Frequency of Sister Chromatid Exchanges in Bloom Syndrome Fibroblasts Reduced by Cocultivation With Normal Cells

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Summary. Cocultivation of fibroblast cells from a male patient with Bloom syndrome (BS) and a female control reduced the rate of sister chromatid exchanges in the BS cells from a mean of 54 SCE per metaphase (range 42—65) to 41 (range 24—59). Medium used to culture control cells for 48 h also reduced the rate of SCE (from 40—65 to 33—54), whereas medium used for only 24 h altered the SCE rate only slightly (to 39—61). Dialyzed medium concentrate with molecular cutoff at 15,000 did not alter the SCE rate. These initial studies suggest that normal cells produce an agent, presumably lacking in BS cells, that is capable of mitigating the chromosomal manifestation of the BS mutation (bl) in bl/bl cells.

Introduction

Recently, Tice et al. (1978) reported that cocultivation of Bloom syndrome (BS) and normal fibroblasts increase the rate of sister chromatid exchanges (SCE) in the controls. They attributed this effect to an agent(s) produced by BS cells and capable of damaging DNA. Aside from the possibility that an increased rate of SCE may not necessarily reflect DNA damage, it appears at least as plausible that BS cells actually lack a gene product, or an active form thereof, that is required for unspecific functions related to chromosomal integrity. The increased rate of SCE could be viewed as a compensatory effect at the chromosomal level. Therefore, the result cited above is unexpected and may in fact be open to differences in interpretation. We examined the rate of SCE in cocultivated BS and control fibroblasts and found that control cells are capable of reducing the level of SCE in BS cells, rather than the reverse.

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Material and Methods

Cell Cultures. Fibroblast cultures from a 14-year-old male with known BS, designated MK-1, Bloom syndrome registry no. 30 (German et al., 1977a), and a 24-year-old female as control, designated CS-1, were derived in 1976 and 1977, respectively. The BS fibroblasts had been stored in liquid nitrogen in their fourth passage. Cells were grown in DME medium (Gibco, Grand Island, New York), supplemented with 10% fetal bovine serum (Gibco) according to standard techniques. The medium was buffered with HEPES 20 mM and NaHCO₃, 0.5 g/l. The cultures were incubated in the presence of 4% CO₂ in air.

Cocultivation. Two forms of cocultivation were carried out: (a) cell-cell in 1:1 proportion, (b) cell-medium, which had supported a culture as outlined below.

For experiment (a), each 25-cm² Falcon plastic flask was seeded with 3 × 10⁶ cells of either BS or control origin. After 4 days growth in medium supplemented with 5-bromodesoxyuridine (BrdU), 30 µg/ml, 4.5 × 10⁵ cells of either control or BS origin were added and cocultivated for another 4 days in the presence of BrdU. All cultures were in log growth phase in their 5th or 11th subculture generation, respectively. For experiment (b), medium previously used for 24 and 48 h to support either BS or control cultures was added to cultures.

Controls. The following experiments served as controls: (1) control cultures and BS cultures alone, without cocultivation, tested at the same time under identical conditions, (2) medium obtained from control cultures added to control cultures, and medium from BS cultures added to BS cultures.

Differentiation of Sister Chromatids. The initial culture, either BS or control, was exposed to BrdU for 4 days as described above to assure total BrdU substitution. Thus, only the responding cells showed sister chromatid differentiation and were studied for effects of cocultivation. In addition, the male BS metaphases could be distinguished from the female control by karyotyping, although formal analysis was done only in BS metaphases showing less than 25 and controls showing more than 20 SCE.

Chromosomal Analysis and Scoring for SCE. Chromosomal preparations were obtained and analyzed for the SCE rate as described previously (Bartram et al., 1976). The SCE rate is expressed as number of visible exchanges per metaphase.

Use of Dialyzed Medium Concentrate. 50 ml medium obtained from controls after 3 days of cultivation without fetal-calf serum were reduced to 2.5 ml using collodion cartridges (Sartorius, Göttingen) with a cutoff point below 15,000 mol. wt. and dialyzed against phosphate-buffered saline. A 10% concentrate was added to BS cells.

Results

As shown in Figure 1, slot 4, we found that bl/bl cells cultured as responding cells for 4 days in the presence of control cells have a reduced rate of SCE (41 per metaphase, with a range of 24—59) compared to bl/bl cells cultured alone (54 with a range of 42—65). Control cells responded to cocultivation with BS cells with a normal rate of SCE (Fig. 1, slot 3). Medium used for 48 h on control cultures and added to bl/bl cultures also reduced their SCE rate (to 41, range 33—54, Fig. 1, slot 10). Medium used for 24 h had only a slight effect, and medium used for 72 h could not support cell proliferation. Medium from BS cells had no effect on normal cells.

The dialyzed medium concentrate derived from control cultures had no effect on the SCE rate of BS cells.