

New Translocations in Human Lymphocytes: A Mutagen Monitoring System

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The human lymphocyte is a premier cell for monitoring chromosome aneuploidy. The lymphocyte is easily obtained, can be studied before and after culture, and has been extensively investigated.

Assays available for lymphocytes include the scoring of chromosome breaks (subjective and laborious), the analysis of chromosome abnormalities such as increase or decrease in number (versus normal background), dicentricity etc., and the micronucleus test (presumable end-state phenomena). We propose the monitoring of somatic chromosome translocations in human lymphocytes. Background data available from North America indicate that the frequency of *de novo* chromosome translocations in Halifax, Portland, Denver, and Atlanta is about 1.7×10^{-3} . The most common translocation arising in lymphocytes is between chromosomes 7 and 14 (with a frequency of 4×10^{-4}). All translocations occurring *de novo* in human lymphocytes tend to appear balanced with no evidence for loss or gain of chromosome material.

Cytogenetic laboratories are processing lymphocytes daily. The resultant photographs and karyotypes are all scorable for *de novo* translocations. Suitable data on exposure to possible mutagenic agents could be collected in advance of these chromosome studies. This would provide a new method for monitoring chromosome changes in the population.

The cost of monitoring lymphocyte chromosomes for somatic translocations would be small, since numerous laboratories study lymphocytes routinely for clinical diagnostic purposes.

There may be merit in availing ourselves of easily available data from a very available species: man.

A major concern of human genetics is to establish and then monitor mutation rates. There are a number of ways the establishment and monitoring of human mutation rates can be approached.

We will illustrate two general models first in relation to meiotic chromosome mutations. Let us take, for example, Down's syndrome, the commonest autosomal chromosome abnormality in newborn babies. (incidence at birth $\cong 650$).

Types of Studies

Prospective Model

We plan to gather data on the incidence of Down's

syndrome at birth. We may select a population sample and then gather prospective data pertaining to parental age, x-ray exposure, drug and chemical experience in that population (before or during pregnancy). Next we screen all babies born to this population for Down's syndrome. Then we calculate the incidence to see if it is in the expected range.

Retrospective Model

We take advantage of data accumulating in cytogenetic laboratories. We will probably not have complete sampling of the population at risk. We will, however, have data from already finished diagnostic cytogenetic studies. Further we will have several different categories of Down's babies: *A* = number of trisomy 21 babies; *B* = number of babies with *de novo* translocations; *C* = number of babies with inherited translocations; *D* = number of babies with trisomy 21/normal mosaicism. From past experience

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we might anticipate that 0.95 of the total will be *A* (trisomy 21), 0.02 of the total will be *B* (*de novo* translocations), 0.02 of the total will be *C* (inherited translocations), and 0.01 of the total will be *D* (mosaics).

We look at the real numbers in the four categories. We set up various ratios such as $B/(A + B)$. The expected ratio of $B/(A + B)$ would be 0.02. If the observed ratio during a period of time rose to 0.03, we would suspect an increase in *de novo* chromosome translocations. Since the altered ratio might equally well reflect a relative decrease in trisomy 21 (nondisjunction), we might then examine the ratio B/C , which would be expected *a priori* to be 1.0. If B/C were observed to be 1.5, this would confirm an increase in *de novo* translocations.

Prospective Versus Retrospective Models

The prospective model is to design research, carry it out and analyze the results. It is neat and clean. It is also time consuming and expensive.

The retrospective model is to take what data are available and look at them. This is less tidy and less elegant, but it is quick and costs little.

Somatic Translocations in Lymphocytes

We here propose the use of retrospective data from diagnostic cytogenetic studies of lymphocytes to establish and monitor the rate of somatic chromosome translocations.

Pilot Study in Denver

We studied lymphocytes cultured in 1974-75 from 300 consecutive individuals (1). Twenty metaphases from each individual were studied for a total of 6,000 metaphases.

The individuals were patients and their relatives seen for genetic counseling in Denver. The diseases for which they sought counseling spanned much of the range of clinical genetics. No individual was known to have had unusual x-irradiation or chemical exposure.

The lymphocytes were cultured with phytohemagglutinin for 72 hr and harvested with colchicine. Cells were fixed routinely onto slides. The slides were Giemsa-banded, scanned under low power microscopy for intact, well-spread metaphases, which were then photographed under higher power. Two or more karyotypes were prepared from each individual.

Four of the 6000 metaphases we studied had a chromosome rearrangement. Each of the four

translocations was found in a solitary cell from a different unrelated individual. All four of the rearrangements involved the same two chromosomes: 7 and 14. The breakpoints in 14 were in band *q*12 in all 4 cells. The breakpoints in 7 were in band *q*13 (1 cell) or *q*33-35 (3 cells). All four translocations appeared "balanced" with no evidence for loss or gain of chromosome material.

Pilot Studies in Halifax and Atlanta

Studies similar to ours were carried out concurrently in Halifax (2) and Atlanta (3). The results are presented, together with those from Denver, in Table 1.

Somatic Translocation Rates in Pooled 3-Center Sample

Rates of occurrence of somatic translocations in the three pilot studies can be calculated. The rates may be of value, since all data from the three centers were obtained in 1974-75 employing similar protocols.

The frequency of 7/14 translocations in the three-center sample was $12/1402 = 1$ per 117 individuals.

The frequency of other (non-7/14) translocations in the three-center sample was $43/1,402 = 1$ per 33 individuals.

The frequency of all *de novo* translocations can also be calculated from Table 1. Per person, there were 55 in 1402 or 1 in 25 to 1 in 26 individuals.

Somatic Translocation Rates in Halifax and Atlanta

As will be discussed below, Denver's cumulative experience with somatic translocations in cultured lymphocytes is now available. Excluding Denver, there were eight translocations involving chromosomes 7 and 14 in 1102 persons or 1 per 138 individuals.

Denver Cumulative Experience

Since chromosome banding was instituted in the Clinical Cytogenetics Laboratory at University of Colorado Medical Center and National Jewish Hospital, approximately 40,000 cells from 2500 persons have been studied and 17 cells with a 7/14 translocation, each from a different person, have been observed.

The rate of occurrence therefore was $17/2500 = 1$ per 147 persons. Cumulative data are not yet available from Denver on other translocations (other than those of the 7/14 type).

Table 1. *De novo* translocations in human translocations.

Center	No. of individuals	Lymphocytes		Translocations		
		Per individual	Total	7/14	Other	All
Denver	300	20	6,000	4	0	4
Halifax	250	20	5,000	3	6	9
Atlanta	852	25	21,300	5	37	42
Totals	1,402	20-25	32,300	12	43	55

Table 2. Rates of occurrence of 7/14 translocations in cultured lymphocytes.

	7/14 Translocations	No. of individuals	Translocations per person
Halifax and Atlanta	8	1,102	1/138
Denver	17	2,500	1/147
All centers	25	3,602	1/144

Discussion

The human lymphocyte is a suitable cell for monitoring mutagenesis. Several features make the lymphocyte favorable: the lymphocyte is easily obtained; the lymphocyte is in suspension as a single cell; the lymphocyte can be studied at various times—immediately after being obtained, after short-term culture, or after longer periods in culture; Chromosomes in lymphocytes can be conveniently studied; the human lymphocyte is a human cell, so extrapolation from another species to Man is not needed.

In view of these and other favorable features, we have proposed the monitoring of somatic translocations in cultured human lymphocytes as a way of detecting environmental mutagenesis.

The 7/14 translocations, which predominate in lymphocytes (1-2), warrant comment. We have observed clones of lymphocytes with translocations in patients with ataxia-telangiectasia (3), an autosomal recessive disorder predisposing to lymphoreticular malignancy. The clones in ataxia-telangiectasia often are marked by 7/14 translocations indistinguishable from those reported here in isolated cells from individuals who do not have A-T. Possible explanations for this include (a) coincidence, (b) non-random production of or selection for 7/14 translocations (1-3), and (c) expression of heterozygosity for ataxia-telangiectasia. We have speculated also about the possible malignant potential of 7/14 translocation lymphocytes (4).

Since the rates of 7/14 translocations in the studies reported here were relatively constant, any increase

in rate would suggest increased exposure to mutagens (or carcinogens).

Other cytogenetic assays are, of course, available and may also be of value with lymphocytes, including: scoring of chromosome breaks (subjective and laborious), scoring deviations in chromosome number (large numbers of cells needed), and the micronucleus assay (the micronucleus presumably betokens an end-stage cell with a fatal chromosome aberration).

Proposal

Cytogenetic laboratories are processing lymphocytes daily. The resultant photographs and karyotypes are scored for *de novo* translocations. A translocation in a lymphocyte can be detected soon after its occurrence. The cost of monitoring lymphocyte chromosomes for translocations is small. Numerous laboratories study lymphocytes routinely for clinical diagnostic purposes. (The average laboratory studies 400-600 individuals per year).

The collection of data from 100 laboratories would thus yield data on about 50,000 individuals per year. One would expect in the neighborhood of about 347 persons in 50,000 (1 per 144) to have a cell with a 7/14 translocation. If, for example, 387 persons were noted to have a 7/14 translocation cell, this would be a statistically significant increase ($p < 0.05$) in the rate of occurrence and should lead to concern about mutagen exposure.

Summary

We propose that human lymphocytes be monitored for *de novo* translocations, especially of the 7/14 type, to help detect environmental mutagens.

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