Abnormal lymphokine production: a novel feature of the genetic disease Fanconi anemia

I. Involvement of interleukin-6

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Summary. The correction of chromosomal hypersensitivity to mitomycin C (MMC) in Fanconi anemia (FA) human lymphoblasts is observed by growth in a medium conditioned by normal human cells. Under the same conditions, the cytotoxic effect of MMC on FA cells is restored to an almost normal level. The addition of interleukin-6 (IL-6) to an unconditioned culture medium increased the resistance of FA cells to MMC cytotoxicity. This correcting effect is partially abolished by addition of an anti-IL-6 antibody to the conditioned medium.

Both lymphoblasts and fibroblasts derived from FA patients demonstrate a reduction in IL-6 production. Moreover, this lymphokine is not induced by tumor necrosis factors α and β (TNFα and TNFβ) in FA cells, as is the case in normal cells. It is suggested that the observed deficiency in IL-6 production may account for one of the major characteristics of FA disease, i.e., the defect in differentiation of the hematopoietic system.

Introduction

Fanconi anemia (FA) is an autosomal recessive genetic disorder clinically characterized by progressive pancytopenia, bone marrow failure, and growth retardation. Affected individuals are predisposed to cancer, especially acute myelogenous leukemia (for review see Schroeder-Kurth et al. 1989). A number of in vitro observations are consistent with the onset of neoplasia. In particular, cultured FA human cells demonstrate a spontaneous chromosomal instability (Schroeder et al. 1964), hypersensitivity to the cytotoxic and clastogenic effects of crosslinking agents such as mitomycin C (MMC), diepoxybutane (DEB) and photoactivated psoralens (Sasaki and Tomonura 1973; Auerbach and Wolman 1976; Weksberg et al. 1979; Ishida and Buchwald 1982), and sensitivity to oxidative stress (Joene et al. 1981; Gille et al. 1987). Also, perturbations in the cell cycle due to the elongation of the G2 phase (Dutrillaux et al. 1982), and anomalies in mutation frequencies and in the pattern of mutations (Papadopoulo et al. 1990a, b), have been observed in FA cells.

Human FA lymphoblasts have been classified, by somatic cell hybridization, into at least two complementation groups A and B (also named non-A) (Duckworth-Rysiecki et al. 1985), which differ in their response to inhibition of DNA synthesis, and in repair capacity, after crosslinking treatment (Moustacchi and Diatloff-Zito 1985; Moustacchi et al. 1987; Papadopoulo et al. 1987). In spite of intensive efforts, however, the molecular basis of the FA defect is still unclear, and no enzyme deficiency consistent with the observed phenotype has been found to date.

In a previous report (Rosselli and Moustacchi 1990), we described the partial correction of the chromosomal hypersensitivity to cross-linking agents in FA group A (FA A) cells following co-cultivation with mouse lymphoma L5178Y cells, which have the same sensitivity to MMC as normal human cells. No complementation was observed for FA group B (FA B) cells. Under such conditions, no alteration in the frequency of chromosomal aberrations was observed in the mouse cell line. These observations suggested that a diffusible “factor(s)” produced by normal mouse cells can correct, at least in part, the chromosomal sensitivity in FA A cells.

In the present study, we demonstrate that it is possible to correct the chromosomal hypersensitivity of human FA A cells by cultivating them in a medium conditioned by normal human cells. In other words, cellular contact is not obligatory for complementation. Moreover, under these conditions, modifications are also observed in the response of cells of the two FA complementation groups to the cytotoxic action of mitomycin C (MMC).

A possible role for proteins involved in regulation of cellular growth and differentiation (i.e., interferons and cytokines) in the above response was suggested to us by the following: (a) failure in the differentiation of the hematopoietic system in FA patients; (b) the existence
of cell cycle perturbations in FA cells. The fact that mouse cells release a factor(s) which is effective in human cells indicated that a non-species-specific protein was involved, eliminating the involvement of interferons. We therefore decided to examine the activity of different cytokines, including IL-6, in FA cells. IL-6 is a multifunctional cytokine which, among other properties, has the capacity to enhance the proliferation and differentiation of B and T cells. It acts synergistically with interleukin-3 (IL-3) to increase the proliferation of hematopoietic progenitor cells. IL-6 is produced by different cell types in response to infection or to tissue injury, and its expression is stimulated in normal cells by a variety of agents, including other cytokines such as tumor necrosis factor α (TNFα) (for general reviews see Seghal et al. 1989; Arai et al. 1990; Hama-Inaba et al. 1990; Van Snick 1990).

We show here that the addition of interleukin-6 (IL-6) to the culture medium increased the resistance of the FA cells to MMC toxicity in a dose-dependent manner. Moreover, relative to normal cells, the concentration of endogenous IL-6 released into the growth medium is low for FA cell lines belonging to both complementation groups. This is true for both lymphoblasts and fibroblasts derived from FA patients.

Our data suggest that the major clinical feature of FA, i.e., the pancytopenia associated with bone marrow failure, might be causally related to the reduced level of IL-6 production in FA, compared to normal, cells.

Materials and methods

Cell lines and reagents

Normal human lymphoblasts AHH-1 (provided by W. Thilly, Massachusetts Institute of Technology) and FA lymphoblastoid cells lines HSC-99 (complementation group A) and HSC-62 (complementation group B) (Duckworth-Rysiechi et al. 1985; Moustacchi et al. 1987) (both provided by Dr. M. Buchwald, Hospital for Sick Children, Toronto, Ont.) were grown in suspension in RPMI 1640 medium (Gibco, USA) supplemented with 12% fetal serum (Boehringer Mannheim, France). Mouse lymphoma L5178Y cells (provided by Dr. Hama-Inaba, National Institute of Radiobiological Sciences, Chiba, Japan) (Hama-Inaba et al. 1983, 1988) were grown in suspension in Fisher’s medium supplemented with 12% fetal calf serum. The normal skin fibroblast cell line 1BR/3 was kindly provided by Dr. C. Arlett (University of Sussex, Great Britain). The two skin fibroblastoid cell lines derived from FA patients used were FA1.50, provided by Dr. R. Voss (see details in Moustacchi and Diatloff-Zito 1985), and FA1.45, provided by Dr. Buchwald (Ishida and Buchwald 1982). These cells grow in MEM supplemented with 12% fetal calf serum.

Pure human recombinant E. coli-derived interleukin-6 (specific activity 2 × 10^6 units/ml) was prepared in our laboratory (U196 INSERM).

Human interleukin-1α (IL-1α) was obtained from Hoffman-La Roche, Switzerland. human interleukin-2 (IL-2) was a gift from Roussel Velog, and human interleukin-1 (IL-3) was from Genzyme, Boston, USA. Human TNFα and tumor necrosis factor β (TNFβ), also named lymphotoxin (LTβ) produced by Genentec was provided by Dr. Adolf from Boeringer-Ingelheim (Vienna, Austria).

A monoclonal antibody directed against the E. coli-derived human IL-6 (a gift from Dr. J. Wijdem, CNRS, Besançon, France) was added to conditioned medium at a dose known to neutralize 1000 units/ml of IL-6.

Preparation of conditioned media

Exponentially growing cells (3 × 10^5 cells/ml) were cultivated for 48 h (final cell concentration about 1.5 × 10^5 cells/ml in the case of normal cells). Cells were eliminated from the medium in two steps (a) centrifugation (15 min at 3000 rpm); (b) filtration with a Millipore filter (0.45 μm), (France). This conditioned medium was utilized for the culture of other cells.

Co-culture experiments and chromosome aberrations analysis

Exponentially growing FA A cells were collected by centrifugation and resuspended in fresh or conditioned medium at 3 × 10^5 cells/ml, or mixed at a ratio of 1:5 with normal mouse L5178Y cells and resuspended in fresh medium (final concentration 3 × 10^5 cells/ml). The cultures were treated with MMC (10 ng/ml) for 24 h starting immediately after the resuspension in fresh or conditioned medium, or after 24 h of supplementary culture in conditioned medium, or during co-cultivation.

Chromosome preparation and aberrations analysis were performed as previously described (Rosselli and Moustacchi 1990). For statistical analysis, Fisher’s exact test or Student’s t-test was used.

Measurement of growth inhibition

Growth inhibition was assayed by seeding 5 × 10^4 lymphoblasts/well in 24 well plates in 1 ml of culture medium. Various concentrations of mitomycin C (MMC) (Sigma, USA) were added to the wells in duplicate. After a growth period of 4 days in the continuous presence of the drug, cells in duplicate wells were pooled and counted in a Coulter counter. Untreated controls were run in parallel. Each experiment was performed at least six times. Growth percentage was calculated as follows:

% Growth =

\[
\frac{100 \times \text{final number MMC-treated cells} - \text{initial cell number}}{\text{final number control cells} - \text{initial cell number}}
\]

Interleukin-6 titration

Lymphoblast cell suspensions were collected by centrifugation at the time indicated and the supernatant was filtered (Millipore, France). This conditioned medium was utilized for the culture of other cells.

Results

We have previously shown that it is possible to correct the FA cytogenetic cellular defect in vitro by co-cultivation with mouse cell (Rosselli and Moustacchi 1990). Here we demonstrate that cellular contact is not necessary for complementing FA A chromosomal hypersensitivity to MMC during co-cultivation with normal human or mouse cells. Indeed, we observed that when the two cell types (normal and FA) were cultivated at a 1:1 ratio in a trans-wells system (Costar) separated by a filter of 0.45 μm and treated with MMC, the frequency of chromosomal aberrations in FA cells was significantly reduced (data not shown). Our earlier observations dealt with a mixture of human and mouse cells, and they are now extended to combinations of only exclusively human...