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DNA flow cytometric measurements in inflammatory and malignant human gastric lesions *

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Summary. 102 gastroscopically taken biopsy specimens which were normal ($n=28$) or showed superficial gastritis ($n=18$), chronic atrophic gastritis ($n=18$), gastric ulcers ($n=19$) and gastric carcinomas ($n=19$) were submitted to FCM analysis. Carcinomatous specimens were readily recognized by either ploidy abnormality or significantly raised S- and G_2+M values. Intestinal-type and diffuse carcinomas could not be distinguished by proliferation kinetics, however, diffuse carcinomas showed a higher rate of aneuploidy. Chronic atrophic gastritis and gastric ulcers, though significantly differing from both normal tissue and superficial gastritis, exhibited similar proliferation characteristics. In gastric ulcers, an S-phase proportion of more than 12% was correlated with histological detection of cellular atypias and a proliferative tendency.

Key words: Flow cytometry – DNA content – Gastric carcinomas – Inflammatory gastric diseases – Cell cycle stage distribution

It is recognized that precancerous conditions and precancerous lesions are of major importance in the genesis of gastric carcinomas. In contrast to chronic atrophic gastritis, the significance of peptic ulcers as a precancerous condition is not generally accepted (Morson et al. 1980). The risk of the development of malignancy can, however, not always be judged from histology (Sprenger and Witte 1980; Weiss et al. 1980). Additional techniques comprising electron microscopy (Goldman and Si-Chun Ming 1968; Riemann et al. 1983), histochemistry (Sipponen et al. 1980), autoradiography (Oehlert et al. 1975), DNA cytophotometry (Wiendl et al. 1974) and radioimmunological methods (Stosiek et al. 1982) have been introduced to define the biological behaviour of gastric conditions likely to undergo malignant change.

DNA flow cytometry (FCM) has become a valuable and reliable tool

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in oncology research (Raju et al. 1980), permitting the rapid and accurate determination of nuclear DNA in large cell populations (Sugarbaker et al. 1979).

Though FCM-investigations have been conducted in both gastric carcinomas (Petrova et al. 1980) and chronic atrophic gastritis (Weiss et al. 1980) exhibiting a considerable rate of aneuploidy and/or an increase of the proliferative compartment, the diagnostic significance of FCM in diseases of the stomach has not been fully assessed (Schwabe and Wiendl 1974; Sprenger and Witte 1980). Considering the potential clinical importance of an additional method supporting the differential diagnosis of inflammatory, regenerative and neoplastic lesions of the stomach, the present study was undertaken to evaluate prospects and pitfalls of DNA-FCM in gastric diseases. The data of FCM analysis were correlated with those of histologic examinations.

Material and methods

Hundred and two biopsies were taken from patients gastroscopically (53 males, 49 females, age 30–74 years), cared for at the Department of Medicine (Head: Prof. L. Demling). Histological examination of the biopsy specimens revealed normal histology ($n=28$, antrum: $n=13$, corpus: $n=15$), superficial gastritis ($n=18$), chronic atrophic gastritis ($n=18$), gastric ulcers ($n=19$) and gastric carcinomas ($n=19$, diffuse type: $n=11$, intestinal type: $n=8$). Each biopsy piece was split into 2 pieces and submitted to simultaneous histological evaluation[1] (Department of Pathology, University of Erlangen, Head: Prof. V. Becker) and FCM-analysis.

The biopsy samples chosen for FCM-analysis were first minced with scissors. Subsequently, according to Barlogie et al. (1978) a single cell suspension was generated by the addition of 3 ml of 0.5% pepsion HCL (Serva, Heidelberg, FRG) and continuous agitation for 5 min at room temperature. Stoichiometric DNA fluorochromation was achieved by transferring the cell suspension into a solution containing ethidium bromide (12.5 µg/ml, Serva, Heidelberg, FRG) and mithramycin (25 µg/ml, Hoechst, Frankfurt, FRG) dissolved in tris buffer (pH 7.4) (Zante et al. 1978). After 10 min of staining 3 drops of 0.1% RNase (Serva, Heidelberg, FRG) were added and an aliquot of the cell suspension was measured in a flow cytometer (ICP-22, Phywe, Göttingen, FRG). Optical arrays were made up of a BG 12 excitation filter, a 590 nm long pass barrier filter and a 450 nm long pass dichromatic mirror. Fluorescence emission of the fluorochromated nuclei, quantified for each cell, was detected by a photomultiplier tube and their pulse height analyzed by a peak detector. After digital conversion, signals were stored in a multichannel cytoanalyzer (IN-96, Intertechnique, Mainz, FRG) according to their relative magnitude. Subsequently, data were displayed in histograms. As routine 20000–100000 cells per sample were analyzed. The remainder of the pepsinized cells were fixed in absolute ethanol, thus enabling later control measurements. As the fluorescence signal was recorded for each cell of the biopsy sample, besides epithelial cells concomitant inflammatory and connective tissue cells were also analyzed.

Cell cycle stage distribution analysis was carried out with computer programs provided by Intertechnique Manufacturer (Mainz, FRG). Correction of the histograms for cellular debris was done using an "exponential fitting" program. Ploidy abnormalities were detected by simultaneous staining and measurement of admixtured human reference lymphocytes. In case of aneuploidy the DNA-index (DI) defined as the ratio of peak channel number of tumour $G_{1/0}$ to normal $G_{1/0}$ cells was calculated (Barlogie et al. 1978).

Statistical evaluation of the data was performed applying the *U*-test of Wilcoxon, Mann and Whitney.[2]

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Table 1. Mean values of the cell cycle distribution analysis in inflammatory and malignant gastric lesions

	$G_{1/0}$ (%)	S (%)	G_2+M (%)
Normal ($n=28$)	90.8 ± 1.5	5.5 ± 1.0	3.6 ± 1.5
Superficial gastritis ($n=18$)	89.5 ± 1.3	6.2 ± 0.9	4.4 ± 0.7
Chronic atrophic gastritis ($n=18$)	85.1 ± 3.0*	9.0 ± 2.1*	5.5 ± 1.1*
Gastric ulcer ($n=19$)	86.0 ± 2.9*	9.1 ± 3.3*	5.0 ± 1.2*
Carcinoma[a] ($n=18$)	77.0 ± 4.6	12.6 ± 1.9	11.4 ± 4.0

* Cell cycle data of the gastric diseases studied differed significantly in each case from normal tissue. Unless marked by asterisks (*) differences of the cell cycle proportions of the disease categories proved significant among another as well. P levels ranging from 0.05 to 0.001

[a] For 1 case of intestinal-type carcinoma, exhibiting 3 aneuploid stem lines, cell cycle stage distribution could not be calculated

Results

With the exception of one case of intestinal-type carcinoma exhibiting 3 aneuploid stem lines, all biopsy specimens were evaluated for both ploidy and cell cycle stage distribution. Cell kinetic data of the different groups are listed in Table 1.

As site-dependency of the cell cycle stages could be ruled out in normal gastric tissue, all those specimens were thus taken as one control group, regardless of the biopsy area.

Carcinomas. Specimens from malignant lesions could clearly be discerned from non-neoplastic ones by their significantly raised S- and G_2+M-values (p levels from 0.05 to 0.001). In addition, occurence of aneuploid stem lines proved to be a tumour marker of high reliability, as heteroploidy could not be demonstrated in benign gastric lesions. Proliferation characteristics between intestinal-type and diffuse carcinomas did not differ significantly. Aneuploidy rates observed with the 2 carcinoma subsets tended to be different, however: with the intestinal type 4 out of 8 biopsies studied were aneuploid (one specimen exhibiting 3 aneuploid stem lines), whereas heteroploidy was observed with 8 of 11 biopsies from the diffuse type carcinomas. Overall aneuploidy incidence amounted to 58% (11 out of 19). DI values ranged from 1.17 (near diploid) to 2.24 (hypertetraploid). A typical histogram obtained from a diffuse gastric carcinoma is shown in Fig. 2.

Chronic atrophic gastritis. The mean percentage of nuclei in S-phase (9.0 ± 2.1) was significantly elevated as compared to normal tissue ($p\leqq0.002$) and superficial gastritis ($p\geqq0.002$). The same holds true for G_2+M-values ($p\leqq0.002$ and $p\leqq0.01$). Notably, cell cycle distribution in chronic atrophic gastritis did not differ from the pattern calculated for gastric ulcers. It has to be emphasized, however, that frank precancerous lesions were not included in this series.

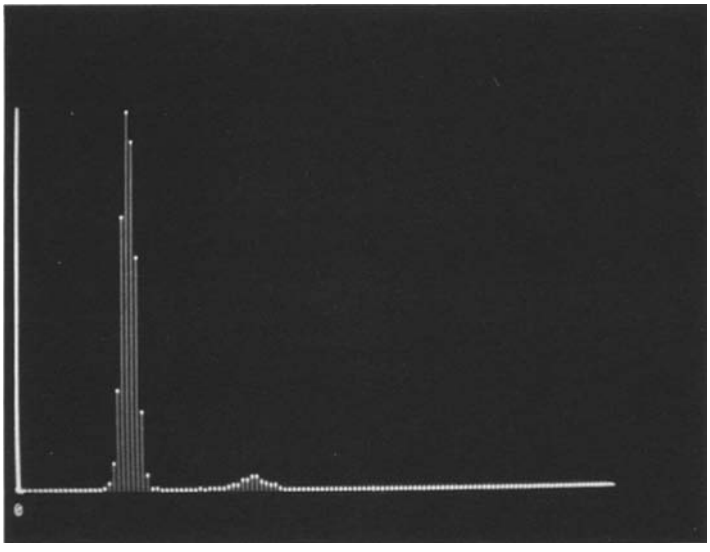

Fig. 1. DNA-histogram obtained from normal gastric tissue. Left peak: $G_{1/0}$ cell population (2 c). *Right peak:* G_2+M cells (4 c). Cell cycle stage distribution analysis: $G_{1/0}$:91.7%, S: 4.81%, G_2+M: 3.47%. *Abscissa:* channel number (relative fluorescence intensity identical in meaning with DNA content). *Ordinate:* number of cells per channel

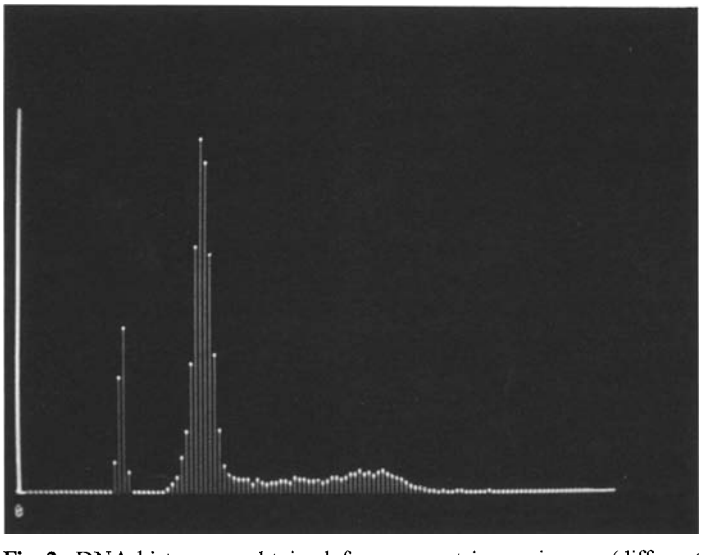

Fig. 2. DNA-histogram obtained from a gastric carcinoma (diffuse type). Peaks from left to right: euploid peak of normal tissue cells ($G_{1/0}$, 2c), heteroploid tumour peak $G_{1/0}$ (DI: 1.81), G_2+M tumour peak. Cell cycle stage distribution analysis of the tumour cell population: $G_{1/0}$: 74.6%, S: 16.1%, G_2+M: 8.64%

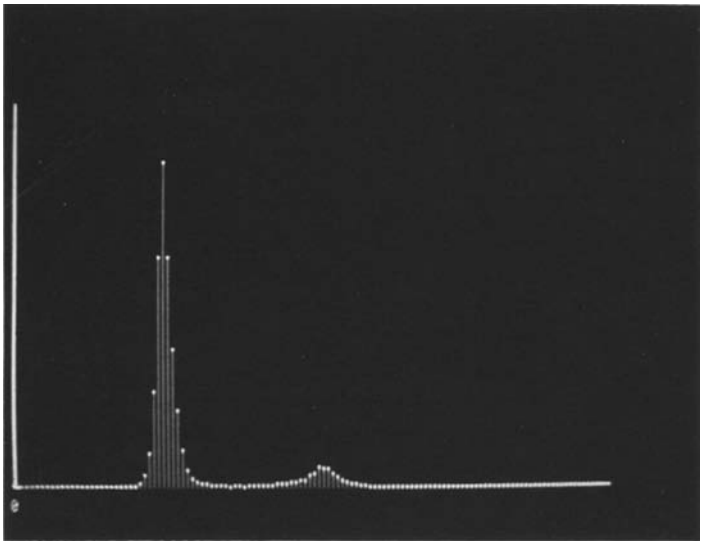

Fig. 3. DNA-histogram obtained from the border of a gastric ulcer exhibiting marked cellular atypia. *Left peak*: $G_{1/0}$ cell population (2c). *Right peak:* G_2+M cells (4c). Cell cycle stage distribution analysis: $G_{1/0}$: 80.6%, S:12.3%, G_2+M:7.0%

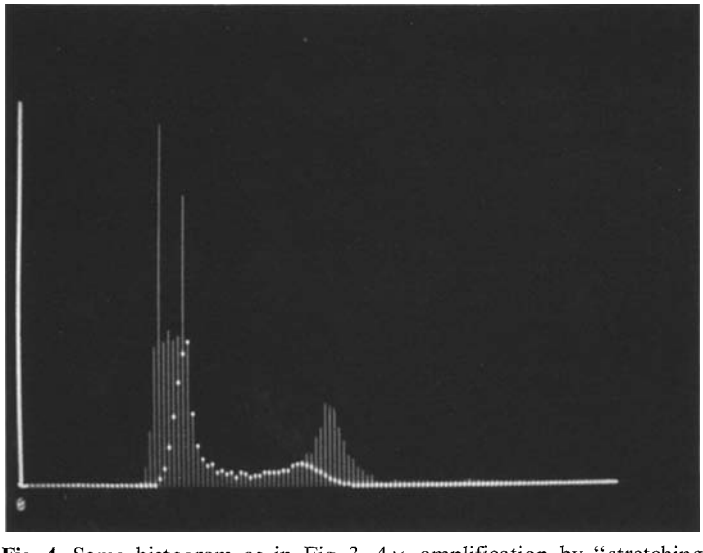

Fig. 4. Same histogram as in Fig. 3. 4× amplification by "stretching" of y-axis. The high S-phase proportion (12.3%) is marked by a dotted line

Border of gastric ulcers. G_1-, S- and G_2+M data significantly differed from those computed for unchanged tissue and superficial gastritis ($p \leqq 0.002$). Three cases among the ulcer group were particularly striking, however: histological examination revealed "marked cellular atypia with proliferation tendency". FCM-analysis showed normal "unimodal" DNA distribution

but S-phase proportions constantly exceeded 12%, thus equaling S-phase values only observed in the carcinoma group (Fig. 3 and 4 resp.).

Superficial gastritis. Cell cycle stage distribution patterns differed significantly from those calculated for all other gastric diseases investigated and from normal tissue (p levels from 0.05 to 0.001).

Comment

In recent years, FCM has become a valuable tool in diagnosis and monitoring of malignant diseases (Raju et al. 1980). For clinical purposes nuclear DNA measurements of isolated cells in suspension are used increasingly. From FCM investigations conducted so far it appears that solid tumours exhibit a considerable rate of aneuploidy (Barlogie et al. 1978; Linden 1982).

Petrova et al. (1980), in a study of 68 gastric carcinomas, found that differentiated adenocarcinomas tended to be aneuploid, while in the group of undifferentiated carcinomas euploid DNA distribution patterns prevailed. Cell cycle stage analysis revealed elevated S- and G_2+M-proportions in both groups as compared to normal tissue (Petrova et al. 1980; Weis et al. 1980; Sprenger and Witte 1980). Aneuploid stem lines in carcinomas of the stomach had already been demonstrated by single cell DNA-cytophotometry, though with this technique only a small amount of cells could be studied (Wiendl et al. 1974; Zank and Krug 1970). Our FCM data confirm these results since carcinomatous specimens could readily be discerned by both ploidy abnormality and increase of S- and G_2+M cycle proportions. No difference was found between the proliferation kinetics of intestinal-type and diffuse carcinomas. Aneuploidy rate in diffuse carcinomas, however, tended to be higher than in intestinal-type ones, though the limited number of cases is far from being conclusive. Reportedly, a different pathogenesis is assumed for diffuse and intestinal-type gastric carcinomas (Lauren 1965). In the intestinal-type, atrophy, metaplasia and dysplasia are considered to be precursors of malignancy whereas precursor lesions are not defined for the diffuse type (Correa 1982). Furthermore, a poorer prognosis is ascribed to diffuse carcinomas. It is, however, not demonstrated by FCM studies, whether or not a correlation exists between the incidence of aneuploidy and the histological grading on one hand and the prognosis on the other. Tribukait et al. (1979), in a study of 41 carcinomas of the bladder, found good correspondance of the histological grading and the degree of aneuploidy, as all highly atypical carcinomas were aneuploid. Similarly, Hofstädter and Ehrlich (1978) noted a strong correlation between the morphological grading in renal carcinomas and the incidence of aneuploidy. No such correlations were detected, however, in neoplasms of the brain (Mork and Laerum 1980), thyroid gland (Tangen et al. 1983), and human prostate (Lämmel et al. 1981).

One shortcoming of DNA measurements has to be noted in this respect, as euploidy detected by FCM fails to reflect cytogenetic data. For instance a tumour with hypodiploid karyotype, as pointed out by Schumann and

Hiddemann (1982), may show a "pseudodiploid" DNA content due to marker chromosomes with increased DNA content. In addition, cytogenetic heterogeneity created by clonal evolution (Nowell 1978) in human solid tumours may lead to "mosaic" subpopulations, thus hindering correlation studies between morphological typing and ploidy behaviour in general (Nervi et al. 1982, Petersen et al. 1981). On the other hand, cellular heterogeneity gets often less pronounced as older subpopulations become extinct with new ones emerging (Vindelov et al. 1982).

Cell kinetic data of the gastric carcinomas in our series not only allowed reliable discrimination from normal gastric tissue but also from superficial gastritis, chronic atrophic gastritis and gastric ulcers. This result is opposed to the findings reported by Schwabe and Wiendl (1974) who, owing to a false-negative rate up to 83%, considered FCM inappropriate for recognition of gastric malignancies. However, analysis of cell cycle stage distribution was not performed by these authors.

The present cell kinetic data in superficial gastritis, gastric ulcers and chronic atrophic gastritis differed significantly from normal tissue. The rise in proliferative activity observed in these diseases is in keeping with former FCM investigations (Weiss et al. 1980) and labelling studies (Steenbeck and Wolff 1971). In 3 cases among the ulcer group, however, S-phase proportions comparable to those of gastric carcinomas were associated with histological features of cellular atypia combined with proliferation tendency. Recently, Steenbeck et al. (1983), from labelling studies, were able to define a sub-group of patients with chronic atrophic gastritis and gastric ulcers exhibiting labelling indices otherwise not observed except in gastric malignancies. Whether such carcinoma-like proliferation patterns herald incipient malignancy remains to be elucidated.

As FCM is restricted to the evaluation of nuclear DNA content, the analysis fails to distinguish per se epithelial and non-epithelial cells. Inevitably almost every biopsy specimen contains both epithelial and non-epithelial cells (e.g. leukocytes, macrophages, fibroblasts) likely to influence the DNA-distribution pattern. Though these cells can hardly account for the occurrence of aneuploidies, they have to be considered when cell cycle stage distributions are calculated. For this reason, an increased S-phase proportion may be due in part to a raised number of mesenchymal cells and thus not be fully attributable to the proliferative activity of the epithelium. It may be mentioned in this respect that a decreased proliferativ activity was found by FCM in the non-inflammatory atrophic human endometrium (Goerttler and Feichter 1982).

In the present study the occurrence of aneuploid stem lines proved to be intimately associated with malignancy. Heteroploidy thus evolved once more as a specific and reliable indice of tumour growth. Aneuploid stem lines have recently been revealed by FCM with in situ-gastric carcinomas (Teodori et al. 1982), in some premalignant oral lesions (Gräßel-Pietrusky et al. 1982), and in minimal invasive cancer of the cervix uteri (Herzog and Ciprian 1983). Unfortunately, specimens of comparable type were not available for our study. Aneuploidy detection by FCM indicates malignant

development unequivocally whereas early premalignant lesions may pass undetected this way. Nevertheless, DNA-FCM can be considered a valuable ancillary technique for the diagnosis of malignant gastric disease, though the introduction of dual-parameter FCM would probably be advantageous particularly in the recognition of early premalignancies.

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