LYMPHOBLASTOGENESIS IN DOWN'S SYNDROME AND ITS INHIBITION BY HUMAN INTERFERON

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1. Introduction

In addition to its antiviral effect, interferon has been reported to have biological effects on cell growth [1,2] and on the immune system [3,4]. DNA synthesis in mitogen-stimulated lymphocytes was shown to be inhibited by interferon [5–7]. In human cells, a gene on chromosome 21 has been implicated in determining the antiviral response to interferon, possibly through control over the interferon receptor [8–10]. It was not clear whether chromosome 21 also plays a role in the non-viral effects of interferon. It therefore seemed of interest to compare the sensitivity to interferon of lymphoblastogenesis in cells trisomic for chromosome 21 (as found in Down's syndrome) as compared to normal (disomic 21) subjects. Incorporation of [^{3}H]thymidine in lymphocytes treated with mitogens and interferon was used as an in vitro measure of cell proliferation resulting from antigen stimulation in vivo.

The results reported indicate that this non-viral effect of interferon on lymphoblastogenesis is also regulated by genes on chromosome 21. The possible role of chromosome 21 in cell–cell interactions and in the immunodeficiency seen in Down's syndrome is discussed.

2. Materials and methods

2.1. *Interferon*

Human lymphoblastoid interferon was prepared by Sendai virus induction of Namalva cells [11]. After inactivation of virus by pH 2 treatment, the crude interferon was partially purified by antibody affinity chromatography [12]. Human fibroblast interferon was prepared by super-induction of foreskin fibroblasts [13].

2.2. *Lymphocytes*

Blood was obtained from non-institutionalized children suffering from Down's syndrome (age, 3 months–8 years) and from normal age-matched controls. Karyotyping was carried out on all Down's syndrome subjects and all were found to be trisomic in chromosome 21. Lymphocytes were prepared by density gradient centrifugation on lymphoprep (Nyegaard and Co., A/S, Oslo) by the method in [14] using heparinised blood diluted in Dulbecco modified phosphate buffered saline (PBS). The lymphocytes were washed 3 times in PBS (250 × g, 10 min) and resuspended in RPMI 1640 medium (Gibco) supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml) and 5% fetal calf serum (Gibco). Fetal calf serum was heat-inactivated (56°C, 30 min) before use.

Incubation of lymphocytes was carried out in triplicate in microtiter plates (Falcon, 3041/2) using 6–10 × 10^4 cells/0.2 ml/well. Mitogens used were phytohemagglutinin (PHA-P; Difco, MI), concanavalin A (con A; Miles Yeda) and pokeweed mitogen (PWM; Gibco, NY). The mitogen concentrations which gave optimal stimulation of lymphocytes were PHA 20 μg/ml, and con A and PWM 5 μg/ml. The incubation was

carried out at 37°C, in a humidified atmosphere of 5% CO_2–95% air. Interferon was used at 0–1600 units/ml. Both mitogen and interferon were added at the start of the 65 h incubation. At the end of this time a 4 h pulse of 1 μCi [^{3}H]thymidine (Israel AEC, Negev; 10–20 Ci/mmol) was given. Cells were then harvested (MASH II harvester Microbiological Associates) on glass fibre filters (Whatman GFC) and washed prior to scintillation counting (Bray's scintillation fluid) in a liquid scintillation spectrometer (Packard).

Table 1
[^{3}H]Thymidine uptake (cpm × 10^{-3})

	Control	PHA	con A	PWM
Normal	0.4 –2.0	46–136	46–76	10 –32
Down's	0.80–1.6	33–130	37–82	0.6–14

[^{3}H]Thymidine incorporation in lymphocytes as a result of mitogen stimulation. Conditions are in section 2, with 100 000 cells/well. Figures given are the range of counts determined in 14 Down's syndrome subjects and 12 controls. For details see section 3

3. Results

3.1. *Stimulation of [^{3}H]thymidine incorporation by mitogens*

Table 1 shows the results of mitogen stimulation of lymphocytes derived from Down's syndrome and from normal subjects, in a series of experiments. As can be seen, both PHA and con A elicit in Down's-derived lymphocytes reactions which are comparable to those obtained with normal cells. In the case of PWM, the stimulation observed in Down's-derived lymphocytes was significantly lower than that in normal cells. The figures given are the range of counts

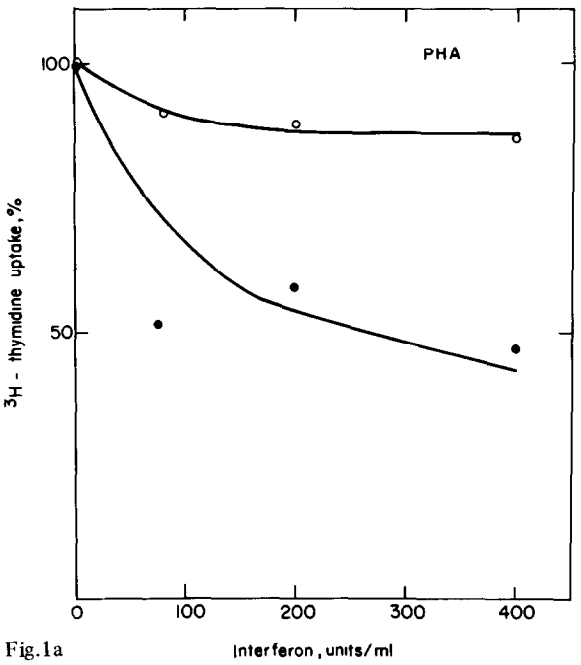

Fig.1a

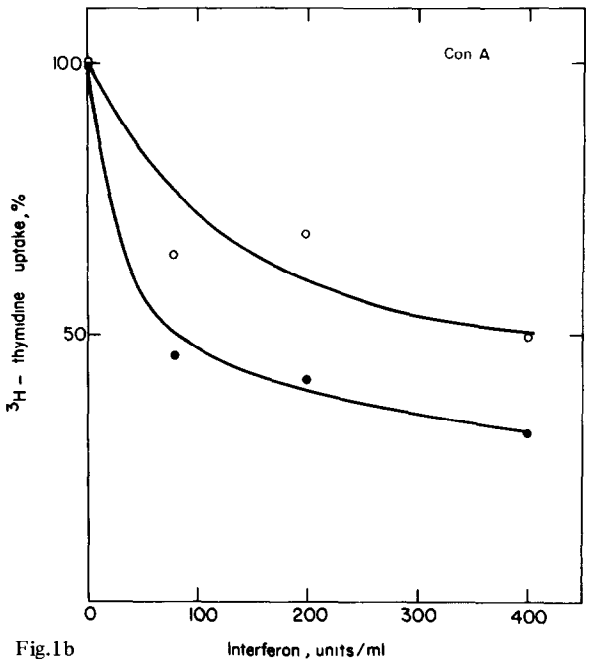

Fig.1b

Fig.1. Effect of interferon on [^{3}H]thymidine incorporation into normal (o) and Down's syndrome (•) lymphocytes on stimulation with mitogens. Lymphocytes were prepared on a lymphoprep gradient and after washing were resuspended in RPM1 1640 medium containing 5% fetal calf serum. Cells were incubated at 100 000 cells/0.2 ml in the presence or absence of mitogen (PHA. 50 μg/ml, fig.1a; con A. 5 μg/ml, fig.1b) and of interferon at concentrations shown. After 65 h incubation, a 4 h pulse of [^{3}H]-thymidine (1 μCi) was given. [^{3}H]Thymidine incorporated into the cells was determined by scintillation counting of the harvested and washed cells.

determined in 14 Down's syndrome subjects and 12 controls, which showed good stimulation with mitogens. The stimulation conditions used in these experiments, see section 2, were found to be optimal conditions for both normal and Down's-derived lymphocytes. Indeed, a series of experiments with varying mitogen concentrations (PHA 2–40 μg/ml; con A 0.5–10 μg/ml; PWM 0.5–10 μg/ml) and variation of incubation time with mitogen (48–96 h), indicated that Down's syndrome and normal lymphocytes are optimally stimulated under the same conditions.

3.2. *Effect of interferon on mitogen stimulation*

The effect of partially purified lymphoblastoid interferon on con A- or PHA-stimulation of [^{3}H]-thymidine incorporation is shown in fig.1. Interferon decreased [^{3}H]thymidine incorporation into DNA in normal and in Down's syndrome lymphocytes stimulated by either mitogen. With con A-stimulated lymphocytes, the concentration of interferon needed to produce a 50% inhibition was about 4-fold less in Down's syndrome lymphocytes trisomic in chromosome 21, than in normal diploid lymphocytes. In PHA-stimulated cells, the extent of inhibition by interferon in Down's lymphocytes was also greater than that observed in normal cells. The PHA-induced response in normal cells was not markedly inhibited, whereas the stimulation in trisomic cells was significantly inhibited by interferon.

Experiments performed with human fibroblast interferon (results not shown) gave similar results to those described, which were carried out with lymphoblastoid interferon.

4. Discussion

The involvement of chromose 21 in determining cell sensitivity to the antiviral effect of interferon in humans has been reported [7,15–17]. We were therefore interested in investigating the effect of interferon on another non-antiviral function in the chromosome 21 associated Down's syndrome.

The property of interferon in inhibiting cell proliferation has now been shown to reside in the same molecule which inhibits virus growth [1,18]. A convenient system for study of the anti-proliferative effects of interferon is mitogen-induced DNA synthesis in human peripheral blood lymphoid cells. This effect of interferon on DNA replication may be closely related to its anti-tumor effect.

The results reported indicate that trisomy in chromosome 21 causes no significant alteration in the response of lymphocytes to the two T-cell mitogens studied, PHA and con A, in [^{3}H]thymidine uptake. Both decreased and increased lymphocyte response in PHA have been reported [19–21]. It is also clear that Down's syndrome lymphocytes are significantly more sensitive to interferon than normal lymphocytes in both the PHA- and con A-induced response. Similar results concerning the effect of interferon on PHA-induced lymphoblastogenesis in Down's syndrome are in [22].

While interferon significantly suppresses the [^{3}H]-thymidine response to con A, there is a less-marked inhibition of the PHA-induced effect. This is of particular interest since data from several laboratories working on both human and murine systems indicate that there are different subsets of the T-cell population which differ in their response to PHA and to con A [23]. These populations have been shown to differ not only in their ontogeny but also in their involvement in different T-cell functions in mammals [24]. Thus, it has been demonstrated that the Ly 1^+ T-cell subpopulation, a high PHA responder, is mainly involved in providing obligatory antigen-specific help to B-cells. In addition, this is the main subpopulation capable of responding strongly to alloantigens associated with major histocompatibility systems. On the other hand, the Ly 2^+ T-cell subpopulation which responds strongly to con A, is mainly involved in the generation of suppressor T-cells and anti-self-modified killer cells. The latter are of prime importance in enabling animals to eliminate virus-infected cells [24]. These data may therefore indicate a differential regulatory role of interferon in regulation of the function of different T-cell subpopulations.

Based on data indicating that chromosome 21 codes for a cell surface component [8], the greater sensitivity to interferon of lymphoid cells with trisomic chromosome 21 may be due to the presence of a larger number of receptors for interferon on these cells. Alternatively, the increased sensitivity may be due to a change in membrane characteristics. The possible involvement of chromosome 21 in such membrane alterations is highly relevant in view of the known

anatomic abnormalities found in Down's syndrome which could well stem from defects in cell–cell interaction during embryogenesis. This hypothesis gains support from the fact that in contrast to the relatively normal response of Down's syndrome lymphocytes to T-cell mitogens, we found a severely impaired response to pokeweed mitogen which is a B-cell mitogen requiring T-cell cooperation [25]. This decreased stimulation by pokeweed mitogen was not due to alterations in the optimal conditions for stimulation of Down's cells by the mitogen since the same effect was demonstrated over a wide range of mitogen concentrations and at various times after mitogen addition.

It may be pertinent to focus attention on the abnormalities accompanying Down's syndrome, as compared to those found in murine cells with abnormalities in chromosome 17 (T-locus mutants) [26]. It is possible that the T-locus-associated genes on chromosome 17 in mice correspond to some of those found in chromosome 21 in man.

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