

Automated methods in cytogenetic surveillance

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During the past 15 years, cytogenetic techniques — the analysis of chromosome aberrations, sister chromatid exchanges, and micronuclei — have been widely used in studies of human exposure to known or suspected carcinogens. Such studies have almost exclusively utilized peripheral lymphocytes because of the easy availability of blood samples and the well-established methods for lymphocyte culture. Structural chromosome aberrations have acquired an important position as a biological measure of exposure to ionizing radiation, and consistent increases in chromosome aberrations have also been found in connection with many chemical exposures. Sister chromatid exchanges and, especially, micronuclei have been utilized much less than chromosome aberrations, but there are several examples of the successful use of these parameters in human cytogenetic monitoring.

Although the cytogenetic methods are very useful tools in experimental studies, their application to human monitoring may sometimes be problematic. One important aspect is the fact that in human (occupational) exposures the expected levels of induced chromosome damage are often relatively low in comparison to positive results in experimental settings. Thus the demand for accuracy is greater in human studies because even small irregularities can have a significant effect on the outcome of the investigation. Therefore, the evaluation of cytogenetic monitoring studies occurs, in most cases, only between reference and exposed groups, individual data being disregarded. This situation is, of course, the state of the art for most biological monitoring methods used at present, but the assays should, eventually, be able to reveal individual exposure as well.

One way of obtaining a better resolution for the cytogenetic monitoring methods is to increase the number of subjects and cells analyzed. In occupational studies, the exposure groups are, however, often too small, and an increase in the number of cells analyzed would make the already tedious microscopic analysis still more time-consuming and expensive. Besides, a more extensive analysis would not remove the subjectivity inherent in any morphological scoring, also for cytogenetic techniques.

It seems that the only realistic way to improve the resolution of cytogenetic monitoring studies is automation. An automatic system would allow the analy-

sis of a high number of cells in a short time. If correctly tuned, such a system would also provide objective and accurate data and increase the sensitivity of the detection. At present, there are two basic approaches available for automation, ie, flow cytometry and image analysis. This paper deals with the possible use of these two techniques for the automation of cytogenetic monitoring. An experimental method for the flow cytometric measurement of micronuclei in the peripheral blood of mice is also described.

Chromosome aberrations and sister chromatid exchanges

Structural chromosome aberrations and sister chromatid exchanges are determined from dividing cells that have been arrested in metaphase by a spindle inhibitor. In principle, the analysis consists of the following three parts: (i) finding the metaphases, (ii) counting the chromosomes, and (iii) checking for chromosome aberrations or counting sister chromatid exchanges. The first step, metaphase finding, has attracted much attention in the automation of cytogenetics, and there are, at present, several excellent and completely automatic systems available for this purpose. Most of the automatic metaphase finders (such as Joyce Loebel Magiscan, Leitz Miamed, Kontron IBAS) use a television camera and operate relatively slowly, but can be left to inspect several microscopic slides during the night. The operator can then call the metaphases up whenever it is convenient. Another approach is provided by Cytoscan (Image Recognition Systems), which uses linear arrays featuring faster metaphase finding than the television-based systems (3). Metaphase finders can accommodate a karyotyping function which enables the user to count the chromosomes and to derive a karyogram from the chromosomes of a metaphase. The automatic chromosome counts may also, in principle, be utilized in the analysis of structural chromosome aberrations. The karyotyping function, which is interactive, has proved to be very useful in clinical cytogenetics in the analysis of metaphases with banded chromosomes, but it may additionally provide some help in the effort to resolve the nature of some complex chromosome changes in the uniformly stained metaphases used for the analysis of induced chromosome aberrations. In general, the karyotyping programs cannot, however, routinely be

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used for the scoring of structural chromosome aberrations.

The automatic scoring of one form of structural chromosome aberration, the dicentric chromosome, is under development — by image analysis — in two laboratories (3, 4), and a completely automatic system may be available within the next few years. One of the approaches (4) employs a custommade system, and the other (3) the commercially available Cytoscan. These lines of research are very promising with regard to the biological dosimetry of radiation exposure, as dicentrics are a major form of chromosome aberration induced in peripheral lymphocytes by ionizing radiation. On the other hand, the automated analysis of dicentrics will not contribute very much to studies of chemical mutagens, because most chemical clastogens tend mainly to produce chromatid-type aberrations. A fully automated analysis of aberrations other than dicentrics will be difficult, and researchers may have to be satisfied with interactive systems. Techniques on the flow cytometric detection of dicentrics in a solution of metaphase chromosomes are also under development and will have applications similar to the methods based on image analysis.

A few systems already exist for the automated counting of sister chromatid exchanges in an image analyzer, but, as they are clearly slower than human scoring and make a lot of mistakes, they do not yet have any practical significance. Automated scoring may not, however, be as difficult for sister chromatid exchanges as for structural aberrations if the speed and accuracy can be improved.

Micronuclei

Micronuclei are formed in dividing human lymphocytes *in vitro* from preexisting chromosome lesions. As a consequence, the identification of the right cell population — in this case those interphase nuclei that have divided once in the culture — is crucial for the assay, because micronucleus frequency will be diluted if cells that have not divided *in vitro* or have divided several times are included.

This problem can be solved by the use of cytochalasin B, which prevents cytokinesis and produces cells where only the nucleus has divided (1). Thus cells that have divided once *in vitro* can be identified by the presence of two nuclei.

The image of a binucleated cell is relatively simple to identify, and efforts have been started to automate the micronucleus assay. In a recent preliminary report, Fenech *et al* (1), however, concluded that the cytokinesis-blocked cells could not be automatically recognized with the computerized image analysis system used due to difficulties in identifying cytoplasmic boundaries. Another method, based on *in vitro* bromodeoxyuridine incorporation and staining with a fluorescent dye specific for deoxyribonucleic acid (DNA) and

Feulgen, proved to be very promising for future automation. The nuclei, also micronuclei, of G1-stage lymphocytes in their second *in vitro* cell cycle were recognized by computerized image analysis through their integrated optical density value, which was half of that for quiescent lymphocytes (1). An automated system for the detection of such nuclei should not be too difficult to build, even as a modification of the commercially available metaphase finders.

Flow cytometric analysis of micronuclei in blood samples from mice

An ideal way of monitoring human exposure would be based on a simple analysis of blood samples, without cell culture or microscopy. One possible choice could be the flow cytometric scoring of micronuclei in blood erythrocytes.

Such a method has been developed for repeated samples of mouse blood (2, 5). Only 20 μ l of blood — enough for several flow cytometric analyses — is normally collected at a time from the tail vein. Erythrocytes are spherized by sodium dodecyl sulfate and fixed with glutaraldehyde, and the samples can be stored for later analysis. Before the measurement, the samples are treated with ribonuclease to get rid of the nonspecific staining of reticulocytes. Each sample is analyzed in the flow cytometer with and without 4,6-diamidino-2-phenylindole (DAPI), a DNA-specific fluorescent dye inducing DNA to emit blue fluorescence after excitation by an ultraviolet laser. The rate of measurement is about 1 000 cells/s, and 50 000 cells are normally scored for a sample both with and without DAPI. The frequency of micronuclei is calculated by a computer program based on the comparison of the unstained and stained samples. According to experiments with such known or suspected genotoxins as benzene, 6-mercaptopurine, mitomycin C, benzo(a)pyrene, ethylnitrosourea, potassium chromate, and bromodichloromethane, the automated scoring is able to recognize micronucleated cells and yields results compatible with manual microscopic scoring. Furthermore, the results suggest that the assay has a better resolution than the conventional manual analysis.

As human spleen is very active in removing micronucleated erythrocytes from blood circulation, the analysis of micronuclei in human blood erythrocytes is not, however, expected to become an important method for human cytogenetic monitoring.

Concluding remarks

The automation of cytogenetic surveillance is, at the moment, in a very interesting phase, as automated metaphase finding is commercially available and completely automated assays for dicentric chromosomes and lymphocyte micronuclei are within reach. How-

ever, for a general adoption of the automated methods, the systems, which are at present very expensive, will have to become available at considerably lower prices.

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