Increased Mutation Frequency following Treatment with Cancer Chemotherapy

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ABSTRACT

The relationship between somatic mutation and cancer was studied by measuring in vivo mutation frequency and in vitro mutability using lymphocytes from 28 untreated adult patients with solid tumors, 14 untreated patients with lymphoma, and 27 untreated patients with solid tumors or lymphoma who had been treated with chemotherapy and/or radiotherapy. In vivo mutation frequency in untreated patients did not differ from that of controls, except perhaps in patients with lymphoma, who showed a slight increase. Lymphocytes from untreated patients with solid tumors or lymphoma did not show a greater increase in mutations induced after X-radiation or UV radiation than did lymphocytes from controls. For all the untreated patients, the geometric mean mutation frequency was $6.72 \times 10^{-6}$, and it was significantly increased to $19.57 \times 10^{-6}$ following chemotherapy and radiotherapy.

The results suggest that excessive systemic exposure to mutagens or inherent susceptibility to mutagenesis are not important etiological factors in at least the majority of patients with cancer. The mutations produced by treatment may be related to the late side effects of therapy such as second neoplasms.

INTRODUCTION

Mutations and mutagenesis may be related to the etiology and treatment of cancer in several ways. Individuals developing cancer may do so as the result of excessive exposure to agents which are mutagens. Also, the existence of disorders of DNA repair such as ataxia telangiectasia and Bloom's syndrome, which are also associated with cancer, raises the possibility that cancer in some individuals may arise as a result of inherited predisposition to mutagenesis consequent to altered mutagen metabolism or impaired DNA repair. Furthermore, many of the agents used in cancer treatment are mutagenic and carcinogenic, and mutagenesis due to these agents may be related to late complications such as the development of second neoplasms and production of genotoxicity.

To investigate these possibilities, we used a newly developed technique (1, 16) for measurement of somatic mutations in lymphocytes at the HPRT locus. The technique is based on enumeration of lymphocyte clones which are resistant to the purine analogue, 6-TG, and the evidence that such clones are mutants is based on their inability to incorporate hypoxanthine, their lack of the gene product HPRT, and the presence in their DNA of structural gene changes at the HPRT locus (1, 16, 20).

Measurement of the base-line frequency of somatic mutation was used as an index both of exposure to mutagens and of the presence of metabolic pathways which result in an excessive number of mutations. Lymphocytes from untreated patients with cancer and lymphoma were exposed to the mutagenic agents X-radiation and UV radiation, and the number of induced mutations was measured as an index of inherent susceptibility to mutagenesis by these agents. The number of mutations present in a group of treated patients was measured as an index of the mutagenic effect of therapy.

MATERIALS AND METHODS

Patients Studied. Mutations were enumerated in 69 adult patients. Each study included a healthy control individual matched for age and sex although, since any one study often involved more than 1 patient, the total numbers in the control and patient groups did not prove to be equal. As shown in Table 1, the untreated-patient group comprised 28 patients with solid tumors and 14 with lymphoma (including myeloma). Studies of in vitro mutagenesis involved study of 7 to 10 untreated patients with carcinoma, 4 to 6 untreated patients with lymphoma, and corresponding numbers of control individuals. Also studied were 9 patients with solid tumors and 18 patients with lymphoma who had been treated with chemotherapy and/or radiotherapy at times in the past ranging from 2 weeks to 12 months. A wide variety of cytotoxic drugs had been used in the patients treated with chemotherapy.

Mutation Assay. Full details of the assay have been given previously (15, 16), but in brief, lymphocytes were separated from peripheral blood using Ficoll-Hypaque and cloned in microwells at limiting dilution in the presence of phytohemagglutinin, irradiated feeder cells, conditioned medium as a source of interleukin 2, and in the presence or absence of 6-TG, $2.5 \mu g/ml$. Cultures were incubated for 18 to 20 days, wells were scored visually for growth using an inverted microscope, cloning efficiencies were calculated using Poisson statistics, and the frequency of mutant cells was calculated as the ratio of the cloning efficiencies with and without 6-TG.

In Vitro Mutagenesis. Susceptibility to a defined exposure of mutagen was measured by exposing freshly isolated lymphocytes to X-irradiation or UV irradiation, culturing for 10 days to allow expression of induced mutations, and finally enumerating the number of induced mutations. X-irradiation was performed using a Philips RT100 machine with conditions of 100 kV, 6 ma with 1.7 mm aluminum filtration, and at a dose rate of 350 rads/min. The radiation dose rate was determined with a 37D X-ray dosimetry (Pitman Industries, United Kingdom). UV radiation with a peak of 254 nm was obtained from a UV germicidal lamp (Ollphant Industries, Adelaide, Australia), and the dose rate was measured with a photometer (International Light IL500 Dextra Industrial Green). For UV irradiation, the separated lymphocytes were washed twice in colorless Eagle's medium, suspended in a volume of 0.5 ml and at a concentration of 1 to 2 x $10^6$ cells/ml, and irradiated with varying doses by varying the time of exposure to UV light. The absorption of UV light by Eagle's medium was 0.2% of the total dose.

After irradiation, the cells were stimulated with phytohemagglutinin and cultured for 10 days in McCoy's medium. Fresh medium containing
10% conditioned medium as a source of interleukin 2 was added every 3 to 4 days.

**Statistics.** Tests for differences between groups were performed on logarithmically transformed data using the one-tailed Wilcoxon rank sum test (4) and the Student-Newman-Keuls test in the analysis of variance program of the statistical package for the social sciences (17).

**RESULTS**

For the studies of solid tumors, the mean age of 23 controls was 50 ± 3 (SE) years, of 28 patients, 59 ± 3 years, and of 9 treated patients, 45 ± 6 years. For the studies of lymphoma, the mean age of 22 controls was 43 ± 3 years, of 14 untreated patients, 52 ± 4 years, and of 18 treated patients, 55 ± 3 years. The geometric mean cloning efficiencies (and range, shown as ±1 SE) for lymphoma controls, untreated lymphoma patients, treated lymphoma patients, solid tumor controls, untreated solid tumor patients, and treated solid tumor patients were 33.3 (40.0 to 27.7), 14.6 (18.8 to 11.4), 6.5 (8.4 to 5.0), 26.5 (31.2 to 22.5), 20.9 (25.2 to 17.4), and 5.1 (7.2 to 3.5) %, respectively. The results for mutation frequencies are seen in Charts 1 and 2. The geometric mean mutation frequency (and range, shown as ±1 SE) for the solid tumor controls was 6.9 (8.2 to 5.8) × 10⁻⁶, for the untreated solid tumor patients, 6.3 (7.4 to 5.3) × 10⁻⁶, and for the treated solid tumor patients, 25.7 (42.0 to 15.8) × 10⁻⁶. The effect of treatment was highly significant (P < 0.005 by the Wilcoxon test). The geometric mean mutation frequency for the lymphoma controls was 5.3 (6.0 to 4.7) × 10⁻⁶, for the untreated lymphoma patients, 7.8 (9.5 to 6.4) × 10⁻⁶, and for the treated lymphoma patients, 24.3 (31.8 to 18.6) × 10⁻⁶. The difference between lymphoma patients and controls did not reach statistical significance (0.1 > P > 0.05), but the difference between treated and untreated patients was highly significant (P < 0.005).

In Table 2 are summarized the results and conclusions from the analysis of variance for untreated patients, for patients treated with chemotherapy alone, and for patients treated with chemotherapy and radiotherapy. Only one patient, who had a normal result, was treated with radiotherapy alone and since there was only one observation, the effect of radiotherapy alone was not analyzed. The results of the analysis showed that both chemotherapy and chemotherapy plus radiotherapy produced a significant increase in mutation frequency over that observed in

<table>
<thead>
<tr>
<th>Types of cancer which were studied</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinoma</td>
<td>5</td>
</tr>
<tr>
<td>Breast</td>
<td>2</td>
</tr>
<tr>
<td>Esophagus</td>
<td>1</td>
</tr>
<tr>
<td>Stomach</td>
<td>1</td>
</tr>
<tr>
<td>Colon and rectum</td>
<td>3</td>
</tr>
<tr>
<td>Lung</td>
<td>4</td>
</tr>
<tr>
<td>Ovary</td>
<td>1</td>
</tr>
<tr>
<td>Unknown primary</td>
<td>3</td>
</tr>
<tr>
<td>Testis</td>
<td>1</td>
</tr>
<tr>
<td>Choriocarcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Squamous cell</td>
<td>2</td>
</tr>
<tr>
<td>Bladder</td>
<td>1</td>
</tr>
<tr>
<td>Melanoma</td>
<td>1</td>
</tr>
<tr>
<td>Uterine sarcoma</td>
<td>2</td>
</tr>
<tr>
<td>Carcinoid</td>
<td>1</td>
</tr>
<tr>
<td>Non-Hodgkin's lymphoma</td>
<td>11</td>
</tr>
<tr>
<td>Hodgkin's lymphoma</td>
<td>2</td>
</tr>
<tr>
<td>Myeloma</td>
<td>1</td>
</tr>
</tbody>
</table>

**Chart 1.** In vivo mutation frequency in patients with solid tumors as measured by the clonal technique. O, chemotherapy; • chemotherapy and radiotherapy.

**Chart 2.** In vivo mutation frequency in patients with lymphoma as measured by the clonal technique. O, chemotherapy; • chemotherapy and radiotherapy; H, radiotherapy.
MUTATIONS AND CHEMOTHERAPY

Table 2
Comparison of in vivo mutation frequency in untreated and treated patients with cancer

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of patients</th>
<th>Mean $\times 10^{-6}$</th>
<th>95% confidence limits for mean $\times 10^{-6}$</th>
<th>Comparison</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>42</td>
<td>6.72</td>
<td>5.01–9.02</td>
<td>Vs. untreated</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>11</td>
<td>19.57</td>
<td>8.02–47.74</td>
<td>Vs. untreated</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Chemotherapy + radiotherapy</td>
<td>15</td>
<td>34.40</td>
<td>19.67–60.17</td>
<td>Vs. chemotherapy</td>
<td>Not significant</td>
</tr>
</tbody>
</table>

untreated patients. Although there was a suggestion that combined therapy resulted in a higher mutation frequency than with chemotherapy alone, the difference between the 2 forms of treatment did not attain statistical significance.

Charts 3 and 4 show the number of mutations expressed after various doses of X-radiation or UV radiation. Neither patients with solid tumors nor with lymphoma differed from controls in their susceptibility to these mutagenic agents.

DISCUSSION

Although carcinogenesis is not equivalent to and may not be due to mutagenesis, there is a close relationship between the 2 processes as evidenced by the fact that carcinogens and mutagens both appear to act through DNA damage and that there is a close correlation between carcinogenic and mutagenic potential (12). The recent recognition that chromosomal translocations or point mutations may result in activation of oncogenes (10) suggests a possible mechanism underlying the relationship. Genotoxicity is believed to be directly related to mutagenesis.

In the present study, most untreated patients with cancer were found to have a normal mutation frequency, although an increased mutation frequency may have been present in patients with lymphoma. The cells which clone and are measured in the mutation assay are all T-lymphocytes of various subclasses (8).

Only a small proportion of cases of non-Hodgkin’s lymphoma arise from T-lymphocytes and, although the cell of origin of Hodgkin’s lymphoma has not been identified, there is little evidence that it is the T-lymphocyte. If the finding of a slightly increased mutation frequency in lymphoma is a true one, then it would suggest that the T-lymphocyte is abnormal in a disease which does not usually appear to primarily involve this cell. The present study and our other cloning studies provide some evidence for this possibility, since the cloning efficiency of T-lymphocytes is below normal in lymphoma (9). One explanation for an abnormality of the T-lymphocyte would be that there had been excessive exposure of T-lymphocytes or their precursors to a mutagenic agent; another would be that the neoplastic line arose from a more primitive cell which gave rise to both T- and B-lymphocytes and which was genetically unstable. However, the in vitro mutation induction studies, which showed no difference between patients with lymphoma and controls, do not support this second explanation but rather suggest that at least most of the circulating T-lymphocytes in lymphoma patients have normal susceptibility to mutagenesis.

Untreated patients with cancer have been studied previously using an autoradiographic technique for measuring HPRT mutations, and somewhat variable results have been obtained. In unpublished studies, we found a normal mutation frequency in 10 adult patients and a moderately increased frequency in 3 of
7 pediatric patients, 1 with neuroblastoma and 2 with lymphoma. Lange and Prantner (11) also observed that mutation frequency was increased in a proportion of pediatric patients with cancer, and Strauss and Albertini (19) observed an increase in some adult patients. However, the autoradiographic technique is very affected by culture conditions and gives unreliable results unless careful attention is paid to technical factors such as stringency of selection (3, 14), and for these reasons we no longer use it.

The finding in the present study of a normal number of mutations in the lymphocytes of untreated patients with solid tumors suggests that these individuals had not been exposed to an excessive systemic load of environmental mutagens and, indirectly, that they were also not unduly susceptible to mutagenesis. The results do not exclude the possibility that specific tissues, such as the gut, might have been exposed to an excessive local concentration of mutagens and that this might have been related to the subsequent development of cancer in those tissues. Study of induced mutations following X-irradiation or UV irradiation of lymphocytes from patients with both solid tumors and lymphoma provided direct evidence against enhanced susceptibility to mutagenesis. To a varying extent, the pathways for repair of DNA damage following chemical mutagens may differ from those for repair of damage following X-irradiation or UV radiation (6, 18), and since chemical mutagenesis was not studied, we cannot exclude the possibility that patients with cancer may be unduly susceptible to mutagenesis following certain chemicals. Apart from this possibility, however, our results provide no evidence that inherited susceptibility to mutagenesis is a factor in the majority of patients who develop cancer.

It might also be argued that the mutation assay is not sufficiently sensitive or is too imprecise to detect either excessive exposure to mutagens or enhanced susceptibility to mutagenesis. For measurement of base-line levels, the statistical uncertainty of the method is relatively high, owing to the small number of mutant clones scored, and replicate studies from our laboratory have shown a coefficient of variation of 53%. However, the statistical uncertainty is much less when measuring induction of mutations by mutagens, since a larger number of clones may be scored. Despite the inherent variability of the method, it has been possible to detect an environmental effect on base-line mutation frequency in a group of nurses and pharmacists occupationally exposed to cytotoxic drugs (2). Owing to inability to obtain material, we have not been able to study lymphocytes from individuals with one of the defined disorders of DNA repair in order to determine the sensitivity of the method in detecting susceptibility to mutagenesis in these syndromes. It should be emphasized, however, that the aim of the present study was to study patients with "common" cancer and not to study cancer families or the rare defined syndromes.

The elevated mutation frequency observed in the present study in patients treated previously with chemotherapy or chemotherapy plus radiotherapy provides direct evidence for a mutagenic effect of cytotoxic drugs in vivo. The low cloning efficiency of lymphocytes derived from treated patients could probably have been an independent effect of DNA damage which produced both mutagenic lesions and potentially lethal lesions such as double-strand breaks and cross-links. Although the data show an increase in mutation frequency at the time of measurement, this increase may not give a precise indication of the number of mutations directly produced by the therapy. The chemotherapy-treated patients were a heterogeneous group treated with various drugs and studied at various intervals following cessation of treatment. The half-life in vivo of cells bearing HPRT mutations is not known, but the mutagenic effects of treatment could well have been underestimated if there had been in vivo selection against such cells. Differing proliferation of nonmutated or mutated cells during the interval between production and measurement of mutations could also lead to a difference between the proportion of cells bearing mutations at the time of therapy and the proportion of cells bearing mutations at the time of measurement.

It is not clear from our data whether radiotherapy alone or interacting with chemotherapy is mutagenic. Certainly, there is abundant other evidence that radiation is mutagenic and the in vitro radiation of lymphocytes in the present study provided further evidence. The mean mutation frequency in patients treated with both radiation and chemotherapy was higher than that in patients treated only with chemotherapy, but the difference was not statistically significant. If there were to be a real difference, it could be due to an independent effect of radiation and/or to some type of interaction between radiation and chemotherapy. However, in unpublished studies of lymphocytes irradiated in vitro, we have found no evidence that irradiation of lymphocytes from patients treated previously with chemotherapy results in more mutations than irradiation of lymphocytes from untreated patients.

Chemotherapy and radiotherapy are known to be associated with several late effects which might conceivably be the consequence of or associated with mutagenesis. There is good evidence that both agents are carcinogenic in patients with both lymphoma and solid tumors (reviewed in Ref. 5), and an increased frequency of abortions and fetal abnormalities in patients with cancer treated previously with cytotoxic drugs and radiotherapy has been reported in one study (7) although not in others. Residual marrow damage may result in persistent cytopenias or even late marrow failure in individuals treated with cytotoxic drugs, and it has been suggested that this phenomenon is the result of damage to DNA of hematopoietic stem cells (13). In view of these possible relationships between therapy for cancer and late complications, the present finding may be important in indicating that following treatment there is an increase in mutations in lymphocytes. Further studies will be needed to show if the degree of increase in mutation frequency is of any value in predicting which patients will develop late complications.

REFERENCES


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