

Problems and Prospects in the Utilization of Cytogenetics to Estimate Exposure at Toxic Chemical Waste Dumps

by Sheldon Wolff*

Introduction

In mammalian cells, one of the most sensitive indicators of exposure to mutagenic carcinogens has been the induction of cytogenetic effects, chromosome aberrations and sister chromatid exchanges (SCEs). Some mutagens, such as ionizing radiations that induce double-strand breaks in DNA are very efficient at inducing chromosome aberrations and can induce them at all stages of the cell cycle. Some other physical mutagens, such as ultraviolet light, cannot induce breaks directly, but form lesions in DNA that lead to the production of chromatid aberrations later when the cells proceed to the S phase. Although a few chemical mutagens can induce double-strand breaks in DNA and thus are radiomimetic or X-raylike, the majority are like ultraviolet light in that they form lesions that are not converted to aberrations until the cells enter S. Unlike X-rays and the few radiomimetic chemicals, these S-dependent agents, be they physical or chemical, are able to induce SCEs very efficiently. In fact, cells exposed to these agents often show large increases in SCEs at doses as low as 1/100 that required to show increases in chromosome aberrations.

Chromosome Aberrations

When cells containing chromosome aberrations divide, the daughter cells often are genetically unbalanced and eventually die. Until cell division occurs, however, the aberrant cell contains its full

genetic complement and can survive. This fact, coupled with the fact that the relation of the induction of aberrations to the dose of radiation is well understood, has made it possible to use the long-lived peripheral lymphocyte, which ordinarily does not divide, as a sensitive integrating biological dosimeter capable of making quantitative estimates of the absorbed dose in man. Because of the success with ionizing radiation, attempts have also been made to use the induction of cytogenetic effects to estimate the exposure to chemical agents. Here, however, we are on more tenuous grounds because most of the agents, being S-dependent, do not produce their effects immediately. During the interval between exposure and the formation of the cytogenetic effects, many factors can influence the final yield. Chief among these is the repair capacity of the cell, for only if the lesions remain in the DNA until the cells enter S will they retain their ability to lead to the types of errors of replication that produce chromatid aberrations or even SCEs.

If the exposure is to the unknown material that might be found in a chemical waste disposal site, many other problems exist. Now questions arise about what chemicals are in the mixture and in what proportions, whether the chemicals need metabolic activation, whether they act synergistically, additively or antagonistically, and whether they might be promoterlike or antipromoterlike. Furthermore, unlike the situation with ionizing radiation, in which the dosimetry is relatively straightforward, the dosimetric problems with chemicals are formidable, for neither their intracellular concentrations nor their biological half-lives are known with any certainty, and in cases of human exposure, questions also arise about the diffusibility of the chemicals and their transport to the cells in question.

*Laboratory of Radiobiology and Environmental Health and Department of Anatomy, University of California, San Francisco, San Francisco, CA 94143.

Difficulty in Assessment

In making cytogenetic surveys of populations exposed at chemical waste disposal sites, we are confronted with the problem of obtaining statistically significant results after very low-level exposures, and then of determining the meaning of such results in regard to health effects in the population as a whole and in the individuals in which the observations were made. We face great difficulties on both counts. In the first place, even with ionizing radiation, the low numbers of aberrations induced at very low doses make it very difficult to obtain significant increases. When the data are considered as a whole in those cases where populations have been surveyed, it has been found that more aberrations are seen as the dose increases. Nevertheless, for statistical reasons, great variability has been found, both between individuals who have been exposed to approximately the same doses, and in repeated samples from the same person. Thus, cytogenetic studies of chromosome aberrations can tell us whether or not a population has been exposed, but it is exceedingly difficult to make an estimation of the dose received by any single individual. Even if we did know the exact yield of chromosome aberrations after low doses from low dose rate exposures, geneticists still could not tell what the biological consequences would be, because most of the aberrations, but not SCEs, are unstable and lead to the death of the cell in which they are found. As a group, however, the exposed cells will have other nonscorable chromosomal changes as well as point mutations, so that the presence of aberrations can signal a potential problem in the population; but quantifying the abnormalities in the surviving, seemingly cytogenetically normal, cells is extremely difficult.

A commonly quoted example of this can be found in the studies carried out on the Japanese populations that survived the atomic bombings of Hiroshima and Nagasaki. These studies have shown that as the dose increased, both the number of chromosome aberrations observed in peripheral lymphocytes and the incidence of cancer increased in the population. Those individuals with the most aberrations, however, were not necessarily those who developed the cancers. Thus, aberrations are an indicator of exposure in the population and, as such, a measure of what we might expect to find, but they cannot be used to predict what will happen to a given individual.

Sister Chromatid Exchange

Although SCEs are easier to score than ordinary chromosome aberrations and are also a more sensitive indicator of damage, their relation to ill health is even more tenuous than is that of chromosome aberrations. For instance, cells with large numbers of SCEs, such as those found in the peripheral lymphocytes of patients with Bloom's syndrome, seem to be no less viable than cells containing fewer SCEs. Nevertheless, because mutagenic carcinogens that produce adducts in DNA also induce SCEs, circumstantial evidence indicates that chemically induced SCEs are a measure of genotoxicity. In fact, it has been found with several chemicals that both SCEs and mutations increase linearly with dose. The ratio of the two, however, changes for each chemical, indicating that chemicals can form lesions that produce SCEs as well as lesions that produce mutations. The relative number of each type, however, varies from chemical to chemical.

Because of its high sensitivity and ease of scoring, the SCE test has provided an extremely valuable method for determining whether or not a compound is potentially genotoxic. These same characteristics have made it attractive to think of using the induction of SCEs in cultured lymphocytes to monitor populations exposed to low chronic doses of hazardous compounds; but several sources of variability make monitoring a population by this method somewhat problematical. For instance, great variability has been found in the baseline SCE frequencies in different people. This variability in presumably unexposed people is far greater than that found for chromosome aberrations, where the yield stays close to 10^{-4} per cell. Some of this variability in SCE frequencies can be attributed to differences in the cells' incorporation of bromodeoxyuridine needed to make sister chromatids stain differentially, some to differences in the sera of individuals, and some to differences in the cells' repair capacity, but these do not account for all of the variability, as well as other areas of uncertainty, before the SCE test can be used for population monitoring with any high degree of confidence.

The numbers of SCEs found after exposure to very large doses of chemicals are not obscured by the variability. Therefore, much research is still needed to sort out this variability, as can be seen in the increased SCE frequencies found in lymphocytes of cancer patients shortly after treatment

with high acute doses of some cytostatic agents. In this instance, the response is somewhat like that with relatively high doses of ionizing radiation (in the order of 10 rads). However, unlike radiation-induced chromosome aberrations, which are permanently formed shortly after exposure, SCEs are not formed in the G_0 lymphocyte, so that there is time for the damage to be repaired before the cell is stimulated to enter S. This leads to a decrease in SCE frequency with time after exposure, which adds another variable in the use of SCEs to monitor populations.

With some chemotherapeutic chemicals, however, it has been found, both in animals and humans, that after multiple exposures the lesions seem to last longer. As yet, it is not known if this persistence will occur after chronic exposures, nor is it known which classes of chemicals might be expected to produce long-lived lesions. Some chemicals, such as cyclophosphamide, can lead to lesions that last through several cell cycles, whereas others, such as *N*-acetoxyacetylaminofluorene, do not. Thus some potentially dangerous compounds might escape detection by this system.

In several attempts to monitor people exposed to suspected toxic agents, very few compounds have been found to elevate the mean number of SCEs above that found in the reference population. Thus, only laboratory workers carrying out hormone analyses and organic chemical research, nurses handling cytostatic compounds, and hospital workers exposed to ethylene oxide have had increased numbers of SCEs in their peripheral lymphocytes. In one case the increase was found only when a comparison was made between exposed nonsmoking individuals and the nonsmoking control, for smoking itself induced an increase in SCEs. The nonsmoking exposed people, smoking exposed people and smoking nonexposed control people did not differ in their SCE yields. Furthermore, there is a considerable overlap in the SCE frequencies found in populations of smokers and nonsmokers, making it exceedingly difficult to make an estimate of exposure in a given person.

Conclusion

The cytogenetic analysis of chromosome aberrations in peripheral lymphocytes of irradiated people

has proved to be a reliable method for determining the amount of exposure because the aberrations are formed in the long-lived lymphocyte while it is still in G_0 . This cell acts as an integrating dosimeter, which allows its use to estimate low doses of chronic radiation. This system should also work well for the limited class of chemical clastogens that are not S-dependent, that is, cause double-strand breaks in DNA and thus produce aberrations at all stages of the cell cycle. For the majority of chemical mutagens, which are S-dependent, however, the utility of the system is far from certain because the DNA repair capacity of the cell can remove most of the adducts before the cells are stimulated to enter S.

Sister chromatid exchanges, which are much easier to score than chromosome aberrations, have provided our most sensitive cytogenetic laboratory test for S-dependent chemicals. They are also an efficacious way to determine exposure of people to high doses of these chemicals, but they are insensitive to S-independent agents such as ionizing radiations. Because of great interpersonal variability of response, variability in different reference populations, and the repair phenomenon just mentioned, it has not yet been proved that SCEs can be used to monitor populations for low-level chronic exposure. Much research and many surveys are needed before we will know whether or not the system will live up to our initial expectations.

It should also be noted that even when cytogenetic tests on peripheral lymphocytes show that the genetic material has been damaged, these tests can only be used to estimate risk in the population. They cannot be used to predict that any given individual will suffer any particular form of ill health.

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REFERENCES

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