Influence of α-interferon therapy on blood lymphoid cells

Studies on antibody production, mixed lymphocyte culture response, mitogen responsiveness and 2'-5'oligoadenylate synthetase activity

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Summary. The influence of natural α-interferon (α-IFN) therapy (3 × 106 units i.m. daily) on blood lymphoid cells was studied in 20 patients with gynecological neoplasias (7 patients with condylomata acuminata and 13 patients with ovarian carcinoma). There was a statistically significant increase in the intracellular levels of 2'-5'oligoadenylate synthetase 1 day after the first injection of IFN and with few exceptions this activity remained increased during 3 months of treatment. In most of the patients, the capacity of blood lymphoid cells to produce IgA, IgG, and IgM following stimulation with pokeweed mitogen was decreased 1 day after the first injection of IFN and with few exceptions it remained low during 6 months of IFN therapy. In most patients there was a decrease in the capacity of lymphoid cells to act as stimulator or responder cells in a mixed lymphocyte culture during IFN therapy. The α-IFN therapy had no major influence on the response of lymphoid cells to mitogens. We conclude, that neither this nor our previous studies on the influence of IFN therapy on immunological functions have given support to the hypothesis that the antitumor action of IFN is mediated by the immune system.

Introduction

The interferon (IFN) system has been implicated in the host's defence against viral and bacterial infections as well as in the defence against tumors [24]. Since IFN is found in the serum and other body fluids of patients with viral infections [19] including AIDS [5], bacterial infections [21], malignant tumors [17], and autoimmune disorders [19] we consider the question of how IFN affects the functions of lymphoid cells to be of major importance. IFN therapy has been shown to induce remissions in a variety of malignant tumors [24]. The mechanism(s) behind these antitumor effects is not known. Studies on the influence of IFN therapy on immunological functions may possibly tell us whether IFN exerts antitumor effects by stimulating host mechanisms against neoplastic cells.

We have previously shown that α-IFN therapy causes changes in a variety of immunological functions [14, 16]. In the work presented here we have studied the influence of natural α-IFN therapy on the following characteristics of lymphoid cells: intracellular levels of the enzyme 2'-5'oligoadenylate synthetase (2'-5'A synthetase), which is induced in most IFN-sensitive cells following exposure to IFN, antibody production in response to pokeweed mitogen (PWM), responder and stimulatory capacity in a mixed lymphocyte culture (MLC), and mitogen responsiveness.

Patients and treatment

A total of 20 patients were studied, 13 with ovarian carcinoma and 7 with condylomata acuminata. The mean age was 59 years for the ovarian carcinoma patients (age range 46–72 years) and 30 years for the patients with condylomata acuminata (age range 22–48 years). A control group consisting of 20 healthy females was included. The mean age of the group acting as control for the ovarian carcinoma patients was 58 years (age range 45–72 years), whereas that of the group serving as control for the patients with condylomata acuminata was 31 years (age range 23–44 years). All patients included in the study received 3 × 106 units of partially purified α-IFN [3, 4] i.m. daily.

Mitogens. The contents of vials containing phytohemagglutinin (PHA, Phytohemagglutinin M, DIFCO, NY, USA) or PWM (Grand Island Biological Co., NY, USA) were dissolved in 5 ml of Eagle's minimal essential medium. These solutions are referred to as 100% PHA and PWM respectively.

Separation of lymphoid cells. During IFN therapy, blood was obtained 24 h after the last injection of IFN. Lymphoid cells were separated from heparinized venous blood on a Ficoll-Hypaque gradient [2] followed by washing. Approximately 90% of the cells were lymphocytes as determined after crystal violet staining, the rest being classified mainly as monocytes.

Freezing and thawing of cells. After Ficoll-Hypaque separation the cells were frozen in liquid nitrogen [27]. After all samples had been collected the lymphoid cells from one patient and one control were thawed simultaneously, washed by centrifugation, and the number of living cells calculated following trypan blue staining. In four samples the proportion of dead cells exceeded 5% and these samples were therefore excluded.
The allogeneic cells used in the MLC experiments came from a frozen stock from a single donor. For every experiment, lymphocytes from this donor were thawed simultaneously with lymphocytes from the patient and the healthy control.

Repeated experiments showed that freezing and subsequent thawing of lymphocytes that had been preincubated in the absence or presence of IFN in vitro did not influence the functions studied (data not shown).

**Assay for 2'-5'A synthetase.** The cytoplasmic levels of 2'-5'A synthetase were determined as previously described [22]. Briefly, 10^6 cells were lysed in 0.1 ml of Nonidet P-40 at 4°C. The samples were then centrifuged for 6 min at 6000 g and the supernatants frozen at -80°C. For the enzyme assay 10 μl of the extract was added to poly(rC)poly(rC) agrose beads and the mixture incubated for 15 min at 30°C. The beads were then washed, the reaction mixture, containing 10 mM 4-(2-hydroxyethyl)-l-piperazine-ethanesulfonic acid buffer (pH 7.5), 5 mM MgCl2, 7 mM dithiothreitol, 10% glycerol, 2.5 mM α32P-ATP (0.1-0.3 Ci/mmol), creatine kinase (3 mg/ml), 10 mM creatine phosphate and poly(rC)poly(rC) (40 μg/ml), added, and the samples incubated for 16 h at 30°C. One unit of bacterial alkaline phosphatase in 140 mM Tris-base was then added. After 1 h at 37°C, 20 μl of water was added, after which the beads were removed by centrifugation. The samples were then run through 0.3-ml alumina columns equilibrated in 1 M glycine-HCl buffer (pH 2) and collected in scintillation vials, which were counted in the 3H channel of a scintillation counter (Packard). The tests were done in duplicate. A standard prepared from normal lymphocytes and a blank containing Nonidet P-40 only was always included in the assay. The nanomoles ATP incorporation in 10^5 cells was calculated.

**Antibody production.** Lymphoid cells (5 × 10^5) were cultured in glass tubes containing 1 ml RPMI 1640 supplemented with glutamine, antibiotics, and 10% heat inactivated fetal calf serum (FCS). The cell mixtures were incubated at 37°C for 7 days in the presence of 3% and 0.3% of PWM or medium alone in a humidified 5% CO2 atmosphere before they were centrifuged at 200 g and the supernatants collected for determination of Ig.

The amounts of IgG and IgM in the culture medium of lymphocytes were determined by enzyme linked immunosorbent assay as described previously [25]. Supernatants obtained in the same experiment were always examined on the same day. The variance of Ig concentration between duplicates was < 10%.

**MLC and mitogen responsiveness.** The proliferative response of lymphoid cells to mitogens and allogeneic cells was measured using a microassay as previously described [20]. In brief, 10^5 lymphocytes were cultured in wells of microtest plates containing RPMI 1640 supplemented with glutamine, antibiotics, and 10% FCS. Mitogens were added to some of the cultures, whereas others received 10^5 allogeneic lymphoid cells that had been pretreated with mitomycin C for 1 h. To other wells no stimulants were added. In the MLC experiments, IFN at a concentration of 100 units/ml, was added to some wells at the beginning of the culture period. The final volume in the wells was 0.2 ml. The cells were incubated in a humidified 5% CO2 atmosphere and after 4 days the cultures received 1.0 μCi of 3H-thymidine (5 Ci mM 3H, Radiochemical Centre, Amersham, England). The cultures were terminated 24 h later and incorporated radioactivity, expressed as counts per minute (cpm), determined by liquid scintillation counting. Mean values of quadruplicate cultures were calculated and expressed as log_{10} cpm.

**Statistical analyses.** Statistical significances were evaluated by paired Student's t-test and by linear regression analyses.

**Results**

### 2'-5'A synthetase activity

Prior to initiation of IFN therapy the mean 2'-5'A synthetase activity of lymphoid cells from the patients was significantly above that of lymphoid cells from the healthy controls (Table 1). This elevated level 2'-5'A synthetase activity was more pronounced in lymphoid cells from the patients with ovarian carcinoma as compared to patients with condylomata acuminate (Table 1). With few exceptions, the intracellular levels of 2'-5'A synthetase were in-

| Table 1. 2'-5' A synthetase levels of lymphoid cells from patients with condylomata acuminate and ovarian carcinoma and from healthy donors. Change during IFN treatment (expressed as percentage of pretreatment value). Figures within brackets denote number of donors tested. Means ± SE are presented |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                 | All patients    | Condylomata acuminate | Ovarian carcinoma | Healthy donors |
| Before IFN^a                    | 275 ± 45 (12)   | 204 ± 25 (4)       | 310 ± 64 (8)     | 103 ± 21 (12)   |
| After 1 day^b                   | 425% ± 122      | 228% ± 115        | 579% ± 181       | 139 ± 191       |
| p < 0.001                       |                 | p < 0.001         | p < 0.001        |                 |
| After 1 week^b                  | 195% ± 50       | 226% ± 29         | 178% ± 79        |                 |
| p < 0.05                        |                 | p < 0.01          |                 |                 |
| After 3 months^b                | 623% ± 969      | 25% ± 473         | 103 ± 12 (12)    |                 |

^a nmol of ATP per 10^5 cells

^b Percentage of pretreatment value