J. Clin. Chem. Clin. Biochem.
Vol. 22, 1984, pp. 935–942

Automated Flow-Cytometric Identification of Colo-Rectal Tumour Cells by Simultaneous DNA, CEA-Antibody and Cell Volume Measurements[1])

By *G. Valet, L. Rüssmann*

Max-Planck-Institut für Biochemie, Martinsried, FRG and

R. Wirsching

Chirurgische Klinik Großhadern der Universität München, München, FRG

(Received April 25/September 25, 1984)

Summary: A new method for the automated flow-cytometric identification of colo-rectal tumour cells was developed. Fresh tissue is cut mechanically to obtain single cell suspensions. The cells are then incubated with antibodies in an indirect immunofluorescence assay for CEA (carcino-embryonic antigen) on the cell surface, and counterstained with the DNA stain propidium iodide. Monosized latex particles are added as internal standard, then cell volume, antibody fluorescence and DNA are measured simultaneously in a FLUVO-METRICELL flow cytometer. A FORTRAN IV computer program was used to determine whether aneuploid cells or cells with high density of CEA on their surface were present in the sample. All relevant data were stored automatically in a self updating data base, which is important for quality control and automated thresholding. The samples were taken from 120 different patients. A tumour sample and a sample of healthy adjacent mucosa of the same patient were available in 88 patients. 97.5% of all tumours and 88.6% of the normal mucosa samples were correctly identified. This shows for the first time that the majority of colo-rectal tumour samples can be identified by a flow cytometric measurement with automated data evaluation. The identification of tumour samples was substantially better when based on the measurement of the three parameters, compared with identification by aneuploidy (59%) or by the CEA antibody alone (91%). It will be possible to automate the measurement of the samples.

Automatische Identifizierung colo-rektaler Tumorzellen mit Hilfe simultaner durchflußcytometrischer Messung von DNA, CEA-Antikörpern und Zellvolumen

Zusammenfassung: Es wurde eine neue Methode für die automatisierte durchflußcytometrische Identifizierung colo-rektaler Tumoren entwickelt. Sie besteht aus der mechanischen Zerkleinerung frischen Gewebes zur Einzelzellsuspension, Inkubation der Zellen mit Antikörpern in einem indirekten Immunfluoreszenzansatz für Zelloberflächen-CEA (carcino-embryonales Antigen), Gegenfärbung mit dem DNA-Farbstoff Propidium-Iodid, Zusatz monodisperser Latex-Partikel als internem Standard und simultaner Messung von Zellvolumen, Antikörper Fluoreszenz und DNA-Fluoreszenz jeder Zelle in einem FLUVO-METRICELL Durchflußcytometer. Mit einem FORTRAN IV Computer-Programm wurde bestimmt, ob in der Probe aneuploide Zellen oder Zellen mit hoher CEA Packungsdichte auf der Zellmembran vorhanden waren. Alle relevanten Daten der Auswertung wurden automatisch in einer selbstergänzenden Datei abgelegt, was für die Qualitätskontrolle und die automatische Schwellensetzung von Wichtigkeit ist. Es wurden Proben von 120 verschiedenen Patienten untersucht. Von 88 Patienten waren sowohl eine Tumorprobe als auch eine Probe der umgebenden, gesunden Darmschleimhaut verfügbar. 97,5% der Tumoren und 88,6% der normalen Mucosa-Proben wurden richtig identifiziert. Dieses Ergebnis zeigt erstmals, daß die Mehrheit colo-rektaler Tumoren mit durchflußcytometrischer Messung und automatischer Datenauswertung identifiziert werden kön-

[1]) Presented at the Kleinkonferenz „Immunologische Diagnostik" der Deutschen Gesellschaft für Klinische Chemie, Hamburg, Juni 1983.

nen. Die Identifizierung der Tumorproben durch die Dreiparametermessung war wesentlich besser als durch Aneuploidie (59%) oder CEA-Antikörper (91%) allein. Die Probenmessung kann in der Zukunft ebenfalls automatisiert werden.

Introduction

The correct identification and characterization of malignant and premalignant cells in smears, effusions or biopsy material is of great importance for tumour diagnosis, therapy and prognosis. The recent advances in flow cytometric instrumentation, the use of microprocessor technology (1), development of flow chambers for automated instruments (2), new staining techniques for the measurement of functional cell parameters in living cells (3–5) and the increasing number of monoclonal and conventional antisera produced against tumour-associated cell surface antigens open new possibilities for an automated identification of tumour cells by flow cytometry. The reexpression of onco-developmental antigens on tumour cells e.g. carcino-embryonic antigen (CEA) (6, 7) or α-foetoprotein (AFP) is of particular interest, because these antigens are biochemically well characterized. The determination of serum CEA-levels for the recognition of tumour relapses is an established clinical procedure (8), and potent antisera are available for this purpose. CEA is secreted by tumour cells and its cellular location can be demonstrated by immune histology using fluorescein-isothiocyanate (FITC) or peroxidase coupled antibodies (9, 10). Although serum CEA is often increased in gastrointestinal tumours, an increase is not seen for all tumours (8). Furthermore, elevated CEA levels may occur in e.g. smokers or during inflammatory disease without the presence of a tumour. CEA is, therefore, not an absolute tumour marker (7, 8). Hitherto, the measurement of FITC-antibody-labeled cells in flow cytometers and cell sorters has been performed mainly in connection with immunological studies for the identification of cellular subpopulations of the immune system. There has been little work on the determination of CEA-antigen in single cell suspensions of solid tumours, due to difficulties of tissue desintegration and preservation of the antigenic determinants on the cell surface.

In addition to changes of the cell surface antigen pattern, malignant transformation is often associated with chromosomal aberrations resulting in measurable cellular aneuploidy. The flow-cytometric measurement of DNA distribution curves has become a routine procedure during the last decade (11–14). Although cellular DNA-aneuploidy is a good indicator of malignancy, it is not very useful on its own for cancer prescreening purposes, because not all tumours are aneuploid.

Few reports are available on combined DNA-immunofluorescence measurements for tumour cell recognition. We have shown in an earlier investigation (15) that the simultaneous flow cytometric measurement of DNA, CEA-immunofluorescence and cell volume is a fast and sensitive method for identifying cervix uteri cancer cells in suspensions. The purpose of this study was to extend this work to gastro-intestinal tumours. The study was also undertaken to explore the conditions for the automation of sample measurement and data evaluation.

Material and Methods

Cells

Between 0.05 to 0.5 g tumour tissue was removed from malignant colo-rectal tumours immediately after surgery. Tumours from 120 sequentially operated patients,

Dukes stage (16) A: 20.1%,
B: 34.1%,
C: 34.1%,
D: 11.7%;

histological grading (17):

2.5% well differentiated adenocarcinoma (I);
72.5% moderately differentiated (II);
13.3% moderately to poorly differentiated (II–III);
11.7% poorly differentiated (III).

A second tissue sample of similar size was taken from the healthy adjacent intestinal mucosa of the same patient. Both samples were immersed in a 0.15 mol/l NaCl solution, buffered with 10 mmol/l Tris/HCl to pH 7.35 (Tris buffered saline), cooled to 0 °C in an ice bath and kept at 0–4 °C during the following procedure until cell fixation. The samples were separately minced once with a *McIlwain* electric tissue chopper (The Mickle Comp., Gomshall, England). The chopper was modified so that five parallel razor blades with 0.8 mm spacing were used as the cutting knife instead of the one blade provided by the manufacturer. The chopped tissue was taken up in 10 ml Tris buffered saline containing 10 mmol/l EDTA in a 50 ml plastic Falcon tube with conical bottom (Becton Dickinson, Heidelberg, FRG). The suspension with the small tissue pieces was quickly sucked 30 to 50 times back and forth in a 1 ml Eppendorf pipette (Eppendorf, Hamburg, FRG) without producing air bubbles. The disposable plastic tip of the pipette was cut for this purpose at the bottom to obtain an opening of 0.5 or 1 mm in diameter in order to let the tissue pieces just freely pass. The cell suspension was filtered through a V2A-steel sieve of 60 μm mesh width, washed twice by centrifugation in 50 ml Tris buffered saline at 300 *g* and resuspended in Tris buffered saline at a cell concentration of 5×10^7/ml. The majority of the cell suspension was fixed at 0 °C for 12 h by addition of an equal amount of a freshly prepared formaldehyde solution (35 g/l Tris buffered saline adjusted to pH 7.35). For immunofluorescence staining, the fixed cells were washed twice with 50 ml Tris buffered saline to eliminate the formaldehyde and resuspended at a cell concentration of 5×10^7/ml in Tris buffered saline containing 15 g/l bovine serum albumin and 40 mg/l of propodium iodide (Sigma Chemicals, Munich, FRG) to stain the cellular DNA. The remaining ali-

quot of fresh cells was centrifuged for 5 min at 300 *g*, resuspended to the original cell concentration in albumin/Tris buffered saline and stained according to the same protocol as fixed cells. 85% of the samples were stained as fixed cells and the remainder as fresh, unfixed cell preparation.

Immunofluorescence

Aliquots (50 µl) of fixed or vital cell suspension were incubated for 12 h at 0 °C in 96 well microtiter plates (250 µl wells), either with 10 µl of an antibody dilution in albumin/Tris buffered saline or with albumin/Tris buffered saline alone as a control. Diluted (1/100) mouse monoclonal anti-CEA antibody (Hybritech, Paesel, Frankfurt, FRG, 1 g/l IgG), or diluted (1/100) rabbit-anti-CEA monospecific antiserum (DAKO, Hamburg, FRG) rendered free of anti-NCA (non specific cross reacting antigen) antibodies by absorption with human spleen and lung tissue (serum kindly provided by Dr. *Lamerz*, Klinikum Großhadern, Munich, FRG, 5.3 g/l IgG) were used to detect cell surface CEA. The assays were washed twice by centrifugation (10 min, 300 *g*) with 200 µl albumin/Tris buffered saline and reincubated with either a fluoresceine isothiocyanate (FITC)-labelled rabbit-anti-mouse or a FITC-labelled goat-antirabbit IgG (both antisera: Paesel, Frankfurt, FRG, 10 g/l IgG, fluoresceine/protein ratio 3.71 (mg/g) and 12.0 g/l IgG, fluoresceine/protein ratio 3.95 (mg/g)) for another 12 h at 0 °C. Both antisera were 1/40 diluted with albumin/Tris buffered saline. The cells were washed twice with albumin/Tris buffered saline and finally resuspended in 200 µl Tris buffered saline containing 40 mg/l propidium iodide. Resuspension of the cells after each wash was effected by a Titertek vibration mixer (Flow-Laboratories, Großmeckenheim, FRG). A sample (5 µl) of porous, NH_2 group-containing, monosized (6 µm diam.) latex particles (kindly provided by Prof. *J. Ugelstad*, SINTEF and University of Trondheim, Norway) were prestained with FITC and added at a final concentration of 2.5×10^5/ml to each assay as internal standard.

Flow-cytometric measurement

The cell volume and the green FITC and the red propidium iodide fluorescence of the double stained cells were determined in a FLUVO-METRICELL flow cytometer (18). The volume of each cell was measured electrically at a current of 0.47 mA in a Tris buffered saline-filled measuring chamber at 22 °C after hydrodynamic focusing of the sample beam through the center of a cylindrical orifice of 85 µm diameter and 100 µm length. The fluorescence was excited by epi-illumination with UV light between 418 and 500 nm from a HBO-100 high pressure mercury arc lamp, after the cell had left the sizing orifice. The red and green fluorescences were separated by a 530 nm dichroic mirror. The green FITC-fluorescence was collected between 500 and 530 nm and the red propidium iodide fluorescence between 550 and 650 nm. The maximum height of each signal was amplified by a 2.5 decade logarithmic amplifier for the cell volume signal and by linear amplifiers for the FITC and propidium iodide signals. The amplified signals of each cell were stored in list mode on magnetic tape and subsequently analysed by FORTRAN IV computer programs described in part earlier (19). The analysis consisted of the calculation and plot of three one parameter distribution curves (cell volume, FITC-antibody and propidium iodide-DNA distribution) (figs. 1, 2), a two parameter FITC-antibody versus propidium iodide-DNA histogram (fig. 4) and a three dimensional cube for visual inspection (fig. 3). The total data evaluation was recently automated. After completion of the measurements, the user only types the word "ANTIGEN" in the computer (INTERDATA 7/32, Perkin Elmer, Oceanport, NJ, USA, 768 kbyte core memory, 200 Mbyte disc, magnetic tape, electrostatic plotter, graphic display) which starts a command-file where all calculation procedures are defined. The user obtains the above mentioned graphs and a data list for each sample. The data list contains the number of antigen positive cells, the mean antigen surface density per cell and the aneuploidy index. The antigen positive cells are the cells beyond a threshold in the FITC-antibody versus propidium iodide-DNA histogram (fig. 4a, b). The antigen surface density of the antigen positive cells was calculated for each cell from the cell volume and the intensity of the FITC-antibody fluorescence, assuming a spheroid shape of the cell. To determine the aneuploidy index 1 (AN1), the product of % cells in $S+G_2/M$ phase times their mean DNA-fluorescence was divided by the product of % cells in G_0/G_1 phase times their mean DNA-fluorescence. The aneuploidy index 2 (AN2) was calculated, when a sample of normal mucosa from the same patient was also available. AN2 is the ratio of AN1 of the tumour divided by AN1 of the mucosa. AN2 was a very sensitive indicator of abnormalities because it indicated minor differences between tumour and mucosa. A sample was judged aneuploid above an AN1 or AN2 of 1.2. All calculated data were automatically stored in a self updating data base which was installed for long time quality control and automated optimation of the decision thresholds. A cell sample was automatically judged malignant by the computer classifier program either when it was aneuploid or when the surface density of CEA-positive cells exceded the normal range.

Results

The identification of samples containing tumour cells was easily possible when aneuploid cells were present. They were visible as a separate peak (fig. 1a) which did not occur in cell samples from the healthy adjacent mucosa (fig. 1b). 71 out of 120 malignant tumour samples (59%) were aneuploid with DNA-indices mostly in the hyperdiploid region (1.15–1.90).

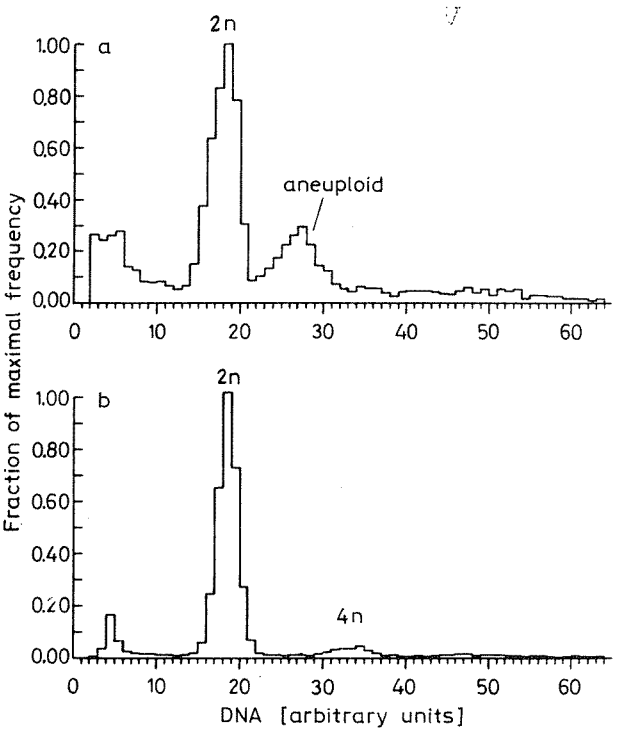

Fig. 1. DNA distribution curve.
a) of an aneuploid human rectum cancer cell sample.
Number of particles measured: 3401.
Maximal frequency: 394.
b) of the adjacent healthy mucosa.
Number of particles measured: 8870.
Maximal frequency: 2243.

The identification of euploid tumour cells was not possible with one-parameter measurements alone. This is apparent from figure 2 where the DNA distribution (fig. 2a, b), the antibody distribution (fig. 2c, d) and the volume distribution curves (fig. 2d, e) of cells of the tumour (fig. 2a, c, d) and the normal mucosa (fig. 2b, d, e) of a patient with a euploid tumour are shown. All curves begin with an exponentially decreasing background in the left part. The respective curves of tumour sample and normal mucosa were quite similar. The shape of the diploid peak of the DNA distributions (fig. 2a, b) was broad with coefficients of variation (CV = 100 · standard deviation/mean value) in the order of 8 to 10%. The CV of the DNA distribution curves was lower (6 to 8%) for fresh cells. The high CV's were due to the intestinal cells and to formaldehyde fixation, but not to instrumental variation because CV's of 1.5 to 1.9% were obtained for ethanol-fixed rat thymocytes. Ethanol fixation caused, however, cell clumping. The diploid peaks of the DNA distribution curves were often left skewed after formaldehyde fixation because the large epithelial cells stained slightly higher with propidium iodide as compared to the

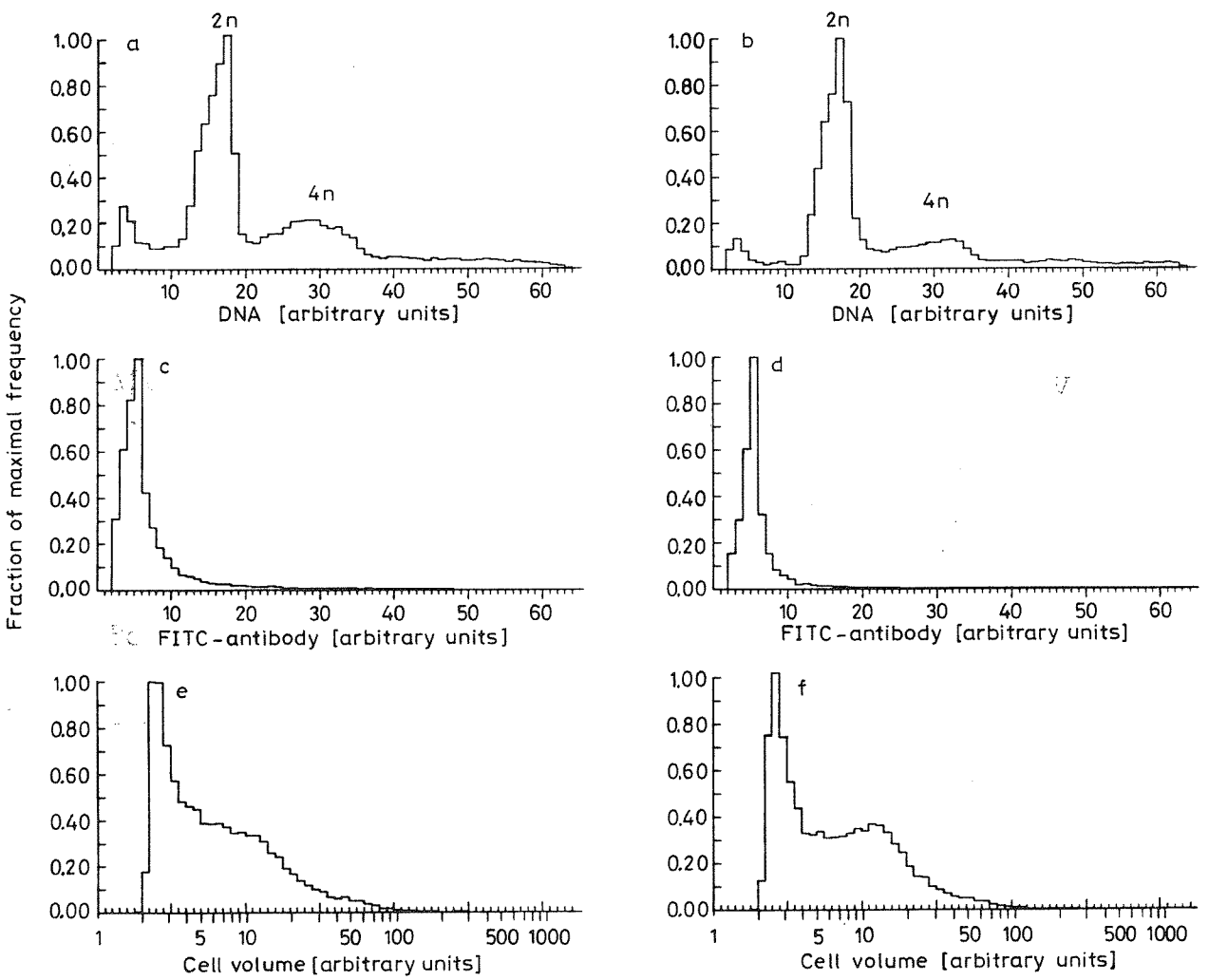

Fig. 2. DNA, FITC-antibody and cell volume distribution of an euploid colon cancer (left) and of the adjacent healthy mucosa (right). All three curves begin with an exponentially decreasing experimental background on the left side of the plot. It is most pronounced in the antibody (c, d) and cell volume distribution (fig. 3e, f).
Between 10600 and 18700 cells per curve were measured.
a) DNA, euploid colon cancer; maximal frequency 1906.
b) DNA, adjacent healthy mucosa; maximal frequency 1547.
c) FITC-antibody, euploid colon cancer; maximal frequency 6286.
d) FITC-antibody, adjacent healthy mucosa; maximal frequency 9496.
e) Cell volume, euploid colon cancer; maximal frequency 2969. One class on the logarithmic volume scale corresponds to 30 fl.
f) Cell volume, adjacent healthy mucosa; maximal frequency 3120.

small inflammatory leukocytes. The left skew is visible in figure 2a, b. Since the identification of euploid tumour cells by the one parameter measurements of figure 2 was not possible one could conclude that flow cytometry is not a suitable method for this purpose.

This was, however, not true, when a simultaneous measurement of the same three parameters was performed. The measurement resolved additional features of the different cell populations (fig. 3a, b). The three parameter measurement distinguishes between cell debris and morphologically intact cells. Only particles with DNA and volume are morphologically intact cells. Cell debris i.e. enucleated cells or broken cells give rise to volume signals but not to DNA signals, and bare nuclei show DNA signals but only very small volume signals which do not appear on the volume scale. Cell debris is significantly present in the tumour sample (fig. 3a) and also in the normal mucosa sample (fig. 3b). Intact, antigen positive cells are visible amongst the cells with large volume in the tumour sample (fig. 3a). This is reasonable because tumour cells occur amongst the large epithelial cells. Some CEA-antigen positive cells were also present in the normal mucosa sample of the same patient (fig. 3b). The intact inflammatory cells at the lower edge of the volume scale are not antigen positive, indicating that the antiserum is free of granulocytes that react with NCA-antibodies. The cluster of calibration particles is also visible. It is situated where no interference with stained cells occurs. The particles serve as internal standard for the calculation of the cell concentration. They are also useful for checking instrument function with regard to cell volume and fluorescence. They allow a precise recalibration of the instrument from day to day, which is important in automated operation for maintaining standard thresholds over a long period of time. The cube display is useful for a quick visual judgment of the three parameter measurement, but not suitable for quantitative evaluation. The quantitative evaluation is performed on two parameter histograms obtained by projecting the content of the cube onto the antibody versus DNA plane (fig. 4a, b). The calibration particles were not projected to simplify the distributions. The solid lines of the graph separate the area of antigen negative and antigen positive cells used by the computer program for the calculation of cell parameters. The substantial amount of cell debris which is strongly positive for the CEA-antibody is again visible. The quantitation would be significantly influenced if the debris were not distinguished from the intact cells by the DNA stain of the cell nuclei. The evaluation of the tumour samples and of the normal mucosa cells of two patients (figs. 1 and 2, 3) is given in table 1. The aneuploidy of the tumour sample of the first patient is

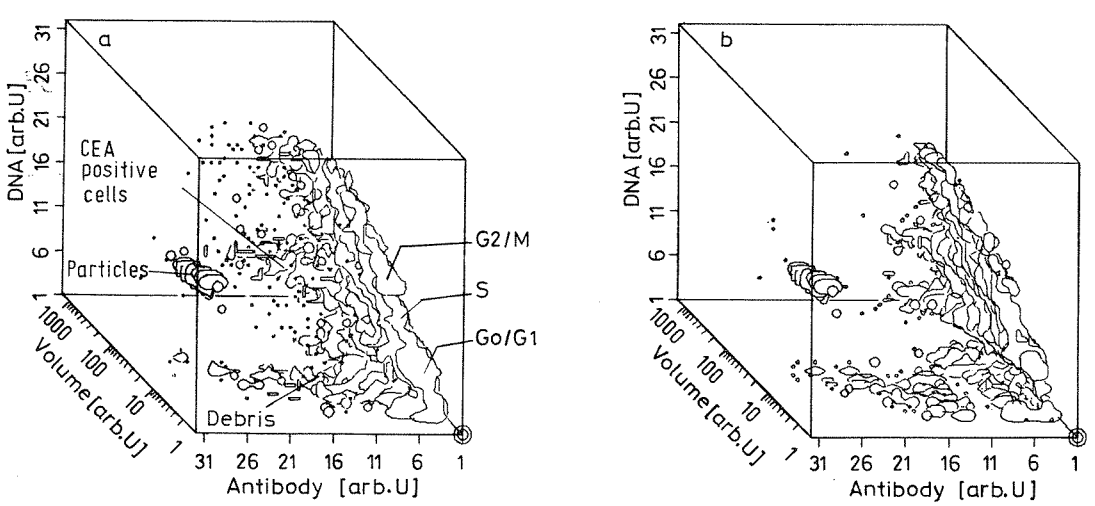

Fig. 3. Three dimensional representation of a simultaneous cell volume, DNA and CEA-antibody measurement of colon cancer cells (a) and cells of the adjacent healthy mucosa (b). The cells are derived from the same samples as those represented in the one parameter distribution curves of figure 2. The increased number of morphologically intact CEA-positive cells in the tumour cell sample (a) is visible. The contour lines surround the areas where particles and cells are located. The channel contents are standardized to the maximum logarithmic channel content and plotted for the 10% level. Channels with 2 and 3 cells are already contoured which means that the location of most of the measured cells is indicated. The calibration particles are used as internal concentration standard and also for day to day standardisation of the fluorescence measurement. One class on the logarithmic volume scale corresponds to 30 fl. 19419 (a) and 11948 particles (b) were measured. The maximum channel contents are 256 and 245 particles respectively. G0/G1, S and G2/M represent the cell cycle phases.

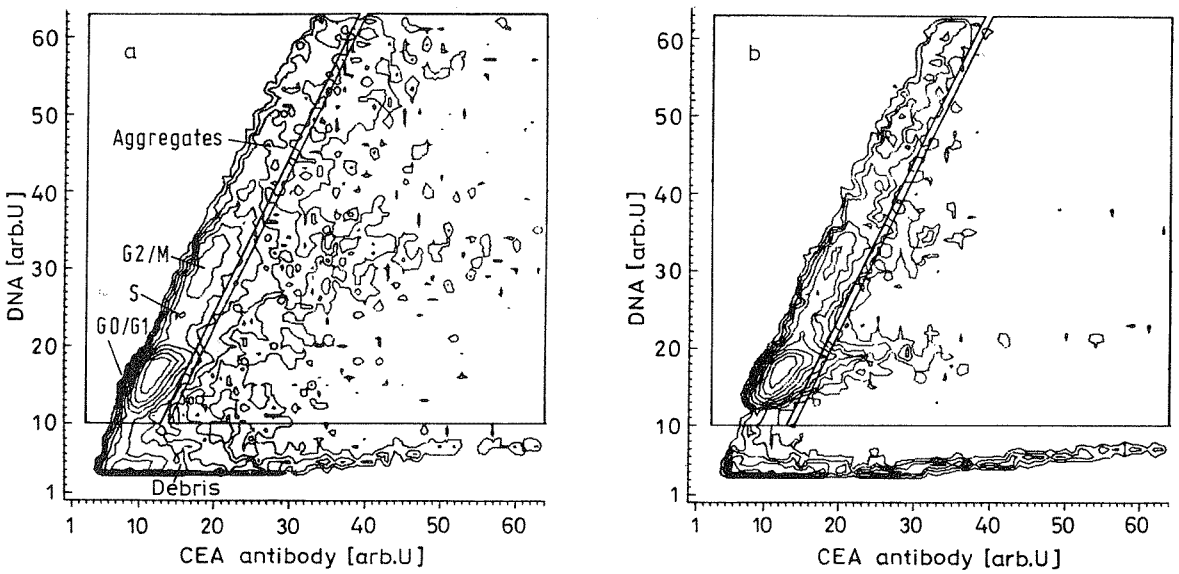

Fig. 4. CEA-antibody versus DNA histogram of colon cancer cells (a) and cells of the adjacent healthy mucosa (b). The graphs were obtained by projecting the respective cube diagrams of figure 3 onto the antibody/DNA plane. The solid lines represent the areas used for the computer evaluation. To the left is the antibody negative and to the right the antibody positive compartment. 16.1% CEA positive cells were found in the tumour sample and 7.9% in the normal mucosa. The graphs were standardized to the maximum logarithmic channel content and contour lines are plotted in 10% linear increments. To simplify the calculation of antibody positive cells the calibration particles of figure 3 were not projected onto the antibody/DNA plane. 18574 (a) and 10665 particles (b) were measured. The maximum channel contents are 523 and 395 particles respectively.

Tab. 1. Calculated parameters from simultaneous CEA-antibody, DNA and cell volume measurement of samples from an aneuploid (fig. 1) and an euploid colo-rectal tumour (fig. 2 to fig. 4).

	CEA-positive cells (%)	Relative antigen density	Aneuploidy index 1	Aneuploidy index 2
Aneuploid				
Rectum carcinoma	15.13	0.653	1.35	3.75
Normal mucosa	4.24	0.406	0.36	–
Euploid				
Colon carcinoma	16.12	0.759	0.842	1.00
Normal mucosa	7.89	0.389	0.840	–

correctly recognized by the AN2 (>1.2). The number of antigen positive cells is abnormally high in both patients and the mean antigen density/cell is substantially increased in the tumour samples. A total of 120 patients with colo-rectal tumours and 88 normal mucosa samples from the same patients have been screened so far by this method. All samples were measurable. The lower number of mucosa samples is due to the fact that some of the tumours were taken from patients prior to cryotherapy without a normal mucosa sample. Also included in the tumour cases are 11 patients from which only metastatic lymph-node material was obtained by abdominal surgery. The mean antigen density on the antigen positive cells of most tumour samples was higher than on the antigen positive cells of the adjacent normal mucosa. 109 (91%) of the tumors were recognized by the CEA-antibody alone. Of the remaining 11 tumours, 8 were aneuploid and thus could be additionally identified. 3 tumours i.e. 2.5% of all tumours were not recognized. The metastatic cells behaved similarly to the tumours. Table 2 contains the quantitative values. The conventional anti-CEA

Tab. 2. Flow-cytometric classification of colorectal tumour and mucosa cells by CEA-antibodies and DNA-aneuploidy.

	Monospecific DAKO-antibody		Monoclonal Hybritech antibody	
	Tumour FN (%)	Mucosa FP (%)	Tumour FN (%)	Mucosa FP (%)
Aneuploidy + % CEA-positive cells	8.3	13.7	6.1	27.5
Aneuploidy + rel. CEA-antigen surface density	2.5	11.4	4.9	18.3

FN = false negative (n = 120 tumour samples)
FP = false positive (n = 88 mucosa samples)

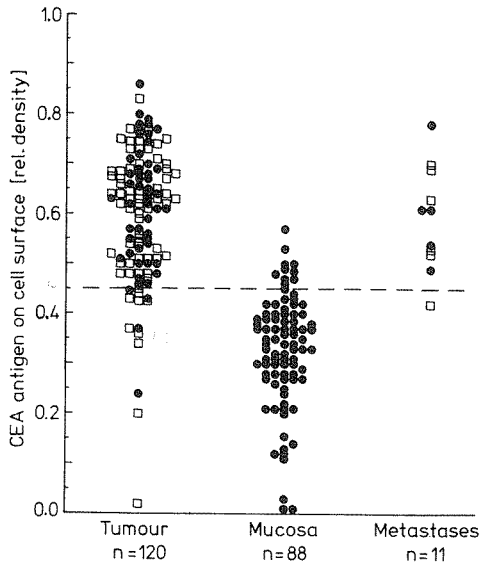

Fig. 5. Mean CEA density on colorectal tumour, mucosa and metastatic cells. The closed symbols represent euploid samples, the open symbols aneuploid tumours. With a fixed borderline at a relative density of 0.45, a fraction of 0.025 of the tumours are false negative and a fraction of 0.114 of the mucosa samples are false positive. For the metastases similar results as for the tumours are obtained.

antibody recognized the cells better than the monoclonal antibody. It is important to note that the recognition of the tumour cell-containing samples was substantially better if the antigen density on the antigen positive cells was compared instead of the number of antigen positive cells alone. Altogether the flow-cytometric method identified correctly 97.5% of the tumours and 88.6% of the mucosa samples in a fully automated way.

Discussion

The most important result of this study is that more than 95% of tumour samples were correctly recognized by an automated flow-cytometric evaluation method. To our knowledge it is the first time that this has been possible. Most flow-cytometric work has been done, so far, on the aneuploidy of tumours. The search for aneuploidy alone is, however, not sufficient for patient screening, since not all tumours are detectably aneuploid (11–14). 59% of the tumours in the present study were aneuploid. This result compares well with values between 50% and 78% given by other investigators (20–22). The measurement of additional cell parameters, such as light scatter and cellular protein (23, 24, 25) do not improve the results very much, because these parameters are not tumour-specific enough.

In the present approach, the first two parameters (cell volume and DNA) were used to identify aneuploid cells, to distinguish the morphologically intact cells from cell debris, and to separate the large epithelial cell compartment with the carcinoma cells (fig. 3a, b) from the small inflammatory cells (lymphocytes, granulocytes). The third parameter, the CEA-antigen of the morphologically intact cells, is important for the recognition of euploid tumour cells. CEA is expressed to a certain extent on normal mucosa cells, mainly on the brush borders, as revealed by light microscopic examination. Although not strictly tumour-specific, the fluorescence signals of the CEA-antibodies shift a significant number of tumour cells into an area of the three parameter cube (fig. 3a, b), where not many cells are located in cases of normal mucosa samples. The cell volume served for calculation of the mean antibody packing density on the cell surface. This is an important aspect of the volume measurement since the cell is used as a minicuvette to obtain standardized data. The validity of such calculations was shown earlier when the electrical charge density on the cell surface of erythrocytes could be correctly predicted by a flow-cytometric measurement (26). It is evident that the simultaneous three parameter measurement offers substantial advantages over one or two parameter measurement.

It can be argued that this method, although superior to other flow cytometric methods is still worse than the normal histo-pathological examination of colorectal tissue sections. This is true at the present time but there are possibilities of improvement. As in the case of the CEA and epithelial membrane antigen (EMA), which are independently expressed on cervix tumour cells (15), it is possible to test antibodies against other tumour associated antigens on colorectal tumour cells (27) for the detection of the remaining 2.5% false negative tumour samples. The automation of a diagnostic flow cytometric procedure is substantially easier than the automated recognition of cancer cells from histological or cytological specimens by computer image analysis. It seems, therefore, of particular interest to further pursue the flow cytometric approach.

There is the practical question of whether automated analytical flow cytometers with automated evaluation can be developed for routine clinical and research application. The operation of a flow cytometer at the present time is comparatively complicated and slow, and typically between 5 and 10 min are required to measure and change a cell sample. The purpose of recent microprocessor (1) and measuring-chamber (2) development was to increase the

speed (30 to 60 s/sample) and simplify the operation of flow cytometers. An important requirement for automation are monosized calibration particles of suitable size, which became available only recently (28). They can be used as internal standard for cell concentration but also for the continuous monitoring of the correct sample flow by the microprocessor data analysis system. The software was adapted for automated operation. The relevant parameters of each measurement are automatically stored in a data bank file which assures the long term stability of the measurements. It will be possible to use automated flow cytometers in the same way as as photometers, thus greatly extending the possibilities for cellular work. In addition to the identification of tumour cells it is, for example, of interest to count blood cells and to determine simultaneously their functional state (29), to measure cellular assays for cytostatic drug testing on patient tumour cells (30), to partially differentiate bone marrow aspirates (31) and to measure radiation damage in bone marrow and blood samples for biological radiation dosimetry (32). Fully microprocessor-controlled flow cytometers are comparatively cheap and will also be accessible to smaller hospitals and to specialized private practice.

Although the prospects for a broader application of flow-cytometry in diagnostic medicine are substantially increasing at the present time, it is obvious that one is still at the beginning of a development. Much further work is needed to elaborate a spectrum of diagnostic methods for cells, comparable to the large number of common clinical chemistry assays. Despite the improvements one has to see the limitations of the method. The tissue architecture has to be destroyed for the preparation of single cell suspensions. Flow cytometry can, therefore, not replace the histopathological examination. Nevertheless, the possibility of performing fast and quantitative biochemistry in single cells is of great interest for the characterization of the alterations of cell metabolism in disease.

References

1. Kachel, V., Schneider, H., Bauer, J. & Malin-Berdel, J. (1983) Cytometry *3*, 244–250.
2. Kachel, V., Glossner, E. & Schneider, H. (1982) Cytometry *3*, 202–212.
3. Haskill, S., Becker, S., Johnson, T., Marro, D., Nelson, K. & Propst, R. H. (1983) Cytometry *3*, 359–366.
4. Valet, G., Raffael, A., Moroder, L., Wünsch, E. & Ruhenstroth-Bauer, G. (1981) Naturwiss. *68*, 265–266.
5. Shapiro, H. M., Natale, P. J. & Kamentsky, L. A. (1979) Proc. Natl. Acad. Sci. USA *76*, 5728–5730.
6. Lamerz, R. & Burtin, P. (1983) Br. J. Cancer *47*, 823–832.
7. v. Kleist, S. & Hohneck, A. (1982) Internist *23*, 10–12.
8. Lamerz, R. & Fateh-Moghadam, A. (1975) Klin. Wochenschr. *53*, 193–203.
9. Rognum, T. O., Fausa, O. & Brandtzaeg, P. (1982) Scand. J. Gastroenterol. *17*, 341–348.
10. Rognum, T. O., Thorud, E., Elgjo, K., Brandtzaeg, P., Orjasaeter, H. & Nygaard, K. (1982) Br. J. Cancer *45*, 921–934.
11. Barlogie, B., Drewinko, B., Schumann, J., Göhde, W., Dosik, G., Latreille, J., Johnston, D. A. & Freireich, E. J. (1980) Am. J. Med. *69*, 195–203.
12. Barlogie, B., Raber, M. M., Schumann, J., Johnson, T. S., Drevinko, B., Swartzendruber, D. E., Göhde, W., Andreeff, M. & Freireich, E. J. (1983) Cancer Res. *43*, 3982–3997.
13. Klein, F. A., Herr, H. W., Sogani, P. C., Whitmore, W. F. & Melamed, M. M. (1982) Cancer *50*, 389–395.
14. Baisch, H., Otto, U., König, K., Klöppel, G., Köllermann, M. & Linden, W. A. (1982) Brit. J. Cancer *45*, 878–886.
15. Valet, G., Ormerod, M. G., Warnecke, H. H., Benker, G. & Ruhenstroth-Bauer, G. (1981) J. Canc. Res. Clin. Oncol. *102*, 177–184.
16. Dukes, C. E. (1932) J. Pathol. *35*, 323–332.
17. Morson, B. C. & Sobin, L. H. (1976) WHO Bull. Nr. 15, p. 1956, Geneva.
18. Kachel, V., Glossner, E., Kordwig, E. & Ruhenstroth-Bauer, G. (1977) J. Histochem. Cytochem. *25*, 804–812.
19. Valet, G. (1980) Flow Cytometry IV, Universitetsforlaget, Oslo, p. 125–129.
20. Frankfurt, O. S., Greco, W. R., Slocum, H. K., Pavelic, S. A., Arbuck, S. A., Pontes, E. J., Huben, R. P., Petrelli, N., Piver, S. M. & Rustum, Y. M. (1983) Clinical Cytometry II, Am. Engineering Found. Conferences, Sea Island, p. 61, Abstr.
21. Hiddemann, W., Wörmann, B., Bassewitz, D., Müller, K. M., Ritter, J., Roessner, A., Hauss, J. & Büchner, Th. (1983) Clinical Cytometry II, Am. Engineering Found. Conferences, Sea Island, p. 67, Abstr.
22. Böhm, N. & Sandritter, W. (1975) In: Current topic in pathology, Springer Verlag, Berlin pp. 151–217.
23. Barrett, D. L., Jensen, R. H., King, E. B., Dean, Ph. N. & Mayall, B. H. (1979) J. Histochem. Cytochem. *27*, 573–578.
24. Stöhr, M. & Goerttler, K. (1979) J. Histochem. Cytochem. *27*, 564–566.
25. Habbersett, M. C., Shapiro, M., Bunnag, B., Nishiya, I. & Herman, Ch. (1979) J. Histochem. Cytochem. *27*, 536–544.
26. Valet, G., Bamberger, S., Hofmann, H., Schindler, R. & Ruhenstroth-Bauer, G. (1979) J. Histochem. Cytochem. *27*, 342–349.
27. Herlyn, M., Steplewski, Z., Herlyn, D. & Koprowski, H. (1979) Proc. Natl. Acad. Sci. USA *76*, 1438–1442.
28. Ugelstad, J., Mork, P. C., Herder Kaggerud, K., Elingsen, T. & Berge, A. (1980) Adv. Coll. Interf. Sci. *13*, 101–140.
29. Valet, G. (1984) Blut *49*, 83–90.
30. Valet, G., Warnecke, H. H. & Kahle, H. (1984) Blut *49*, 37–43.
31. Valet, G. & Kahle, H. (1984) Blut submitted.
32. Valet, G. & Kahle, H. (1984) Biological Radiation Dosimetry, Konferenz Bundesgesundheitsamt, Schloß Reisenburg, Dez. 1983. BGA-Schriften, MMV Verlag, München, in press.

Prof. Dr. G. Valet
Max-Planck-Institut
für Biochemie
Am Klopferspitz
D-8033 Martinsried