Sister Chromatid Exchanges and Chromosome Aberrations in Children after Treatment for Malignant Lymphoma

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ABSTRACT

Sister chromatid exchanges and chromosomal aberrations were investigated in lymphocytes from 11 children with malignant lymphoma after cessation of treatment. Chemotherapy combined with radiation was administered, terminating between 4 months and 13 years before chromosome analyses were performed.

The frequency of sister chromatid exchanges per chromosome was the same, 0.20, in the patients and a matched control group, although there was a significant heterogeneity between individuals.

The number of cells with chromosome abnormalities increased from 2.9% in control children to 4.8% in treated patients, a nonsignificant increase. Only translocations showed a significant increase.

INTRODUCTION

The application of an intensive treatment with radiation and multiple-drug chemotherapy has in recent years clearly improved the survival of patients with cancer. It has been reported that the treatment seems not to cause any long-standing defects on the immune system (8), which is in accord with the observations of Blomgren et al. (3) on 5 of the children in this investigation (L6 and L8 to L11). It is known, however, that secondary tumors occur in these patients (5, 20, 37). It is also of interest to investigate the damage induced in the genetic material, because these children can be expected to survive long enough to reproduce. At present, however, there are no appropriate methods for investigations of damage induced in the human germ cells; therefore, extrapolations from somatic cells must be used.

We have investigated the chromosomal damage induced in somatic cells in a group of children treated for non-Hodgkin’s lymphoma with radiation and chemotherapy. The methods used were investigations of the rates of chromosomal aberrations in Q-banded chromosomes and of SCE’s in peripheral lymphocytes as compared to those of matched control children.

MATERIALS AND METHODS

The study encompassed 11 patients treated for non-Hodgkin’s lymphoma during childhood. At present, they are all considered clinically disease free after cessation of antitumor therapy (period of time between 4 months and 13 years). Details of the clinical data for individual patients, type of treatment, and period of follow-up are presented in Table 1. Chemotherapy has been given as follows: VCR, 2 mg/sq m weekly during the first 6 weeks and then 2 injections every 2 months; prednisolone, 60 mg/sq m daily for a period of 6 weeks and then every 2 months during 1 week; 6-mercaptopurine, 50 mg/sq m daily; SX, 300 mg/sq m once a week; procarbazine, 100 mg/sq m daily for a period of 10 days every 2 months; mustine, 6 mg/sq m i.v. on Days 1 and 10 every 2 months. The radiation treatments have been given with 60Co γ-irradiation (dose fraction ranging from 85 to 200 rads each time). Conventional X-rays have been used only in Patient L11.

Healthy volunteers (school children) matched for age and sex with each patient served as controls.

Peripheral lymphocytes were stimulated with phytohemagglutinin and cultured in Roswell Park Memorial Institute Medium 1640 (Flow Laboratories Svenska AB, Solna, Sweden) supplemented with autologous serum and L-glutamine (Flow Laboratories). For scoring of chromosomal aberrations, the cells were cultured for 48 hr. For scoring SCE’s, the cells were cultured for 72 to 96 hr in the dark in the presence of 40 μM 5'-bromo-2'-deoxyuridine (Sigma Chemical Co., Saint Louis, Mo.) and 10 μM fluorodeoxyuridine (Calbiochem, Stockholm, Sweden). All SCE cultures were handled under a red photosafe lamp. Slides were prepared according to our standard method (12).

For scoring of SCE’s, the slides were stained according to the fluorescence plus Giemsa method (31) and analyzed directly on a television monitor combined with a microscope, an arrangement which was specially constructed for direct contrast enhancement of the microscopic picture. For each individual, 20 to 25 metaphases were scored.

For scoring of chromosomal aberrations, the slides were stained in quinacrine mustard (0.05 mg/ml) (Sterling-Winthrop, Rensselaer, N. Y.) (7), and suitable metaphases were photographed. The photographic negatives were analyzed in a television set equipped for contrast enhancement (6). Only cells with 45 or more centromeres were analyzed.

For each individual, about 100 metaphases were scored. Gaps, defined as nonstaining regions less than the width of a chromatid, were not included in the aberration counts. In this context, aneuploid cells are defined as those with more than 46 centromeres, inasmuch as chromosomes are easily lost from the metaphase plates during slide preparation. The number of aneuploid cells in Tables 2 and 3 thus is lower than the real frequencies.

RESULTS

The mean frequency of SCE’s was the same, 0.20/chromosome, corresponding to 9.2 SCE’s/cell, in both the patient...
and the control group (Tables 2 and 3). Between individuals, however, a heterogeneity was found (patients, $\chi^2_{10} = 51.8, p < 0.001$; controls, $\chi^2_{10} = 53.8, p < 0.001$).

Two of the patients, L3 and L5, were shown to be smokers during the course of the investigation, and their controls were not matched as regards smoking habits. Smoking is a factor that has been shown to influence the SCE rate (17), but the SCE frequencies of our 2 smokers do not seem to be extremely high.

The number of aberrant numerical and structural metaphase plates increased, although not significantly ($\chi^2_1 = 3.61, p > 0.05$), in the patients compared to the controls (Tables 2 and 3). If, however, the total number of structural aberrations was compared in patients (47 of 818 metaphases) and controls (18 of 787 metaphases), the increase was significant ($\chi^2_1 = 11.47, p < 0.001$), depending on a clustering of aberrations, particularly translocations in a number of cells in 3 of the patients who had received the same kind of treatment as the others. Translocations also were the only kind of aberrations that showed a significant increase (20 of 818 in patients compared with 3 of 787 in control individuals).

There was no correlation between the frequencies of chromosomal breaks and SCE's in patients ($r = -0.03$) and controls ($r = 0.17$).

The 18 translocations that could be classified as to break points belonged to the following types: tandem, 9; dicentric, 7; reciprocal, 1; and robertsonian, 1. Only 2 of the dicentrics were of such a type that they were expected to be accompanied by an acentric fragment which also was found. The other 5 dicentrics seemed to involve 2 intact chromosomes. The tandem translocations involved one intact chromosome fused to an acentric fragment which also was found. The other 5 dicentrics seemed to involve 2 intact chromosomes. The tandem translocations involved one intact chromosome fused to a segment of the other chromosome. With the resolving power of present-day microscopy and staining techniques, it cannot be unequivocally determined whether these translocations were not, in fact, reciprocal.

The 11 deleted chromosomes classified all lacked the expected acentric fragment, thus indicating that the cell, after induction of the aberrations, had undergone at least one division between treatment and the division which was studied.

The acentric fragment was probably lost during this previous division.

The break points occurred almost exclusively in pale Q-bands, but a few were also seen in bright Q-bands and at interfaces between bands.

**DISCUSSION**

SCE's. In our investigation, no effect on SCE's was observed. Aronson (1) also found no increase in SCE rate in childhood cancer patients after treatment. An increase in SCE number seems to return to normal in a relatively short time, and this may explain our result, because our investigation was conducted at least 4 months after treatment was concluded. Raposa (32) investigated lymphocytes from patients treated with SX. During treatment, there was a higher incidence of SCE's; but, about 10 days after termination of treatment, the SCE frequency returned to normal. Nevstad (27) observed an increase in SCE frequency after injection of Adriamycin in a cancer patient, but after 1 month the SCE frequency was down to control level. Lambert et al. (19) found increased SCE levels persisting for 2 to 4 months after treatment with 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea.

Radiation is known to cause a very modest increase in SCE frequency (30). Littlefield et al. (22) treated G0 lymphocytes in vitro with 150 and 300 R 60Co γ-radiation and found no increase of the SCE rate in the second division. Thus, an exposure in vivo to radiation would not give an increased SCE frequency in cultured lymphocytes. Lambert et al. (18) also could not relate radiotherapy to increased SCE frequencies in patients treated for malignant diseases.

Among the cytostatics given to our patients (Table 1), SX has been shown to give elevated SCE rates in vitro (16, 32), while Lambert et al. (18) reported SCE frequencies in vivo within control range. With VCR, Stoll et al. (38) obtained a decrease of SCE in human lymphocytes in vitro. However, Santesson et al. (33) believe that the results of Stoll et al. show the normal value for B-cells. The B-cells should be more tolerant than T-cells to 5'-bromo-2'-deoxyuridine treatment;
thus, B-cells were the only cells to reach the second generation at the time of harvest.

Chromosome Aberrations. It is noticeable that the amount of radiation and cytostatics given during therapy to these patients does not result in a more marked increase of chromosome aberrations. Our results are in accord with those of Schinzel and Schmid (34), with a nonsignificant increase in overall aberration rate. When we considered only structural aberrations, however, the increase was significant, depending on the large number of translocations in the patients compared to control persons. Other authors, e.g., Miller et al. (23), found a 10-fold increase in posttreatment patients compared to controls. However, the aberration frequency of the control persons is 10 times lower than that of our material. The investigation of Miller et al. was made on G-banded chromosomes. Bridge and Melamed (4) found little increase in chromosome aberrations in cancer patients treated with single drugs and somewhat greater increase in patients treated with combined therapy. In patients treated with both chemotherapy and radiotherapy, a marked increase was noted.

Among the cytostatics used for treating our patients, SX (9, 24, 25), 6-mercaptopurine (26, 29), and VCR (11) gave an increased number of chromosome aberrations in somatic cells of treated patients.

Chromosome aberrations induced in circulating lymphocytes in humans in vivo have been shown to persist for more than 20 years (2). Thus, we would still expect aberrations resulting from treatment which was terminated 4 months to 13 years ago to be visible in our patients. The heavily damaged cells in 3 of our patients may be such persistent cells. There seemed to be no correlation between frequency of chromosome aberrations and length of the interval between treatment and chromosome analysis. This is in agreement with Miller et al. (23), who found no decrease in aberration frequency with increasing posttreatment time.

It thus seems as if an increased rate of SCE's indicates acute DNA damage, while an increase of chromosome aberrations may also be indicative of damage having occurred several years previously.

In contrast to this, Dobos et al. (9) found that the rate of chromosome aberrations in children treated with SX for autoimmune diseases returned to normal 6 to 7 months after therapy.

The lack of correlation between SCE's and chromosome breaks may partly be explained by the fact that SCE's and chromosome aberrations return to background levels at different rates. The finding also seems reasonable because there is no consistent relationship between the ability of various agents to induce structural aberrations and SCE's (41), inasmuch as the 2 mechanisms of induction probably are different (15, 40, 42) and since the sites of induction are not related (13, 35, 36).

The majority of aberrations were of chromosome rather than chromatid type. Irradiation during G0, the stage in which most circulating lymphocytes exist in vivo, results in chromosome

### Table 2

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<th>Deletions</th>
<th>Inversions</th>
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Chromosome Studies after Lymphoma Treatment

breaks. The cytostatic drugs acting when the chromosomes have replicated during S phase give rise to chromatid breaks. When the cells then undergo a subsequent division, the resulting aberrations will be of the chromosome type. Thus, some of the cells with chromosome damage induced by cytostatic treatment of our patients are likely to have gone through at least one division in vivo. The lack of accompanyingacentric fragments in metaphases with deleted chromosomes further suggests this.

Although the present investigation is concerned with damage in somatic cells, not only is the problem of secondary tumors, being somatic cell events, important. In addition, these children can be expected to survive long enough to reproduce; therefore, damage induced in the germ line is also of interest. However, at present, there are no methods available for investigations of human germ cells; therefore, extrapolations from somatic cells must be performed. The only kind of aberration that showed a significant increase was translocation (Table 2).

In humans, reciprocal translocations are the most frequently occurring among structural aberrations in the general population (10, 14, 28). The translocations giving rise to dominant lethals are of minor importance in this context because they would be recognized as spontaneous abortions or not even noticed. Reciprocal translocations, on the other hand, are transmissible and may give rise to unbalanced chromosome complements in the next generation. Depending on the amount of chromosome gain or loss, there will be varying degrees of fertility damage or loss.

One of the females in this investigation gave birth to 3 healthy children after termination of treatment. No excess of congenital abnormalities in 293 pregnancies of 146 cancer patients treated in childhood with X-rays and various kinds of chemotherapy was found (21). Vogel and Jäger (39) estimated the genetic load due to cytostatic treatment to be very low, but, in their series of 663 patients, reproduction was found to be far below that expected for the population in question. However, as far as we know, these are the only investigations of this kind performed, and further studies are thus needed.

ACKNOWLEDGMENTS

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