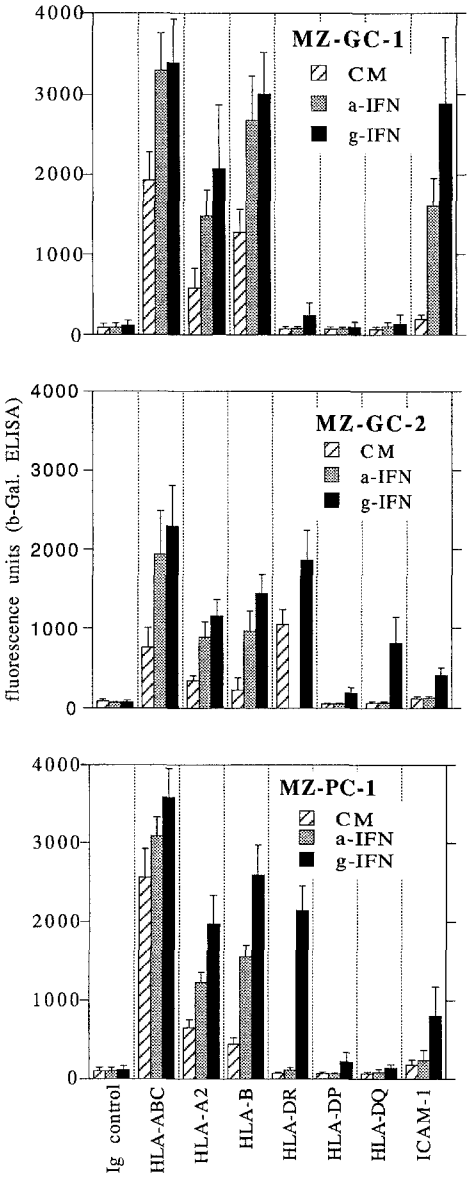

Fig. 9 Differential expression and induction of HLA antigens and ICAM-1 by pancreatic and gastric carcinoma cell lines. Tumour cell lines were grown with culture medium (CM), 5000 U/ml α-interferon (a-IFN) or 100 U/ml γ-interferon (g-IFN) for 4 days. The antigen expression of the cells was tested with the respective antibodies by a β-galactosidase-ELISA. Test values were derived from a Dynatech microfluor reader and expressed as fluorescence units. Each column represents the mean of at least two experiments, each with triplicate values. Bars are showing 95% confidence intervals of the means

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