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Original article

Immuno-chemotherapy of advanced colorectal cancer with alpha-2a interferon and 5-fluorouracil. Immunopharmacological studies

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Summary. Twelve patients with metastatic colorectal cancer received alternating cycles of low immunomodulating doses of alpha-IFN + 5-Fluorouracil (5-FU) or 5-FU alone. Hematological, biochemical and physical evaluation showed that both treatment cycles were well tolerated. However, transient fever and moderate flu-like symptoms were observed following alpha-IFN administration. Treatment with 5-FU alone produced long-lasting inhibition of CD8+ T lymphocytes, but did not depress NK activity (NKA). Combined treatment with alpha-IFN produced a short-term increase of NKA and antagonized the effect of 5-FU on CD8+ cells on day 5 of the cycle. Parallel studies on *in vitro* models showed antiproliferative effects of 5-FU on PHA-stimulated MNC and confirmed the preferential inhibition of CD8+ cells. Pretreatment with alpha-IFN did not reverse the effect of 5-FU on CD8+ lymphocytes, but partially protected MNC from the toxic effects of the drug. This was presumably due to the cytostatic effects induced by alpha-IFN on MNC before exposure to the cycle-specific antineoplastic agent. This investigation suggests that alpha-IFN could play a positive role in immuno-chemotherapy of colorectal cancer through multiple mechanisms not entirely related to direct antitumor effects of the agent.

Key words: alpha-interferon, 5-FU, colorectal cancer, immuno-chemotherapy

Introduction

A number of studies support the concept that tumors expressing antigens different from those of normal tissues are capable of eliciting immune responses in the autochthonous host [1].

Preclinical investigations have shown that the therapeutic effect of antineoplastic agents can be potentiated by immune responses of the host against immunogenic tumors [2, 3]. Moreover, it was found that significant amplification of the antitumor effect of anticancer drugs can also be supported by natural immunity (NI) *in vivo* [4].

It has been demonstrated that interferons (IFNs) increase various types of immunity such as antigen-dependent cell-mediated cytotoxicity [5] and NI [6, 7]. Therefore, it could be anticipated that the immuno-enhancing effects of IFNs on NI would result in the amplification of antitumor activity of antineoplastic agents against chemo-sensitive, NI-susceptible cancer cells. Moreover, IFNs are expected to attenuate drug-mediated depression of immunity against pathogenic microorganisms, thus being of potential value in host protection against infectious diseases.

A number of clinical trials have been performed using very high doses of IFNs utilized as a single drug in chemotherapy-like phase I protocols (i.e., at maximum tolerated doses, [8]). However, high doses of IFNs were found to be markedly immunodepressive, presumably through the activation of suppressor cells [9]. It follows that low rather than high doses of IFNs should be used for a rational approach to immuno-chemotherapy regimens based on the combined effects of *in vivo* IFN-activated NI and antineoplastic agents.

On this basis a study was designed to test whether the administration of low doses of IFN to cancer patients would affect NK cell activity (NKA), an *in vitro* function possibly related to NI *in vivo* [10].

Patients with advanced colorectal cancer were subjected to cycles of treatment with low immunomodulating doses of recombinant alpha-IFN + 5-fluorouracil (5-FU). The results presented in this report show that alpha-IFN induced significant NKA increase which was detectable after 5-FU administration, and that 5-FU alone did not afford significant inhibitory effects on NK cell function. Moreover, *in vitro* studies carried out on peripheral mononuclear cells (MNC) collected from healthy donors show that the antiproliferative effects of alpha-IFN resulted in limited but significant protection of MNC from the cycle-specific cytotoxic activity of 5-FU.

Materials and methods

Patients and treatment schedule. Twelve patients with

metastatic colorectal cancer entered this study.

Eligibility criteria included (a) age less than 75 years; (b) biopsy-proven malignancy of the colon or rectum and unresectable metastases; (c) measurable metastatic lesions; (d) no previous chemotherapy for at least 6 months; (e) performance status, according to Karnofsky, greater than 50%; (f) no other active cancer; (g) adequate hepatic function, i.e., bilirubin < 2 mg/100 ml, SGOT less than three times normal; (h) adequate renal function, i.e., BUN ≤ 30 mg/100 ml or creatinine ≤ 1.5 mg/100 ml; (i) no cardiac diseases; (j) adequate bone marrow function (wbc count ≥ 4000 mm^3, hematocrit ≥ 30% and platelet count ≥100,000/mm^3; (k) informed consent signed by the patient.

The treatment schedule of immuno-chemotherapy, described in detail in Fig. 1, was designed according to

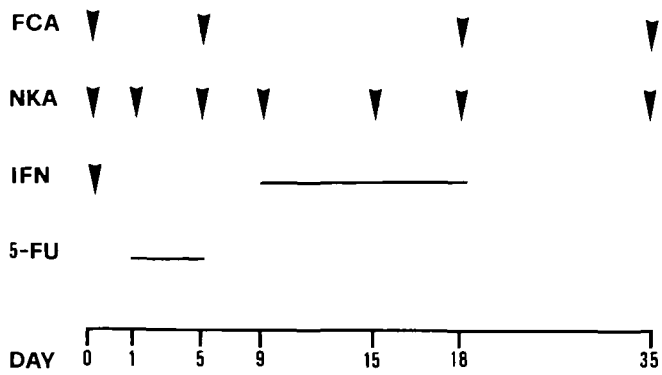

Fig. 1. Scheme of treatment and immunological tests in colorectal cancer patients subjected to alpha-IFN + 5-FU or to 5-FU alone in alternating cycles. Each cycle was repeated at 5-week intervals until the onset of progressive disease: alpha-IFN (3×10^6 IU/m^2 im, on day 0 and 0.5×10^6 IU/m^2 daily, from day 9 through day 18); 5-FU (450 mg/m^2 iv bolus, daily for 5 days) NKA = Natural Killer Activity tested on blood samples on day 0 (i.e., before the first alpha-IFN administration), on day 1 (i.e., after alpha-IFN before chemotherapy), on day 5 (i.e., at the end of chemotherapy), on days 9, 15, 18 (i.e., before, in the middle and at the end of daily treatment with alpha-IFN).

FCA = Flow Cytometric Analysis concerning CD4+, CD8+ and CD16+ lymphocyte subpopulations.

the following sequence: (a) step 1, NI stimulation by a single moderate dose of alpha-IFN; (b) step 2, chemotherapy according to the conventional 5-FU protocol, beginning 24 h after alpha-IFN administration (i.e., at the time of NI peak response to alpha-IFN treatment [11]) to generate immuno-chemotherapy synergism [4]; (c) step 3, recovery of NI function, possibly depressed by chemotherapy [12], by administration of minimal daily doses of alpha-IFN.

Blood samples for NKA assay were obtained from all patients on days 0 and 1 (i.e., after alpha-IFN, before chemotherapy), on day 5 (i.e., at the end of chemotherapy), on days 9, 15 and 18 (i.e., before, in the middle, and at the end of daily treatment with alpha-IFN).

Drugs and reagents. Recombinant interferon alpha-2a (alpha-IFN) was kindly provided by Roche (Roferon-A, Roche, S.p.A. Italy) in lyophilized vials containing 10^6 IU/vial; 5-fluorouracil (5-FU, 250 mg/vial, Roche, Italy) and PHA (PHA-A, Difco, Detroit, MI, USA) were commercially available.

Recombinant IL-2 was kindly provided by Hoffman – La Roche, Basel, Switzerland.

Tumor cells. The human cell line K562 [13], was maintained in tissue culture in RPMI-1640 medium (Flow Lab., McLean, VA, USA) supplemented with 10% fetal calf serum (FCS) (Flow Lab.), 2 mM glutamine (Flow Lab.) and 50 μg/ml gentamycin (Flow Lab.) (hereafter referred to as 'complete medium', CM).

Preparation of Mononuclear Cells (MNC). Peripheral blood MNC were separated on a Ficoll-Hypague gradient from heparinized whole blood obtained from cancer patients, washed twice in RPMI-1640 medium containing 2% FCS, 10 mM Hepes (Flow Lab.), and 50 μg/ml gentamycin (hereafter referred to as 'washing medium', WM) and suspended in CM.

Labeling of target cells for cytotoxicity assay. K562 target cells were suspended in 0.1 ml of FCS, labeled with 100 uCi $Na_2{}^{51}CrO_4$ (Amersham International Plc, Amersham, Bucks, UK), and incubated in a 5% CO_2 humidified atmosphere for 1 h at 37°C. After incubation, the cells were extensively washed with WM and resuspended at the desired concentration in CM.

Cytotoxicity assay. Effector MNC in 0.1 ml of CM were plated in quadruplicate in U-bottomed 96-well microtiter plates (Greiner C. A. Nurtigen, Germany) by making serial two-fold dilutions, starting at a concentration of 2×10^6 cell/ml. Labeled target cells (2×10^4) were added in a volume of 0.1 ml to give effector to target cell ratios ranging from 100:1 to 12.5:1. The plates were then incubated at 37°C in a 5% CO_2 humidified atmosphere for 4 h. At the end of incubation the plates were centrifuged at 250xg for 10 min and 0.1 ml of supernatant was collected and counted in a gamma-scintillation counter (5142, Packard Instruments Co, Downers Grove, IL, USA).

The means of quadruplicate samples were expressed as the percentage of specific lysis according to the formula:

$$\% \text{ specific lysis} = \frac{\text{test counts/min-autologous counts/min}}{\text{total counts/min}} \times 100$$

where test counts/min is the mean radioactivity in counts/min released in the presence of effector cells, autologous counts/min is the mean radioactivity in counts/min released by target cells incubated with 2×10^5 unlabeled autologous cells in place of effector cells, and total counts/min is the total amount of ^{51}Cr incorporated in target cells.

Evaluation of cytotoxic activity of effector cells in terms of Killed Cells (KC). The cytotoxic activity of effector cells was calculated using a data processing technique developed in our laboratory [14]. All values were expressed in terms of number of target cells lysed (i.e., 'killed') by a fixed number of effector cells, as follows:

$$KC\ (m) = \frac{m \times T \times n\%}{En \times 100}$$

where KC (m) is the number of target cells killed by m effector cells, T is the total number of target cells present in each well, En is the number of effector cells present in each well at the effector:target cell ratio selected for calculation, and n% is the specific lysis produced theoretically by En effector cells. The n% value is extrapolated from the best-fit curve obtained by plotting the different percentages of specific lysis vs ln of the number of effector cells/well, at graded effector:target cell ratios. In the present study KC(m) was calculated for $m = 10^6$ cells (i.e., KC (10^6)), and $En = 2 \times 10^6$ cells (i.e., at effector:target cell ratio of 100:1).

Statistical analysis. Differences in cytolytic activity of effector cells were evaluated taking into account the percentage of specific cytotoxicity at all effector:target ratios. Therefore, P values were calculated using covariance analysis performed on the regression of the percentage of specific ^{51}Cr release over the ln of the number of effector cells. Differences in the means of other parameters (i.e., KC (10^6) relative to NK activity of patient MNC, or number of lymphocyte subpopulations in cancer patients) were statistically evaluated according to paired Student's t-test analysis.

Flow Cytometric Analysis (FCA). Analysis was performed using a FACS analyzer (Becton Dickinson, FACS Systems, Mountain View, CA, USA), with a mercury lamp as light source. Gates and thresholds were selected by using electronic volume to exclude red blood cells. Data usually collected as 'list mode' were obtained from 10,000 cells for each analysis. Processing was performed by Consort 30 Software (Becton Dickinson) running on a HP 9.000/217 computer (Hewlett-Packard Co., Fort Collins, CO, USA). The proportion of cells showing background fluorescence was selected, by marker setting, according to unstained control samples and allowing the computer to quantitize the positive cells with subsequent subtraction of background fluorescence.

Results

1. In vivo studies

A total of 12 patients entered this study, and underwent 2–7 alternating cycles of immuno-chemotherapy vs chemotherapy alone, as described in Materials and methods.

No treatment-related deaths or life-threatening toxicities were detected. Hematological toxicity was moderate and the nadir of blood cell count was observed on days 15–19 of treatment for all patients. In addition, leukopenia, but not thrombocytopenia (data not shown), was significantly more pronounced in patients treated with alpha-IFN+5-FU than in those who received 5-FU alone (see footnote of Fig. 2). No significant variation from 'baseline' (i.e., hematological value

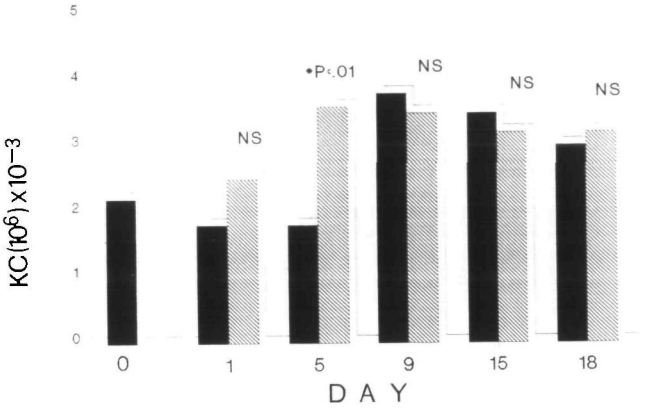

Fig. 2. NKA expressed in terms of mean KC (10^6) of MNC collected from patients on different days (see protocol, Fig. 1), 5–19 observations per group. ■ pooled data of all patients on chemotherapy cycles (i.e., cycles with 5-FU alone); ▨ pooled data of all patients, on immuno-chemotherapy cycles (i.e., cycles with alpha-IFN + 5-FU).

* $P < 0.01$ according to Student 't' test analysis comparing mean value with reference to 5-FU alone to that with reference to alpha-IFN + 5-FU. A similar analysis conducted on day 1 did not reach significant difference ($0.1 < P < 0.05$). Mean KC (10^6) values of day 1 or of day 18 were not significantly different from that of day 0. On day +5 mean KC (10^6) value with reference to alpha-IFN + 5-FU, but not that relevant to 5-FU alone was significantly higher ($P < 0.05$) than that of day 0. On days 9 and 15 all mean KC (10^6) values were significantly higher ($P < 0.05$) than on day 0.

MNC counts relevant to 5-FU vs alpha-IFN + 5-FU cycles did not differ significantly from day 0 through day 15. On day 18 mean MNC counts expressed in cells/mm^3, ± standard error, were 1691 ± 155 (5-FU cycles) and 1021 ± 91 (alpha-IFN + 5-FU, $P < 0.05$) respectively. However, this difference did not result in a statistically significant difference between the 2 groups when the NKA per ml of blood is considered.

on day 0) in erythrocyte counts or hemoglobin was found on day 18.

Complete recovery of white blood cell (WBC) and platelet counts occurred within 30 days of each cycle, regardless of the drug regimen used (data not shown).

Alpha-IFN administration was followed by fever and moderate or minimal flu-like symptoms, muscle pain and weakness. Antipyretic therapy with Paracetamol was given for pyrexia greater than 38.5°C. Hyperpyrexia occurred in all patients 2–5 h after administration of 3×10^6 IU/m^2 of alpha-IFN. Gastrointestinal toxicity and mucositis were moderate in 10 and severe in 2 patients, not, however, requiring treatment suspension but only supportive therapy.

Clinical responses consisted of 1 'complete response' (i.e., complete disappearance of all lesions)

lasting more than 3 years (this patient is still alive, although with progressive disease), 1 'partial response' (i.e., reduction of more than 50% of all measurable lesions) lasting 430 days, 5 'stable disease' (i.e., a less than 50% reduction of all measurable lesions, without appearance of new lesions) lasting from 60 to 200 days, and 5 'progressive disease'.

Peripheral blood MNC were collected from all patients and tested for NKA. The results, illustrated in Fig. 2, show that: a) treatment with 5-FU alone did not significantly impair NK cell function; b) on day 5, treatment with alpha-IFN was associated with higher NKA than that found in patients on day 0 or following treatment with 5-FU alone; c) on days 9 and 15 but not on day 18, NKA was significantly higher than on day 0. However, no significant difference was found between 5-FU and alpha-IFN + 5-FU treatment.

Phenotypic analysis of MNC (Table 1) shows that:

Table 1. Lymphocyte subpopulations of patients during chemotherapy or immuno-chemotherapy cycles.

Lymphocyte subpopulation[a]	Day 0	Day 5		Day 18	
		CC	ICC	CC	ICC
CD4+	458±65[b] (23.9)	741±91[d] (40.7)	465±49[c] (29 6)	612±113 (36.2)	330±51[d,c] (32 4)
CD8+	203±42 (10.6)	109±24[d] (6)	173±28[c] (11)	98±29 (5.8)	87±20[d] (8.5)
CD4/CD8 ratio	2.26	6.8	2.69	6.2	3.79
CD16+	103±29 (5.4)	69±16 (3.8)	75±23 (4.8)	37±21[d] (2.2)	68±22 (6.7)

[a] Phenotypic analysis of MNC was performed on days 0 (i.e., 'basal value for both cycles'), 5 or 18 during chemotherapy cycles (CC, i.e. 5-FU treatment alone), or immuno-chemotherapy cycles (ICC, i e. alpha-IFN + 5-FU treatment).

[b] Mean number (± standard error) of positive cells/μl of blood (9–14 observations per group). In parenthesis the percentage of positive cells with respect to total MNC.

[c] Significant difference ($P < 0.05$) between ICC and CC.

[d] $P < 0.05$ (with respect to day 0).

(a) chemotherapy alone produced an early and long-lasting decrease in the number of CD8+ MNC. By contrast, a significant increase in CD4+ MNC count occurred on day 5, resulting in a substantial increment in CD4+/CD8+ cell ratio; (b) alpha-IFN administration before 5-FU treatment produced a complete inhibition of the changes of CD4+ and CD8+ cell counts induced by 5-FU on day 5. However, the drop in CD8+ cell count observed on day 18 after treatment with 5-FU alone did not differ significantly from that observed in immuno-chemotherapy cycles; (c) 5-FU treatment induced a marked decrease of the number of CD16+ cells on day 18. In this case alpha-IFN pretreatment did not afford a statistically significant protection.

2. *In vitro studies*

The results of the *in vivo* studies showed that alpha-IFN + 5-FU depressed MNC count more than did 5-FU alone on day 18 (see footnote of Fig. 2). Two main mechanisms could explain these results: (a) alpha-IFN induces cytostatic effects on peripheral MNC precursors [15]. In this case MNC recovery after 5-FU-induced myelosuppression is simply delayed, but non-proliferating MNC precursors could be actually protected from the toxic effects of 5-FU, a cycle-specific cytotoxic drug; (b) alpha-IFN sensitizes MNC precursors to the toxic effects of 5-FU. In this case no protection, but rather additive or synergistic myelotoxic effects, could be afforded by the agents used in combination. Complete recovery of WBC counts, including MNC and platelets, occurred in all patients at the end of alpha-IFN + 5-FU cycles (data not shown). This suggests that the low doses of alpha-IFN utilized in the present study do not potentiate the myelotoxic effects of 5-FU.

To test this hypothesis, MNC of healthy donors were subjected to a sequential treatment *in vitro*, designed according to the following 4-step schedule (Fig. 3): (a)

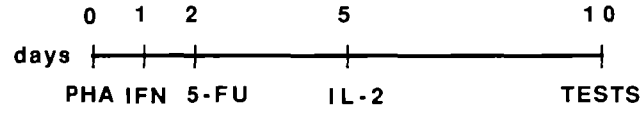

Fig. 3. Treatment schedule of normal MNC *in vitro*.
PHA, 4 μg/ml; IFN, alpha-IFN, 10^3 U/ml; 5-FU, 5-fluorouracil 180 μg/ml for 1 h at 37°C; IL-2, 100 Cetus Units/ml.
Tests: cell count, evaluation of NK cell activity, and flow cytometric analysis for CD3+, CD4+ and CD8+ cells.

treatment with PHA (day 0) to induce MNC proliferation; (b) exposure to alpha-IFN (day 1), presumably resulting in cytostatic effects; (c) exposure to 5-FU (day 2) which is expected to produce cytotoxic effects in proliferating cells; (d) second treatment with IL-2 (day 5) to induce expansion of IL-2R+ cells not irreversibly inhibited by drug pretreatment. A cell count assay for NK cell activity and phenotypic analysis were performed on day 10 of culture. Technical details of the *in vitro* experiments are described in the footnote of Fig. 3.

The results of a representative experiment (Table 2) show that: (a) alpha-IFN produced a modest but significant reduction of cell growth; (b) marked inhibitory effects were caused by treatment with 5-FU alone; (c) sequential treatment with alpha-IFN + 5-FU resulted in a total number of viable MNC significantly higher than that found in samples treated with 5-FU alone; (d) NK cell activity in the control group was much higher than that found on day 0, as expected on that basis of IL-2-induced boosting of natural cytotoxic activity and expansion of IL-2R+ cell population; (e) no significant difference in the relative cytolytic activity was detected in any of the treated groups; (f) a slight increase or reduction of the percentage of CD3+ cells over that of the control was produced by alpha-IFN or by 5-FU (alone or in combination with alpha-IFN), respectively; (g) no substantial changes in the percentage of CD4+ cells were found in treated groups as compared with

Table 2. Effect of alpha-IFN, 5-FU or alpha-IFN + 5-FU on polyclonally-stimulated MNC *in vitro*[a].

Parameter[b]	Control	Alpha -IFN	5-FU	Alpha -IFN + 5-FU	p[c]
FCC ($\times 10^{-6}$)	6.0	5.3*	0.43**	0.55**	<0.01
KC(10^6)($\times 10^{-1}$)	5.0	5.0	4.5	5.0	NS
% CD3+ cells	43	48*	35*	37*	NS
% CD4+ cells	28	30	32	33	NS
% CD8+ cells	18.5	19.5	11.5*	11*	NS

[a] MNC were treated *in vitro* according to the treatment schedule described in Fig. 3.

[b] All tests were performed on day 10 of culture FCC, 'final' cell count, i.e. geometric mean of the number of total MNC generated by 10^6 cells, as determined in 4 separated samples. The use of geometric means is dictated by the observation that cell count values follow Poisson distribution. The inital MNC preparation (i.e. on day 0) showed NK activity corresponding to 1.6 KC(10^6)($\times 10^{-3}$).

[c] P, probability calculated according to Student's 't' test analysis, comparing mean values referred to 5-FU group vs those referred to alpha-IFN + 5-FU group. NS, not significant.

* $P < 0.05$; ** $P < 0.01$, according to Student's 't' test analysis, comparing mean values of the control vs those of treated groups.

the untreated control; (h) 5-FU produced a considerable reduction of the relative number of CD8+ cells. This effect was not antagonized by alpha-IFN pretreatment.

Discussion

Treatment of advanced colorectal cancer with a number of chemotherapeutic regimens has been largely disappointing, since essentially no drug combination has been found to be consistently superior to 5-FU monochemotherapy [16]. However, recent data suggest that high-dose 5-FU combined with alpha-IFN produces high remission rates despite marked toxicity [17], although other clinical trials have not fully confirmed these results [18].

Amplification of the antineoplastic activity of 5-FU by alpha-IFN has been demonstrated in experimental conditions in which the host's immune system was not involved [19]. However, additive or synergistic effects between chemotherapy and antitumor NI have been demonstrated in experimental models *in vivo* [4] and *in vitro* [20]. Therefore, it is reasonable to hypothesize that the high response rate obtained in colorectal cancer following treatment with high-dose 5-FU + alpha-IFN [17] could be due, at least in part, to an IFN-mediated increase of NI acting in synergism with 5-FU chemotherapy.

Previous investigations performed by our group [21] showed that CMF chemotherapy depressed NKA of peripheral blood MNC in breast cancer patients. *In vitro* studies showed that NI impairment was presumably due to cyclophosphamide, with 5-FU or MTX unable to affect NKA. In the same study beta-IFN antagonized either *in vivo* or *in vitro* the depressive effects of chemotherapy on the NK function.

In the present investigation 5-FU did not show inhibitory effects on NKA in cancer patients, confirming the result of the previous *in vitro* experiments [21]. In addition, combined treatment with alpha-IFN+ 5-FU resulted in an early and significant increase of NKA of patient MNC. A similar transient increase which occurred on days 9 and 15 after 5-FU administration cannot be explained on the basis of the present findings.

Drug treatment would probably have been followed by a decline of suppressor cells acting on the NK function. Alternatively, 5-FU could have preferentially spared cytolytic NK cells *in vivo*, suppressing other compartments of MNC population.

The present study also revealed that *in vivo* treatment with 5-FU results in a temporary increase of CD4+ cells and in a long-lasting deficiency of CD8+ cells. No data are presently available to explain this phenomenon. The hypothesis that the drug would influence the number of circulating CD8+ cells through a redistribution mechanism without affecting their total number does not appear to be supported by the *in vitro* experiments. Actually the results illustrated in Table 2 point out that the *in vitro* inhibitory effects of 5-FU on proliferating T cells are preferentially directed against the CD8+ lymphocyte subset. The finding that *in vivo* treatment with alpha-IFN can reverse, at least temporarily, the effect of 5-FU on CD4+/CD8+ populations cannot be easily explained. The data available from the literature concerning the effect of IFN on human T cell subpopulations are contradictory. In adult patients with cutaneous T-cell leukemia, the topical injection of IFN restored the normal CD4/CD8 ratio [22]. Moreover, melanoma patients treated with recombinant alfa-IFN showed a decrease from pretreatment levels in the suppressor T cell (CD4+/CD11+) subset and a dose-dependent decrease in the helper/inducer (CD4+, Leu 8+) T-cell subset [23]. However, in patients with B chronic lymphocytic leukemia the number of CD57+ lymphocytes increased after alpha-IFN treatment without changes in the remaining T-lymphocyte (CD2-CD4 and CD8) and NK (CD16 and CD11b) subpopulations [24].

Treatment cycles with sequential alpha-IFN + 5-FU administration resulted in a more profound depression of WBC (data not shown) and MNC counts (Fig. 2) than did those in which 5-FU alone was used. As previously mentioned, this finding could suggest that the alpha-IFN + 5-FU combination may provide additive or synergistic toxic effects on WBC/MNC precursors and mature proliferating targets. However, the complete recovery of WBC/MNC counts found at the end of all cycles (data not shown) does not favour this interpretation.

The *in vitro* studies performed in the present investigation provide information on the mature compartment of the immune system, leaving open the question of precursor cells. The results, illustrated in Table 2, actually show that alpha-IFN would, at least in part, antagonize rather than increase 5-FU-mediated suppression of PHA-stimulated proliferating MNC. It is

possible that alpha-IFN, added on day 1 of culture, temporarily inhibited MNC proliferation induced by PHA, as confirmed by the lower cell count of IFN-treated MNC compared to that of controls on day 5 (data not shown) and on day 10 (Table 2). Therefore, it is reasonable to assume that a cell cycle-specific agent such as 5-FU produced less toxic effects against IFN-treated MNC than against fully proliferating untreated cells.

In conclusion, the present study suggests that alpha-IFN, apart from its direct antitumor effects, could play a role in the immuno-chemotherapy of colorectal cancer mainly through its effect on the NI compartment of the immune system. It also cannot be ruled out that in certain schedule-dependent conditions alpha-IFN would protect mature proliferating MNC from the toxic effects of cell cycle-specific antitumor agents.

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