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Preservation of immune effector cell function following administration of a dose-intense 5-fluorouracil-chemotherapy regimen

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Abstract. In a phase II clinical trial of 5-fluorouracil (5FU) plus *N*-(phosphonacetyl)-L-aspartate (PALA) therapy administration, a number of slowly developing clinical responses were observed. Because of this, a variety of immune parameters were sequentially studied in 21 patients on this trial. Of the 21 patients studied, 20 provided sufficient samples to compare baseline with subsequent values, 10 of the 20 patients responded to treatment. Responders and non-responders did not differ in any studied parameter at baseline. After 2 months of therapy, non-specific monocyte cytotoxicity (NSMC), antibody-dependent monocyte cytotoxicity (ADMC) and natural killer (NK) activity were higher in the entire study population, but these increases were not statistically significant. When responders and non-responders were evaluated separately, it was apparent that the trend was due solely to the changes observed in the responding patient population. When mean lysis values for each patient group were determined for each studied time point, it was possible to generate a mean area under the cytotoxicity/time curve (AUC) for each studied parameter. NSMC and ADMC did not differ in responders and non-responders. However, NK activity was significantly greater by mean AUC analysis ($P = 0.006$) in the responding group; NK activity was maintained in the responders, but decreased in non-responders. When lymphocyte and monocyte expression of the surface markers β_2-microglobulin, HLA-DR, CD56, HNK-1, CD16 and interleukin-2 receptor were evaluated, there were no differences among responders and non-responders at baseline by mean AUC analysis or when comparing baseline with non-baseline values. It is concluded that although baseline immunological characteristics do not identify patients who are likely to respond to weekly 5FU and PALA, treatment is not associated with deleterious effects on the immune effector function parameters evaluated in this study, there being no effects on expression of a variety of associated cell-surface molecules.

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Introduction

5-Fluorouracil(5FU)-based chemotherapy is widely considered to be associated with suppression of immune function [16, 14, 23, 13, 1, 18, 3, 4, 25]. Only a few reports have described augmentation of immune function associated with this drug [5, 8, 12]. Recently, Ardalan et al. reported encouraging clinical responses in a phase I–II study using 5FU and *N*-(phosphonacetyl)-L-aspartate (PALA) for a variety of gastrointestinal malignancies [2]. On the basis of this, we initiated a phase II trial of this combination in patients with metastatic, measurable colorectal carcinoma. An encouraging response rate was observed in this study [17]. Interestingly, a number of clinical responses occurred gradually, with some responses continuing to evolve as long as 6 months following the initiation of therapy. This suggested that immunological mechanisms may have contributed in part to some of these responses, even though 5FU has been felt to be immunosuppressive.

To test this hypothesis and examine the effects of this dose-intense 5FU-based regimen on immune effector cell function, peripheral blood mononuclear cells were obtained and analyzed at monthly intervals from patients treated in this clinical trial. The expression of a variety of cell-surface structures and the ability of purified mononuclear cells to mediate lysis of allogeneic tumor targets were evaluated.

Materials and methods

Patients. All patients enrolled into this study had metastatic or unresectable adenocarcinoma of the colon and rectum, measurable tumor and an ECOG performance status of 0 or 1. Treatment was administered weekly, beginning with 250 mg/m^2 PALA given intravenously by rapid i.v. infusion. After 24 h a 24 h continuous intravenous infusion of

2600 mg/m^2 5FU was begun. Patients were evaluated for toxicity and response monthly. Responders were defined as those who experienced at least 50% reduction of the sum of the bidimensional products of all measurable lesions for at least 4 weeks. Of the 37 patients enrolled onto this study, 21 had blood taken for studies of immune function; 1 patient had baseline studies only. Of the 21 patients, 10 responded objectively, with a median time to response of 2.5 months. Several responses were unusual since they evolved over an extended period of time.

Cell isolation: Peripheral blood mononuclear cells were obtained from 20 ml heparinized blood by density centrifugation of Ficoll/Isopaque gradients as described [6]. Peripheral blood monocytes and lymphocytes were separated by reversible adherence of monocytes to gelatin and autologous-plasma-coated plates as described [7]. The purity of the monocyte preparations was greater than 80% in almost all samples; lymphocyte purity exceeded 95%. Since the preservation of monocyte function following prolonged frozen storage has proven to be variable, all studies were performed on freshly isolated cells.

Cellular phenotyping. All the antibodies used for phenotyping lymphocytes and monocytes were obtained from Becton Dickinson, Mountain View, California, except for β_2-microglobulin. Anti-(human β_2-microglobulin) conjugated to fluorescein isothiocyanate was produced by ICN Immunobiologicals, Lisle, Ill. Lymphocytes and monocytes were separately suspended at 10^6 cells/100 µl RPMI-1640 medium containing 10% fetal calf serum. Cells were incubated with the respective fluorosceinated antibodies for 30 min at 4°C. Following incubation, the cells were washed twice cold phosphate-buffered saline, and fixed with 1% paraformaldehyde in phosphate-buffered saline. Analysis was performed by flow cytometry (FACScan; Becton Dickinson, Sunnyvale, Calif.).

Cytotoxicity assays. Non-specific monocyte cytotoxicity (NSMC) was determined using 18-h ^{111}In-release assays, as described [28]. Briefly, purified monocytes were seeded into 96-well plates at a density of 5×10^5 cells/well in a total volume of 100 µl. ^{111}In-labeled cells of the SW948 colorectal carcinoma line were added to the wells to yield effector-to-target ratios of 50:1. The cells were incubated for 18 h and 100 µl supernatant was removed and assayed for radioactivity release using a Beckman 4000 gamma counter. Antibody-dependent monocyte cytotoxicity (ADMC) was calculated by a similar assay, with 10 µg/well murine monoclonal antibody 17-1A added to each well. This antibody binds to a 33-kDa cell-surface antigen expressed by SW948 cells and mediates ADMC against this cell line [29]. ADMC results are reported as the total percentage lysis, without subtracting the NSMC contribution. Natural killer (NK) activity was assessed by an 18-h ^{111}In-release assay using the erythroleukemia line K562 as the target. Except for the target line, the assay was performed identically to the NSMC assays. Although NK activity is commonly determined using 4-h assays, 18-h time points were used so that the monocyte and lymphocyte samples could be processed in parallel. In our laboratory, 18-h ^{111}In- and 4-h ^{51}Cr-release assays yield equivalent results (data not shown). To assess lymphokine-activated killer (LAK) activity, peripheral blood lymphocytes were activated by incubating 2×10^6 cells/ml in RPMI-1640 medium containing 10% fetal calf serum supplemented with 1000 units/ml human recombinant interleukin-2 (rIL-2) (Cetus). Following 72-h incubations, the activated cells were washed and added to ^{51}Cr-labeled cells of the Daudi line (targets) at varyious effector-to-target ratios. The cells were incubated for 4 h, following which aliquots of supernatants were removed and radioactivity release determined as for the natural killer assays. The formula for calculation of cytotoxicity was:

$$\text{cytotoxicity (\%)} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}} \times 100$$

Spontaneous release never exceeded 30% in evaluable assays. Lytic units were calculated as described [19].

Data analysis. A multivariate model of patient data was used in order to estimate the median and mean area under the cytotoxicity/time curve (AUC) for any studied immune parameter [27]. Since these assays were associated with varied interpatient and intrapatient results, this multivariate model was employed to estimate an AUC of a treatment cohort rather than that of a single patient. This method quantifies the data sets for each treatment cohort, and facilitates statistical comparison of these cohorts. The value of this study is predicated on the assumption that the analysis of cumulative effects of therapy will be more accurate, and possibly more biologically meaningful, than analysis obtained at selected times out of the entire data set. Thus, mean AUC values for NK activity reflect the cumulative NK activity over time for each treatment cohort. Higher mean AUC values connote greater overall NK activity. When median data were used to find median AUC values, the computed variance was multiplied by 1.571 ($\pi/2$) to approximate the resulting larger variance of the estimate.

The statistic:

$$z = [\text{AUC}(1) - \text{AUC}(2)] / [var\ \text{AUC}(1) + var\ \text{AUC}(2)]$$

was treated as normal, mean 0 varian 1 for comparing AUC, values.

Table 1. Baseline immunological parameters

Studied parameter	Lysis of target cells (%)		
	All patients	Responders[a]	Non-responders[a]
NK[b]	14.6 ± 3.7[c]	10.5 ± 5.8	12.4 ± 4.5
NSMC[d]	16.1 ± 4.2	11.1 ± 4.1	23.4 ± 8.1
ADMC[e]	30.7 ± 6.1	28.2 ± 5.1	34.0 ± 12.8
IL-2R[f]	10.4 ± 2.6[g]	6.9 ± 2.1	16.6 ± 5.7
CD3[h]	51.1 ± 7.6	44.7 ± 11.6	62.8 ± 4.3
HNK1[h]	23.5 ± 6.8	23.0 ± 10.2	24.4 ± 6.9
NKH1[h]	17.8 ± 3.2	13.8 ± 3.0	24.9 ± 6.4
CD16[h]	13.2 ± 2.2	12.8 ± 2.3	13.9 ± 5.0
β-Microglobulin[h]	392.8 ± 84.4[i]	360.9 ± 82.8	450.0 ± 196.9
HLA-DR[h]	150.1 ± 54.6	197.6 ± 81.1	64.7 ± 23.6

[a] Response is defined as a greater than 50% reduction of the sum of the bidimensional products of measurable lesions following therapy, lasting for at least 4 weeks. In no parameter studied did responders and non-responders differ statistically at baseline

[b] Natural killer activity was determined by lysis of ^{111}In-labeled K562 targets as described in Materials and methods

[c] Results for NK, non-specific monocyte cytotoxicity (NSMC) and antibody-dependent monocyte cytotoxicity (ADMC) presented as the percentage specific target lysis ± SEM at 50:1 effector-to-target ratios

[d] Non-specific monocyte cytotoxicity against SW948 targets

[e] Antibody-dependent monocyte cytotoxicity against SW948 targets using the murine IgG2A monoclonal antibody 17-1A

[f] Lymphocyte cell-surface expression of the 50-kDa subunit of the interleukin-2 receptor

[g] Results expressed as percentage of positive cells ± SEM for IL-2R, CD3, HNK1, NKH1, CD16

[h] Lymphocyte cell-surface expression of these molecules

[i] Results expressed as mean channel of fluorescence ± SEM for β_2-microglobulin and HLA-DR

Results

Baseline parameters

The results of parameters studied in all patients at baseline are summarized in Table 1. Since nearly half of the patient population responded to this chemotherapy regimen, an adequate sample of both responders and non-responders was available for analysis. There was no difference between the groups in any of the studied cytotoxicity parameters (NK, NSMC, ADMC) or in the percentage of cells expressing natural-killer-associated antigens (HNK-1,

Table 2. Influence of 5-fluorouracil (5FU) and *N*-(phosphononacetyl)-L-aspartate (PALA) therapy on effector cell cytotoxicity

Cytotoxic activity	Percentage lysis at baseline (n^d)	Percentage lysis at 2 months (n^d)
NSMC[a]		
All patients	16.1±4.2[b] (15)	28.5± 9.8 (9)
Responders	11.1 (9)	37.1 (6)
Non-responders	23.4 (6)	11.2 (3)
ADMC		
All patients	30.7±6.1 (16)	41.9±17.4 (18)
Responders	28.2 (9)	46.5 (6)
Non-responders	34.0 (7)	28.2 (2)
NK		
All patients	12.5±5.2[c] (17)	40.9±18.2 (11)
Responders	16.9 (9)	56.1 (7)[e]
Non-responders	7.6 (8)	14.3 (4)

[a] NSMC, non-specific monocyte cytotoxicity; ADMC, antibody-dependent monocyte cytotoxicity; NK, natural killer activity. See Materials and methods for experimental details

[b] Results for NSMC and ADMC expressed as percentage lysis ± SEM of SW948 targets at 50:1 effector:target ratios

[c] Results for NK expressed as lytic units contained in 10^7 effectors, using 20% lysis of K562 targets as the basis for calculations

[d] *n*, number of patients contributing to each data point

[e] No statistical differences were found between groups or time points in any of the three types of assay

NKH-1, CD-16). Neither class I nor class II antigen expression differed on lymphocytes of the responding and non-responding patients; in addition, no difference in the expression of these molecules on treated patients' monocytes were observed (data not shown). There was a trend for enhanced expression of the CD3 molecule and the 50-kDa chain of the interleukin-2 receptor expressed by T cells in non-responders but these differences did not reach statistical significance. This extensive battery of studies did not identify the subgroup of individuals who went on to respond to weekly 5FU and PALA.

Serial evaluations of cellular cytotoxicity and surface phenotype

Monthly peripheral blood samples were obtained from all patients on therapy. The ability of treated patients' monocytes and lymphocytes to lyse allogeneic targets was assessed as shown in Table 2. Because non-responding patients tended to go off study rapidly, we chose to compare cytotoxicity results at baseline and at 2 months. As can be seen, there was maintenance of cytotoxicity potential of treated patients' cells in all three assays, with trends toward enhancement in all three assays. Interpatient variability contributed to wide standard deviations of each tested mean. Nonetheless, it is apparent that the in vitro cytotoxic potential of treated patients' monocytes and lymphocytes was not deleteriously affected by weekly therapy with 5FU and PALA. As Table 3 shows, the data variability is not explained solely by an inability of non-responders to carry out cell-mediated lysis after 2 months of therapy.

Median area under the curve analysis of treatment effects

To take into account all of the cytotoxicity values obtained in these patients, the mean area under the curve over time was determined for each of the three cytotoxicity assays in the responding and non-responding patients. Figure 1 compares NK activity profiles of responders and non-responders. The mean percentage of cell lysis at an effector:target ratio of 50:1 was determined from all patient samples obtained in each group at each study time point. Mean values were connected and the area under the curve was then determined as described in Materials and methods. Variances of the mean determinations never exceeded 5% of the calculated values. Examination of Fig. 1 not only demonstrates the enhanced mean AUC in responders, but also shows that this enhancement is largely due to maintenance of NK activity over time, in contrast to the pattern seen in non-responders. It should be noted that the mean AUC values described here represent the percentage lysis observed per month on study. Thus, the enhancement seen in responders cannot be attributed to the longer period of time during which cytotoxicity assays were obtained in these patients. Of 10 clinically responding patients who had multiple samples obtained, 7 had overall augmentation of NK activity, in contrast to only 2 of 6 clinically non-responding patients with multiple available blood samples. The other 4 patients exhibited decreases in NK activity.

The NK difference between responders and non-responders was significant ($P = 0.036$) by mean AUC analy-

Table 3. Biological effects of 5FU and PALA therapy in responders and non-responders[a]

Cytotoxic activity	Percentage lysis in responders		Percentage lysis in non-responders	
	Baseline (*n*)[e]	2 months (*n*)	Baseline (*n*)	2 months (*n*)
NSMC[b]	11.1[c] (9)	37.1 (6)	23.4 (6)	11.2 (3)
ADMC	28.2 (9)	46.5 (6)	34.0 (7)	28.2 (2)
NK	16.9 (9)[d]	56.1 (7)	7.6 (8)	14.3 (4)

[a] No statistical differences were found between groups or time points in any of the three types of assay

[b] NSMC, non-specific monocyte cytotoxicity; ADMC, antibody-dependent monocyte cytotoxicity; NK, natural killer activity. See text for details

[c] Results expressed as mean percentage specific lysis of SW948 target cells

[d] Results expressed as lytic units/10^6 cells using 20% lysis as the standard

[e] *n*, number of patients contributing to each data point

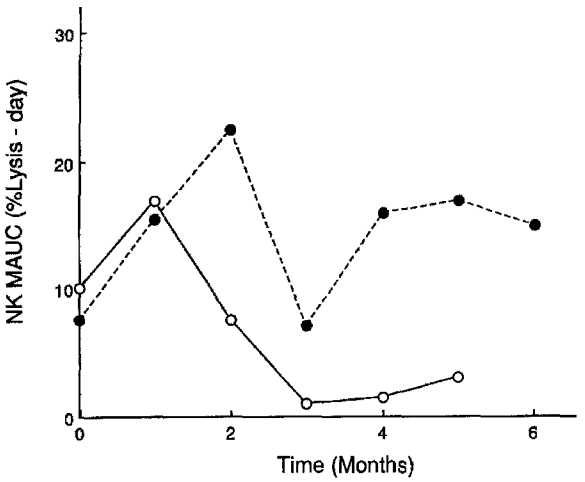

Fig. 1. The median area under the curve (*MAUC*) was calculated by determining the median percentage lysis of K562 targets by patiens' lymphocytes during fluorouracil (5FU) and *N*-(phosphonacetyl)-L-aspartate (PALA) chemotherapy. Each point represents the median lysis value for all patients (– – – –, responders; ——, non-responders) at each studied time point. The median AUC for NK activity of each group was determined as described in the text to estimate the cumulative effects of therapy on this biological parameter in those patients receiving 5FU and PALA. The median AUC (expressed as percentage lysis × day) was 0.159% for responders and 0.067% for non-responders ($P = 0.036$)

sis. As shown in Table 4, responders and non-responders did not differ with regard to NSMC or ADMC activity by this method of analysis. When expression of a variety of cell surface markers was assessed by using the mean AUC method of data analysis, there were no significant differences among the responders and non-responders (Table 5). As with the cytotoxicity analyses, treatment had no significant effects on the cell-surface expression of any of the studied parameters.

Treatment effects on in vitro cellular responses to interleukin-2

These cytotoxicity and phenotypic studies evaluated the ability of freshly obtained monocytes and lymphocytes to express cell-surface molecules and mediate cytotoxicity directed against allogeneic tumor targets. To assess the ability of these cells to undergo activation following appropriate stimulation, peripheral blood lymphocytes were incubated in human rIL-2 for 72 h and their ability to lyse ^{51}Cr-labeled Daudi cells was determined (Table 6). Weekly 5FU and PALA therapy did not impair the ability of treated patients' lymphocytes to respond in vitro to exogenous human rIL-2. No differences were noted between clinically responding and non-responding patients.

Discussion

In this study, weekly chemotherapy with 5FU and PALA was associated with no evidence of progressive compromise of the studied immunological parameters. Deterioration of the studied immune parameters was observed only in patients with progressive disease and natural killer activity was the most sensitive tested indicator. While these data do not demonstrate immunological mechanisms of antitumor response, the lack of treatment-associated immunological deterioration is of interest.

Considering the extent of the literature regarding the immunosuppressive properties of 5FU, the demonstration that this patient group maintained the studied immune parameters is noteworthy. The wide interpatient variability in response in the assays used made it difficult to demonstrate significant augmentation of any of the studied immune parameters, although all of the cytotoxicity parameters that were studied rose over time (Table 2). This maintenance of immune function in patients treated with 5FU and PALA

Table 4. Mean AUC analysis of sequential cytotoxicity assays in patients receiving 5FU and PALA therapy

Cytotoxic activity	Mean AUC		
	Responders	Non-responders	*P*
NSMC	18.8[a]	17.4	>0.1
ADCC	23.1	20.1	>0.1
NK	15.9	6.7	0.036

[a] Results expressed as percentage lysis × month at effector : target ratios of 50 : 1

Table 5. Mean AUC analysis of sequential cell-surface antigen changes in patients receiving 5FU and PALA therapy

Cytotoxic activity	Mean AUC		
	Responders	Non-responders	*P*
NKH-1	18.0[a]	20.2	>0.1
IL-2R	9.0	15.8	0.092
CD16	10.2	10.6	>0.1
HNK1	17.4	31.6	>0.1
β_2-Microglobulin	300.9[b]	293.8	>0.1
HLA-DR	46.1	34.0	>0.1

[a] Results for NKH1, IL-2R, CD16 and HNK1 expressed as percentage positive cells × month

[b] Results for β^2-microglobulin and HLA-DR expressed as mean channel of fluorescence × month

Table 6. Effects of 5FU and PALA therapy on the ability of patients' peripheral blood mononuclear cells to generate lymphokine-activated killer activity in response to interleukin-2

Treatment status	Percentage lysis + SEM (*n*)
Untreated[a]	64.7± 4.8[b] (6)
1 Month	90.0± 1.8 (3)
2 Months	50.6±16.9 (4)
3 Months	40.9±12.2 (5)
4 Months	59.1±11.7 (2)
5 Months	43.3±14.8 (4)

[a] Peripheral blood mononuclear cells from normal control donors were used in this group

[b] Percentage specific lysis of ^{51}Cr-labeled Daudi cells at 50:1 effector:target ratios

may be explained by our selection of a relatively novel panel of assays to assess immune function. Had we chosen other functional assays, such as induction of delayed-type hypersensitivity, the results may have led to different conclusions. However, the most likely explanation for our results is that the patient population studied contained enough clinically responding patients so that the confounding effects of progressive disseminated disease did not negatively bias the results.

The data presented in this study do not exclude the possibility that other measures of immune responsiveness, or the development of such responses, may be adversely affected by the chemotherapy regimen employed here. However, these data clearly show that the chemotherapy used here does not adversely effect the studied parameters of effector function. Analysis of responders and non-responders is always fraught with potential biases. However, there were no obvious intrinsic differences between these populations at baseline, and no evidence that immunological mechanisms underlie the clinical responses observed in this clinical trial.

PALA is known to perturb nucleotide pools in normal and malignant lymphoid cells, but the effects of PALA on immunity are not known. Additional studies will be required to determine the influence of PALA therapy on the expression of cellular cytotoxicity in the setting of 5FU therapy. However, since NK activity declined in patients with clinical progression, the results of this study do not support the interpretation that the maintenance of NK function in clinically responding patients was due to immunological enhancement by PALA. Both immunosuppressive [16, 14, 23, 13, 1, 18, 3, 4, 25] and immunopotentiating [5, 8, 12] properties have been attributed to 5FU in murine [13, 1, 18, 3, 4, 8, 12] and human [16, 23, 5] studies. Among the reported immunosuppressive effects of 5FU are decreased or delayed humoral responses to bacterial antigens [14, 13, 18, 4], loss of established and induced delayed cutaneous hypersensitivity [14], decreased T-lymphocyte-mediated cytotoxicity [13, 3, 25], inhibition of NK-mediated cytotoxicity [1], diminished in vitro T cell blastogenesis in response to phytohemagglutinin [16, 3, 4] and purified protein derivative [16], decreased allogeneic tumor rejection [18], and induction of tolerance to antigen in vitro. Immunopotentiating effects reported include the enhancement of delayed cutaneous hypersensitivity [5, 8] and the inhibition of suppressor T cell activity [12]. There is presently no consensus regarding which of these immunomodulatory properties predominates in man. Interpretation of these studies is limited by (a) variability in the immune parameters studied, (b) variability in the doses and schedules of 5FU administration, (c) the inability to distinguish completely the effects of malignancy on immune functions from those of 5FU and (d) the wide inter-subject variability of responses to the various tests utilized, with limited numbers of subjects in each study.

Evaluation of the immunological consequences of any treatment is hampered by the innate variability of such assays because of interpatient variability and the presence of immune derangement associated with the underlying disease for which treatment is given [11]. For example, the inability to identify statistically significant differences in responding patients at 2 months compared with baseline (Table 3) may be explained by the two individuals who failed to mount any response in the NSMC, ADMC and NK assays at either baseline or 2 months (data not shown). In small patient samples such as the one studied here, such outlying, consistently inactive peripheral blood samples may obscure otherwise valid biological effects. To address these issues, and to insure that all of the data obtained were incorporated into the analyses, mean AUC values were obtained. Mean AUC analysis has the additional value of summing cytotoxicity or phenotypic trends with a single number, thus facilitating statistical comparisons. By using mean values, outlying consistently low or high values at any given time will influence the determination of the mean value at that time, without necessarily obscuring the contribution of the remainder of the data. Thus, the mean AUC may more accurately reflect the true biological events occurring over time.

Mean AUC analysis (Fig. 1, Table 4) clearly demonstrates that natural killer activity was maintained in responding patients for prolonged periods of time. The decrease in NK activity seen in non-responders was probably due to their progressive disease, and suggests this as an explanation for some of of the negative immunomodulatory effects previously attributed to 5FU since clinical responses have been infrequently observed in the past with 5FU therapy. As NK activity rose in responders, this parameter was not modified by treatment, but by response. While these data are consistent with the hypothesis that the antitumor response permitted maintenance of the studied immune parameters, we cannot exclude the possibility that some early, undetected augmentation of immune function discriminated between those patients destined to respond or fail clinically. However, the latter scenario is not felt to be an important mechanism of 5FU-chemotherapy-based clinical responses. Use of the mean AUC method, and the observation that varied results were obtained from different patients whose samples were assayed in parallel, reduce the likelihood that the observed differences were related to assays being performed on fresh, rather than batched samples. However, these data do not formally exclude the possibility that decreases in tumor burden and 5FU treatment have countervailing effects on NK activity, so that progressive disease merely unmasks the immunosuppressive effects of 5FU therapy. Formal exclusion of this latter scenario will require frequent monitoring early in the course of treatment and precise knowledge of the relevant immunological mechanisms to be studied.

5FU and PALA chemotherapy had no effects on the ability of treated patients' lymphocytes to generate lymphokine-activated killer (LAK) activity following in vitro exposure to human recombinant interleukin-2 IL-2 (Table 6). Although statistically significant enhancement ($P = 0.001$) was seen in the lysis of Daudi cells following 1 month of therapy, it is perhaps more interesting to note that there was no substantial diminution in the ability of treated patients' lymphocytes to generate LAK activity over a reasonably prolonged period of time.

There is increasing interest in combining 5FU-based chemotherapy with immunotherapy in the treatment of metastatic colorectal carcinoma and other gastrointestinal

malignancies. Clinical responses have been observed with murine monoclonal antibody therapy in patients with colorectal [21, 22] and pancreatic [24] adenocarcinomas. High-dose interleukin-2 and LAKA cell therapy has induced clinical objective responses in approximately 10% of patients with metastatic colorectal carcinoma [20]. Although there is no clear role for the recombinant human interferons in gastrointestinal malignancies, preliminary evaluations of 5FU combined with interferon α have shown promising results [26]. In the adjuvant treatment of Duke's stage C colon carcinoma the combination of 5FU with levamisole, an antihelminthic agent with immunomodulatory properties [10], produces improved 5-year disease-free and overall survival, reducing the expected cancer mortality by 33% [15]. Individually, neither 5FU nor levamisole produces this therapeutic benefit. Levamisole does not enhance the cytotoxicity of 5FU against colon carcinoma in vitro [9]; thus it is possible that immunological mechanisms underlie its therapeutic activity in combination with 5FU in the adjuvant setting.

In summary, we find no evidence of immunological suppression with this dose-intense regimen utilizing 5FU. Previous studies that implicated 5FU as an immunosuppressive agent in humans may have been negatively biased by the progressive disease associated with these treatment regimens. These observations may be of particular importance in the design of trials integrating 5FU and antitumor vaccines, where preservation of immune function will be critical to therapeutic success.

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