Timing and Specificity of the Cognitive Changes Induced by Interleukin-2 and Interferon-α Treatments in Cancer Patients

LUCILE CAPURON, PHD, ALAIN RAVAUD, MD, PHD, AND ROBERT DANTZER, PHD

Objective: Neuropsychological changes develop in patients treated by cytokine immunotherapy with interleukin-2 (IL-2) and interferon-α (IFN-α). However, the time course of appearance of these effects remains unclear, and their precise nature is still incompletely characterized. The objective of this study was to assess and characterize the early cognitive changes induced by IL-2 and IFN-α in cancer patients at the end of the first week of treatment and to investigate the subsequent evolution of these changes. **Methods:** The study was conducted in 47 cancer patients who received subcutaneous IL-2, administered alone ($N = 17$) or with IFN-α ($N = 7$), or IFN-α alone, administered subcutaneously at low doses ($N = 7$) or intravenously at high doses ($N = 16$). An automated battery of neuropsychological tests (Cambridge Neuropsychological Test Automated Battery) was used to measure reaction time, spatial working memory, and planning tasks. Cognitive tests were performed before treatment (day 1) and after 5 days (day 5) and 1 month of treatment. **Results:** On day 5, patients treated with IL-2 alone had impaired spatial working memory and lower accuracy of planning abilities. In contrast, patients treated with IFN-α did not show any impairment in performance accuracy in these tasks but showed longer latencies in the test of reaction time. Most of these early alterations persisted at the end of the first month of treatment without any obvious sign of worsening. **Conclusions:** These findings suggest the existence of early differential neuropsychological changes in patients treated with IL-2 and IFN-α. **Key words:** cancer immunotherapy, interleukin-2, interferon-α, side effects, neuropsychology, cognitive performance.

CANTAB = Cambridge Neuropsychological Test Automated Battery; IL-2 = interleukin-2; IFN-α = interferon-α; IV = intravenous; MC-MT = multiple-choice movement time; MC-RT = multiple-choice reaction time; SC = subcutaneous.

INTRODUCTION

The synthesis and release of proinflammatory cytokines by activated monocytes and macrophages during the course of the host response to infection are accompanied by a wide range of alterations in brain functions (1). Most prominent among these changes are an increase in the thermoregulatory set point responsible for the development of fever (2), an activation of the hypothalamic-pituitary-adrenal axis (3), behavioral depression, fatigue, weakness, and feelings of malaise (4). Administration of proinflammatory cytokines peripherally or directly into the brain induces similar symptoms (5–7). The possibility that cytokines also affect higher brain functions and cause changes in cognition was investigated by Smith et al. (8, 9), who demonstrated that administration of IFN-α to volunteers mimics the increase in reaction time that occurs during infection with influenza viruses. Similarly, we previously showed that, compared with healthy control subjects, individuals with flu-like syndrome displayed significant memory alterations that were not related to fever in tests specific for higher cognitive processing (10). Impairments in learning and memory have also been reported in animals injected with various cytokines, although the nature and intensity of the observed effects seem to be dependent on the cytokine and its mode of administration (11–13).

Most of the available data concerning the effects of cytokines on cognition come, however, from clinical studies performed in cancer patients treated with high doses of IFN-α and IL-2. One of the first studies investigating the neuropsychiatric side effects of cytokine treatments was conducted by Denicoff et al. (14) on cancer patients receiving very large doses of IL-2 and lymphokine-activated killer cell therapy. Results from this study showed that 50% of treated patients (22 of 44) displayed severe cognitive changes (spatial and temporal disorientation and impaired cognitive efficiency in psychometric tests) that were already observable at the end of the first week of treatment. These disturbances were reversible after cessation of therapy. A study of patients with chronic myelogenous leukemia treated with IFN-α showed that treated patients were less efficient in tests of cognitive speed, verbal memory, and executive functions than similar patients who had not undergone IFN-α therapy (15, 16). The authors of this study concluded that the pattern of cognitive changes observed in patients receiving IFN-α was suggestive of frontal-subcortical brain dysfunc-

From INSERM U394 (L.C., R.D.), Neurobiologie Intégrative, Institut François Magendie, Bordeaux Cedex, France; Department of Psychiatry and Behavioral Sciences (L.C.), Emory University School of Medicine, Atlanta, Georgia; and Institut Bergonié (A.R.), Comprehensive Cancer Center, Department of Medicine, Bordeaux Cedex, France.

Address reprint requests to: Dr Lucile Capuron, Department of Psychiatry and Behavioral Medicine, Emory University School of Medicine, 1639 Pierce Dr., Suite 4000, Atlanta, GA. Email: lcapuro@emory.edu

Received for publication November 30, 1999; revision received August 22, 2000.

0033-3174/01/6303-0376

tion. More recently, patients receiving immunochemotherapy combining IL-2, 5-fluorouracil, and leucovorin were found to display impaired performance in reaction time, picture recognition, and vigilance tasks and in various psychometric tests compared with control patients who received chemotherapy alone (17, 18). These results can be interpreted to suggest that frontostriatal alterations develop in patients treated with cytokines.

In most of the studies available, the neuropsychological effects of cytokine treatments were investigated either after several months of therapy or after intravenous administration of cytokines, which is known to increase the risk of toxicity. Moreover, these effects were generally observed only when they were severe, and they have not been systematically studied. Since the introduction of immunotherapy, treatment modalities have been modified to minimize general toxicity in cancer patients. For instance, use of the subcutaneous route, mainly for IL-2, is associated with reduced toxicity (19). However, the exact impact of the presently available treatments on cognition has not been thoroughly evaluated, especially at early stages of treatment. We previously reported that cytokine immunotherapy with IL-2 is associated with the appearance of depressive symptoms during the first days of treatment (20). In the present study, we tested the possibility that cognitive alterations also develop at early stages of treatment.

The objectives of the present study were 1) to investigate and characterize the early cognitive changes associated with IL-2 and IFN-α treatments in cancer patients, 2) to assess the evolution of these changes at later stages of treatment, 3) and to investigate the influence of treatment modalities on the appearance of these effects. The CANTAB battery, a well-standardized test for a wide range of cognitive functions (21, 22), was selected for these purposes.

METHODS

Patients

Forty-seven consecutive cancer patients (mean age, 50 years; SD, 14 years) participated in the study from July 1997 to July 1999. Patients were included in four distinct therapeutic protocols (Table 1). Seventeen patients with metastatic renal cell carcinoma were treated with IL-2 alone according to the Sleijfer protocol (IL-2 patients) (23). Seven patients with renal cell carcinoma received both subcutaneous injections of IL-2 and IFN-α (IL-2+IFN-α patients) (24). Seven patients with renal cell carcinoma were treated with low doses of IFN-α delivered subcutaneously (SC IFN-α patients). Sixteen patients with high-risk metastatic melanoma received high intravenous doses of IFN-α (IV IFN-α patients) according to the Kirkwood protocol (25).

This study was approved by the local committee for the protection of patients in biomedical research (CCPPRB Bordeaux A). All patients who participated in the study were adults, and all gave informed consent.

Patients treated with neuroleptics or presenting brain metastasis, neurological troubles, or severe visual disturbances were excluded from the investigation.

Cognitive Assessment

Cognitive assessment was performed using the CANTAB battery, which includes computerized tests presented on a touch-screen IBM computer, ensuring a standardized form of testing with instantly available data and detailed recording of accuracy and speed of responses (26).

Three specific tests from CANTAB were used to assess the cognitive performance of patients included in the study. These tests were chosen for their specificity in terms of cognitive functions involved and for their feasibility. All tests from CANTAB were culture- and language-free and have been shown not to be sensitive to gender (27).

Reaction time test. Besides providing a screen for the ability to acquire and perform a motor skill, reaction time was used to com-

TABLE 1. Characteristics of Patients and Treatment They Received

	IL-2	IL-2 + IFN-α	SC IFN-α	IV IFN-α
Primary cancer	RCC[a]	RCC	RCC	Melanoma
Therapeutic protocol				
Treatment	IL-2	IL-2	IFN-α	IFN-α
Dosage (first month)	Week 1: 18 MIU, D1–D5 Weeks 2–4: 9 MIU, D1, D2 18 MIU, D3–D5	Weeks 1 and 3: 18 MIU/m^2, D1–D5 IFN-α Weeks 1–3: 6 MIU, D1, D3, D5	Weeks 1–4: 18 MIU, D1, D3, D5	Weeks 1–4: 20 MIU/m^2, D1–D5
Route	SC	SC	SC	IV
Number of patients	17	7	7	16
Age (±SD), y	56 (11)	51 (10)	57 (10)	41 (15)
Gender (men/women)	13/4	5/2	4/3	9/7

[a] D1 = day 1; D3 = day 3; D5 = day 5; IV = intravenous; MIU = millions of international units; RCC = renal cell carcinoma; SC = subcutaneous.

pare the performance of patients in single- and multiple-choice reaction time tasks (SC-RT and MC-RT, respectively). The test was divided into five stages; each stage required increasingly complex chains of responses. In the first stage, the subject had to touch the screen when a yellow dot appeared in the center, being neither too soon nor too late (SC-RT). In the second stage, the choice reaction time task was introduced (MC-RT). At this level, the yellow dot could appear in any one of five locations. Successful subjects were introduced to a touch key, allowing one to distinguish reaction time (MC-RT) and movement time (MC-MT) for the subsequent stages.

Spatial working memory test. The test of working memory and strategy performance is a self-ordered searching task that is sensitive to frontal-subcortical dysfunction (28). In this test, the subject had to search through a spatial array of colored boxes for blue "tokens" in each box and use the tokens to fill an empty column on the right side of the screen while not returning to boxes where a token was previously found. The number of boxes increased from three and four to six and then eight, with practice and test trials at each level of difficulty. The number of between-search errors, the time to complete trials, and a strategy score derived from the number of search sequences in the six- and eight-boxes problems were measured as indices of performance. The strategy score retraced the "route" previously used by subjects in searching through the spatial array of boxes (29). One possible strategy was to follow a predetermined sequence, beginning with a particular box and then returning to start each new sequence with the same box as soon as a token was found (30). The lower the strategy score, the more efficient was the subject's performance.

Stockings of Cambridge test. This test of spatial planning was selected because of its high sensitivity to frontal dysfunction (31). It was based on the "Tower of London" test and contained incremental levels of complexity. The subject was shown two displays containing colored balls and had to use the balls in the lower display to copy the pattern shown in the upper one. The subject was told not to make the first move until he was confident that he could execute the entire sequence needed to solve the problem. The time taken to complete the pattern, the number of perfect solutions, and the average number of moves required were taken as measures of the subject's planning ability. Better performance corresponded to a lower number of ball moves used to copy the pattern. This copying task was followed by a task controlling for motor performance, in which the upper display moved one ball at time, repeating the moves made by the subject in the corresponding previous planning phase. The difference in time taken to initiate each problem was taken as an index of the additional time taken to plan the solution of the copying (initial thinking time), as distinct from the corresponding yoked following task. For data analysis, trials were dissociated according to their level of complexity: easy problems (two to three moves) and complex problems (four to five moves) (32, 33).

The mean duration for execution of the three tests was approximately 30 minutes. The order in which tests were presented to subjects was counterbalanced for each patient at each time of testing.

Time and Conditions of Testing

Patients were tested with the CANTAB battery on the first day of hospitalization before initiation of treatment (day 1), on the fifth day of treatment (day 5), and at the end of the first month of treatment (1 month). Because the first cycle of treatment associating IL-2 and IFN-α (IL-2 + IFN-α) included only 3 weeks of therapy (see Table 1), this last evaluation was conducted at the end of the third week of treatment for patients from this group. For other patients, the evaluation at 1 month of treatment was conducted at the end of the fourth week of therapy.

Cognitive assessments were performed at approximately the same time of day for all patients, usually in the morning. Evaluations were not done when patients experienced grade 2 or above fever (>38°C), headaches, myalgia (from moderate to severe and treatment-resistant), or vomiting (from transient to intractable) as defined by WHO criteria (34). Neuropsychological assessments were always conducted by the same investigator (L.C.), and the medical follow-up of the patients always by the same physician (A.R.).

Number of Patients Evaluated for Each Cognitive Test at Each Time of Testing

Table 2 shows the number of patients evaluated at each time of testing according to each cognitive test. For 5 of the 47 patients who participated in the study, data on reaction time were not available because of problems with data recording. Moreover, when a patient did not understand the instructions for a test or did not achieve the simplest level of the task, the test was aborted and the results were excluded from data analysis. This was the case for some patients included in the study, as shown by the missing measures in the spatial working memory and Stockings of Cambridge tests.

Thirty-two of the 47 patients included in the study were evaluated again at the end of the first month of treatment. Patients who were not tested at 1 month of treatment had stopped therapy before or were unable to be evaluated at this time of treatment for medical reasons.

Because of the relatively low number of patients in each treatment group and to avoid loss of statistical power, comparisons were carried out independently between day 1 and day 5 and between day 1 and 1 month. However, we checked that this procedure did not result in any bias and that the same changes were observed when all data for the three time points were compared.

Clinical and Biological Survey and Other Measured Variables

Patients were always hospitalized during the first 6 days of treatment. Blood pressure, body temperature, and heart rate were measured during these 6 days of treatment to allow evaluation of general toxicity (34). Other toxic events, such as asthenia (as mea-

TABLE 2. Number of Enrolled Patients by Test Session and Cognitive Test

Test	IL-2			IL-2 + IFN-α			SC IFN-α			IV IFN-α		
	D1[a]	D5	M1	D1	D5	M1	D1	D5	M1	D1	D5	M1
Reaction time	14	14	8	7	7	5	7	7	5	14	14	9
Spatial working memory	16	15	10	7	7	5	7	7	5	16	16	11
Stockings of Cambridge	17	15	11	7	7	5	7	7	5	15	15	10

[a] D1 = day 1; D5 = day 5; M1 = 1 month.

sured by the Karnofsky index, Ref. 35), headaches, myalgia, nausea, and vomiting, induced by treatment during the first month of therapy were measured according to the WHO scale (34). Biological parameters, such as leukocyte count and transaminases, were checked two or three times weekly. A thyroid function test was performed 2 months after the initiation of treatment.

At each time point of the neuropsychological evaluation, a number of psychological variables were measured to control their influence on the patients' cognitive performance. Mood was assessed using the Montgomery and Asberg Depression Rating Scale (36). Quality of sleep was evaluated using a visual analog scale corresponding to a four-item, 100-mm scale without any graduation, on which the patient was asked to place a mark, with items indicative of disturbed sleep at the left end (difficult to sleep, sensation of a short sleep, bad sleep, unable to stay awake, and sensation of eyes closing during the day) and items indicative of good sleep at the right end (easy to sleep, sensation of a long sleep, good sleep, and very awake during the day) (37). The general mean value of the visual analog scale, corresponding to the sum of the four-item scores divided by the number of subscales (ie, 4) was used for data analysis, with lower scores corresponding to impaired sleep and higher scores to undisturbed sleep (37). In addition, a list of drugs with psychotropic effects, such as antidepressants, hypnotics, and morphine-like drugs, was made for all patients at the start of the study and during treatment.

Statistical Analysis

Before treatment the homogeneity of cognitive performance in the different treatment groups was checked by analysis of variance. Post hoc comparisons (Newman-Keuls test) were performed when appropriate. For several CANTAB measures, treatment groups were not comparable before the initiation of immunotherapy (results presented below). This initial difference was related to a significant difference in the age of patients ($F(3,43) = 5.20$, $p < .01$). More precisely, patients in the IV IFN-α group were significantly younger than patients from other therapeutic groups (Newman-Keuls test, $p < .05$) (see Table 1). In view of this initial difference between groups and the low number of patients in some treatment groups, we used Wilcoxon nonparametric analysis on ranks (repeated measures) to compare the cognitive performance of patients between day 1 and each peri-therapeutic evaluation within each treatment group. In addition, we checked that gender, education level, and socioeconomic status had no effect on the cognitive performance of patients before treatment.

Because some of the patients tested on day 5 could not be evaluated at 1 month for the reasons described earlier, we performed two distinct analyses with repeated measures, the first to compare day 1 with day 5 and the second to compare day 1 with 1 month.

Analyses were performed for each level of difficulty of the tests. Results are displayed in tabular form.

RESULTS

Effects of IL-2 and IFN-α on Reaction Time

Baseline measure. There was no difference in reaction time at baseline (day 1) between treatment groups except for the MC-RT test (stage 2) ($F(3,38) = 3.48$, $p < .05$), in which IV IFN-α patients were more rapid than patients from other treatment groups (Newman-Keuls test, $p < .05$). This difference was related to the younger age of these patients because it disappeared when the mean reaction times were adjusted for age.

Effects of cytokine immunotherapy. Cytokine therapy altered reaction time in a manner that was dependent on the modalities of treatment and the level of difficulty of the task.

When tested in the simple-choice task (stage 1), patients reacted faster on day 5 than on day 1 regardless of which treatment they received. This decrease in reaction time between the two sessions was, however, only significant in IL-2 patients ($T = 2.67$, $p < .01$) (Table 3). This improvement was no longer present at 1 month because these patients displayed reaction times comparable to those measured on day 1 ($T = 0.28$, $p > .05$) and higher than those measured on day 5 ($T = -2.24$, $p < .05$). In other treatment groups, SC-RT results at 1 month were also comparable to those measured on day 1.

When tested in the multiple-choice task (stage 2), IV IFN-α patients displayed significantly higher reaction times on day 5 than on day 1 ($T = -2.29$, $p < .05$). This slowing tended to persist at 1 month of treatment ($T = -1.84$, $p = .06$). In other treatment groups, MC-RT remained stable during treatment. Analyses separating reaction and movement times (stage 5) in the multiple-choice task showed that at 1 month of treatment both

TABLE 3. Effects of Cytokine Immunotherapy on Reaction Time After 5 Days and 1 Month of Treatment[a]

	IL-2		IL-2 + IFN-α		SC IFN-α		IV IFN-α	
	D1/D5[b]	D1/M1	D1/D5	D1/M1	D1/D5	D1/M1	D1/D5	D1/M1
Reaction time, ms								
Simple choice	956/601**	876/835	1198/927	1001/677	961/795	978/874	766/668	794/696
Multiple choice	879/875	887/900	982/992	917/900	822/827	868/973	727/845*	749/843***
MC-RT	369/400	351/369	375/380	369/394	356/371	352/363	346/345	337/426*
MC-MT	645/684	705/759	628/677	572/588	562/643*	593/788*	564/622***	593/650*

[a] Compared with day 1.
[b] D1 = day 1; D5 = day 5; M1 = 1 month; MC-MT = multiple-choice movement time; MC-RT = multiple-choice reaction time.
* $p < .05$; ** $p < .01$; *** $p < .10$.

reaction time ($T = -2.07$, $p < .05$) and movement time ($T = -1.96$, $p = .05$) were impaired in IV IFN-α patients, whereas on day 5, only movement time tended to be impaired ($T = -1.66$, $p = .09$). Patients in the SC IFN-α group displayed a slowing in movement time ($T = -2.37$, $p < .05$) that persisted at 1 month of treatment ($T = -2.02$, $p < .05$). In the IL-2 and IL-2+IFN-α groups, no change occurred in these measures during treatment.

In summary, IFN-α impaired reaction time early in the course of treatment, especially when patients had multiple choices (ie, when the task was more difficult). This impairment was more pronounced in IV IFN-α patients, and it was not apparent in IL-2 or IL-2+IFN-α patients.

Effects of IL-2 and/or IFN-α on Spatial Working Memory

Baseline measure. There was no difference in performance at baseline in spatial working memory between treatment groups regardless of the level of difficulty of this task. However, SC IFN-α patients displayed significantly higher (ie, poorer) strategy scores than IV IFN-α patients before treatment.

Effects of cytokine immunotherapy. Cytokine immunotherapy altered spatial working memory abilities early during the course of treatment in a way that depended on treatment modalities and level of difficulty of the task.

When the task was easy, ie, when the number of boxes to search was low (four to six boxes), performance of patients remained stable regardless of which treatment they received.

In contrast, when the task was more difficult (ie, when the number of boxes to search increased to eight), IL-2 patients made significantly more between-search errors on day 5 than on day 1 ($T = -2.07$, $p < .05$) (Table 4). This impairment was not related to alteration in search strategy because strategy did not change during treatment and had no effect on the time necessary to complete trials. At 1 month of treatment, the number of between-search errors made by IL-2 patients in the eight-box search task remained high even if the difference with respect to day 1 was not significant.

In other treatment groups, performance was not impaired in complex trials regardless of the time of treatment. IV IFN-α patients were more efficient on day 5 than on day 1 ($T = 2.15$, $p < .05$). This transient improvement was certainly due to practice effects, as observed in studies on test-retest reliability of CANTAB measures in control subjects (38).

In summary, IL-2 immunotherapy was accompanied by impaired spatial working memory performance as soon as the fifth day of treatment, especially when the level of difficulty of the task was high. This impairment did not appear in other treatment groups.

Effects of IL-2 and/or IFN-α on Planning and Problem-Solving Abilities (Stockings of Cambridge test)

Baseline measure. Before treatment, IL-2+IFN-α patients made significantly more moves than IL-2 patients to solve easy and complex planning problems ($p < .05$).

Effects of cytokine immunotherapy. Cytokine immunotherapy impaired planning and problem-solving (Table 5) abilities in a way that was dependent on the mode of treatment and level of difficulty of the task.

IL-2 patients displayed impaired performance on the Stockings of Cambridge test as soon as the fifth day of treatment. In these patients, the number of perfect solutions (ie, problems solved in a minimum of moves) for all the trials significantly decreased between day 1 and day 5 ($T = 2.07$, $p < .05$), and this impairment persisted at the end of the first month of therapy ($T = 2.05$, $p < .05$). Secondary analysis showed that the

TABLE 4. Effects of Cytokine Immunotherapy on Spatial Working Memory After 5 Days and 1 Month of Treatment[a]

	IL-2		IL-2 + IFN-α		SC IFN-α		IV IFN-α	
	D1/D5[b]	D1/M1	D1/D5	D1/M1	D1/D5	D1/M1	D1/D5	D1/M1
Strategy score	32.3/32	31.8/31	35.4/33.8	35.4/32	37/36.4	33.4/33	31.4/30.5	33.4/33.5
Between-search errors (*N*)								
4 boxes	1.3/0.9	0.4/1.3	0.7/1.3	0.6/0	1.6/2.6	1/1.4	1/0.1*	1.1/1.1
6 boxes	7.3/5.6	7.6/3.9	8.3/8.7	5.4/5	13.1/14.7	11.2/11.8	5.4/3.8	6.4/7
8 boxes	18.7/26.1*	15.7/22	19.1/21.3	20.6/18.6	25.6/23	19.8/21.6	15.7/11.3*	15.2/16.9
Time to complete trial, s								
8 boxes	69/63***	63/63	79/65***	62/52	74/65***	73/76	59/51*	57/50

[a] Compared with day 1.
[b] D1 = day 1; D5 = day 5; M1 = 1 month.
* $p < .05$; *** $p < .10$.

TABLE 5. Effects of Cytokine Immunotherapy on Stockings of Cambridge Test Responses After 5 Days and 1 Month of Treatment[a]

	IL-2		IL-2 + IFN-α		SC IFN-α		IV IFN-α	
	D1/D5[b]	D1/M1	D1/D5	D1/M1	D1/D5	D1/M1	D1/D5	D1/M1
Total perfect solutions	8.87/7.60*	8.91/7.09*	7.14/8.57*	6.80/8.20***	7.57/7.29	8.40/8.20	8.07/8.33	8.10/8.80
Easy problems								
Average moves	2.51/2.61	2.52/2.56	2.75/2.60	2.75/2.50	2.64/2.67	2.65/2.70	2.66/2.55	2.65/2.52
Initial thinking time, s	3.92/2.40*	3.57/2.74***	4.08/2.41*	3.84/1.88***	3.66/2.35	4.11/3.86	3.52/1.5**	3.81/1.4**
Subsequent thinking time, s	0.94/0.45*	1.17/0.5*	0.33/0.43	0.21/0.68	1.29/0.9	1.55/0.65*	1.04/0.1**	1.25/0.2**
Complex problems								
Average moves	5.41/6.2**	5.34/6.18*	6.35/5.67*	6.6/6.8	6.16/6.48	5.55/5.77	5.87/5.98	5.81/5.86
Initial thinking time, s	5.93/5.69	5.70/5.68	4.04/4.99*	4.68/5.47	6.25/4.86	9.12/6.65*	4.93/4.58	4.56/3.37*
Subsequent thinking time, s	3.67/3.84	4.32/3.34	2.59/1.49*	3.07/3.01	2.51/2.13	2.58/1.67	2.98/2.49	2.87/1.41*

[a] Compared with day 1.
[b] D1 = day 1; D5 = day 5; M1 = 1 month.
* $p < .05$; ** $p < .01$; *** $p < .10$.

impairment measured in these patients was more pronounced when the level of difficulty of the task was higher. In complex problems (four to five moves), IL-2 patients displayed lower performance on day 5 than on day 1, because their average moves significantly increased between the two times of evaluation ($T = -2.82$, $p < .01$). This impairment persisted at 1 month of treatment ($T = -2.41$, $p < .05$). In contrast, performance of IL-2 patients in easier trials was unimpaired during treatment, and the time they took to plan the first (initial thinking) and subsequent moves of problem solving was unaffected by the treatment regardless of the level of difficulty and the duration of treatment.

In other treatment groups, performance on the Stockings of Cambridge test was not impaired during treatment. In IL-2+IFN-α patients, the number of perfect solutions was even significantly higher on day 5 than on day 1 ($T = -2.06$, $p < .05$) and tended to remain higher at 1 month of therapy ($T = -1.89$, $p = .06$). In complex trials, these patients made significantly fewer moves to solve problems on day 5 compared with day 1 ($T = 2.21$, $p < .05$), but this improvement was detrimental to planning times because initial thinking times significantly increased between the two periods of evaluation ($T = -2.19$, $p < .05$). At 1 month of treatment, performance of IL-2+IFN-α patients in complex problems returned to baseline.

In summary, IL-2 immunotherapy impaired planning abilities and resulted in lower performance accuracy, especially at high levels of difficulty of the task. This impairment developed as soon as the fifth day of treatment and persisted at 1 month of therapy.

Other Signs of Toxicity and Their Relationships With Cognitive Alterations

Table 6 summarizes the other clinical changes that occurred in patients during the first month of treatment. Patients treated with IL-2, administered alone or with IFN-α, displayed depressive symptoms as soon as the fifth day of treatment, and these symptoms persisted at 1 month of treatment. Depressive symptoms occurred only at the end of the first month of treatment in IFN-α patients, especially when the cytokine was administered at high doses by the intravenous route. Sleep disturbances were reported by a large number of patients during treatment, with higher frequency in IL-2 and IL-2+IFN-α patients. Measurement of toxicity according to the WHO scale demonstrated that toxic events such as headaches, myalgia, nausea, and vomiting were more frequent in IL-2 and IL-2+IFN-α patients, whereas leucopenia and changes in hepatic functions occurred mainly in IV IFN-α patients. Fever and asthenia were apparent in a large number of patients, with higher frequency in patients included in therapeutic protocols based on IL-2 administration.

Analysis of correlations (39) demonstrated that depressive symptoms and sleep disturbances occurring during treatment were not associated with any of the cognitive changes measured during therapy, whatever the time of evaluation. However, the slowing observed on day 5 in MC-RT in IV IFN-α patients was positively correlated with the intensity of the depressive symptoms they displayed at 1 month ($R = 0.76$, $p = .01$). This result indicates that cognitive slowing may be one of the first signs, in term of dynamics of appearance, of IFN-α–induced mood disturbances.

Spearman correlations showed no significant relationship between signs of toxicity measured by the WHO scale and cognitive alterations measured during IFN-α or IL-2 treatments. In the same way, asthenia measured in the WHO scale by the Karnofsky index was not correlated with cognitive changes.

Twelve of the 47 patients (25.5%) took hypnotics before the start of the study. During treatment this

TABLE 6. General Toxicity Induced by IL-2 and IFN-α Treatments

	IL-2		IL-2 + IFN-α		SC IFN-α		IV IFN-α	
	D5[a]	M1	D5	M1	D5	M1	D5	M1
Depressive symptoms[b]								
%	70	100	100	100	40	75	50	90
p	<.05	<.01	<.05	<.05	>.10	>.10	=.07	<.01
Sleep alterations[c]								
%	72	55	88	83	17	55	44	30
p	>.10	>.10	.07	>.10	>.10	>.10	>.10	>.10
Asthenia[d]	18	50	70	60	0	60	12	10
Fever[d]	93	NR	100	NR	43	NR	32	NR
Headache, myalgia[d]	6	0	14	20	0	0	12	9
Nausea, vomiting[d]	12	8	33	20	0	0	6	0
Transaminases[d]	5	0	33	0	0	0	18	45
Leucopenia[d]	0	0	0	0	0	0	50	60

[a] D5 = day 5; M1 = 1 month; NR = data not recorded.
[b] Measured with Montgomery and Asberg Depression Rating Scale. % = percentage of patients with higher scores during treatment (compared with day 1); *p* = statistical significance of changes in scores (compared with day 1).
[c] Measured with visual analog scale. % = percentage of patients with higher score for sleep alterations during treatment (compared with day 1); *p* = statistical significance of changes in score (compared with day 1).
[d] Toxicity measured by WHO scale. Numbers correspond to the percentage of patients experiencing a toxicity grade ≥ 2 for each of the listed symptoms.

proportion increased to 65% of patients. Spearman correlations showed that the use of hypnotics before or during treatment had no effect on the magnitude of the cognitive changes observed during treatment or on the cognitive performance measured before treatment. The number of patients using analgesics (5 of 47) or antidepressants (3 of 47) before and during immunotherapy was too small to statistically assess the influence of such substances on the magnitude of the cognitive alterations that occurred during treatment. Finally, none of the cognitive alterations observable at 1 month was associated with thyroid dysfunction measured 1 month later.

DISCUSSION

The present study reports the differential effects of IL-2 and IFN-α on cognitive functions, which are summarized in Table 7. Although there is always the risk of statistical bias due to the number of comparisons, the results show coherent patterns of alterations, between and within tests and between treatments. The results also agree with the hypothesis that the neuropsychological effects of cytokine therapy develop early and show clear dissociations between the effects of IL-2 and those of IFN-α. These findings provide new insight into the mode of action of cytokines on brain functions.

IL-2 patients showed impaired performance in spatial working memory and planning tasks as soon as the fifth day of treatment, a combination of impairments that is not surprising because spatial working memory is an important component of task planning. The alterations observed in IL-2 patients in these two tasks appeared at higher levels of difficulty, because these patients made more errors when the number of items to memorize in the spatial working memory task was higher, and in solving complex problems in the planning task. This result is consistent with previous data showing that IL-2 therapy induces disturbances in cognitive tasks involving high attentional resources (14, 17, 18). Moreover, IL-2 patients displayed no significant cognitive slowing on any task performed.

Several studies using CANTAB have shown that patients with frontal lobe damage perform poorly in spatial working memory and planning tasks. In the planning task, these patients take more moves to solve the problems and solve fewer problems perfectly than control subjects. In contrast, patients with temporal damage are unimpaired in this task (28). The resemblance between the pattern of impairment of frontal patients in the planning task and that observed in IL-2 patients is suggestive of an alteration of cognitive processes depending on frontal substrates during IL-2 therapy. This hypothesis is in accordance with previous findings showing that IL-2 therapy induced lengthening in P300 latencies and altered electroencephalographic frequencies, especially in frontal regions (40, 41). However, IL-2 patients did not perform exactly like patients with frontal lobe damage, who showed impaired accuracy and strategy search in spatial working memory tasks (31). The impairment of

TABLE 7. Summary of Effects of IL-2 and IFN-α Treatments on Cognitive Abilities After 5 Days and 1 Month of Treatment[a]

	IL-2		IL-2 + IFN-α		SC IFN-α		IV IFN-α	
	D5	M1	D5	M1	D5	M1	D5	M1
Reaction time								
Simple choice	U	U	U	U	U	U	U	U
Multiple choice	U	U	U	U	U	U	I	T
MC-RT	U	U	U	U	U	U	U	I
MC-MT	U	U	U	U	I	I	T	I
Spatial working memory								
Strategy score	U	U	U	U	U	U	U	U
Between-search errors								
4 boxes	U	U	U	U	U	U	U	U
6 boxes	U	U	U	U	U	U	U	U
8 boxes	I	U	U	U	U	U	U	U
Time (8 boxes), s	U	U	U	U	U	U	U	U
Stockings of Cambridge test								
Total perfect solutions	I	I	U	U	U	U	U	U
Easy problems								
Average moves	U	U	U	U	U	U	U	U
Initial thinking time	U	U	U	U	U	U	U	U
Subsequent thinking time	U	U	U	U	U	U	U	U
Complex problems								
Average moves	I	I	U	U	U	U	U	U
Initial thinking time	U	U	I	U	U	U	U	U
Subsequent thinking time	U	U	U	U	U	U	U	U

[a] Compared with day 1.
[b] D1 = day 1; I = significantly impaired; M1 = 1 month; MC-MT = multiple-choice movement time; MC-RT = multiple-choice reaction time; T = trend for impairment ($.05 < p < 0.10$); U = unimpaired.

IL-2 patients only in performance accuracy is similar to the impairment exhibited by patients with temporal lobe damage or amygdalo-hippocampectomy (28). Taken together, these data indicate that IL-2 may interfere with several neural circuits, including those in the frontal, temporal, and subcortical areas. Brain imaging techniques are needed to identify brain areas that are functionally altered by IL-2. In the only study conducted on patients who developed neurologic abnormalities while receiving massive doses of IL-2, all patients had abnormal brain imaging findings in areas including the frontal, parietal, and thalamic regions (42). In another type of investigation, functional neuroimaging studies using positron emission tomography in normal subjects performing the Stockings of Cambridge task demonstrated that complex planning was associated with the activation of a distributed network of prefrontal, parietal, and occipital cortical areas and subcortical areas such as the right thalamus and the head of the caudate nucleus (32, 33). A similar circuit seems to be involved in the functional neuroanatomy of depression (43), which could explain why patients treated by IL-2 develop both depressive symptoms and cognitive disturbances.

In terms of mechanisms, IL-2 immunoreactivity and binding sites have been identified in several areas of the rodent brain, including the hippocampus, frontal cortex, striatum, septum, and hypothalamus (44–46). Moreover, several alterations of neurotransmitter function have been observed in animals treated with IL-2, including altered dopamine, serotonin, norepinephrine, and acetylcholine functioning in brain areas as diverse as the nucleus accumbens, hippocampus, and prefrontal cortex (11, 47). The mechanisms mediating the effects of peripheral IL-2 on the brain, however, remain unclear and might involve intermediate mediators at the level of the blood-brain barrier or activation of afferent neural pathways (48). It is also possible that these effects are mediated by the production of other cytokines, such as IL-1, IL-6, or tumor necrosis factor-α, which have been shown to be involved in neurological disorders (49, 50).

In contrast to IL-2, IFN-α treatment had no effect on spatial working memory and planning tasks. IV IFN-α patients performed even better on the spatial working memory task after 5 days of treatment, consistent with practice effects observed in control subjects after repeated measures with the CANTAB battery (38). Treatment with IFN-α was mainly accompanied by an increase in response latencies on the reaction time task, especially when this cytokine was administered intravenously and when the task involved higher atten-

tional resources. This finding is consistent with other studies showing that IFN-α therapy for chronic myelogenous leukemia disturbed motor speed and dexterity (15). In animals, chronic administration of IFN-α depressed motor activity and inhibited dopaminergic activity (51, 52). An important result from the present study is that the psychomotor slowing observed as soon as the fifth day of treatment in IV IFN-α patients was associated with the depressive symptoms that developed after 4 weeks of treatment. This result may suggest that psychomotor slowing is one of the first symptoms of IFN-α–induced depressed mood.

The performance of IL-2+IFN-α patients was unaltered during the first month of therapy. This result is surprising because these patients received higher doses of IL-2 than patients treated with IL-2 alone. Several explanations can be proposed to account for this. First, IFN-α might oppose IL-2 effects on cognitive functions. However, even if cognition and mood reflect independent mechanisms, previous results showing that both IL-2 and IL-2+IFN-α therapies induced early depressive symptoms, which were more pronounced in IL-2+IFN-α patients (20), do not support such a hypothesis. Second, it is possible that the number of patients included in this group was too small to detect significant cognitive changes during treatment. However, it is important to note that even if these patients solved the complex problems from the Stockings of Cambridge task more efficiently after 5 days of treatment compared with baseline, this was done at the expense of an important cognitive effort revealed by increased planning times. Finally, the lack of improvement with practice in these patients compared with IFN-α patients may suggest that IL-2+IFN-α patients are less able to perform optimally during treatment.

Assessment of the cognitive changes that occur under cytokine immunotherapy both early and at later stages of treatment gives information about the time course of these effects. Most of the cognitive changes observed after 5 days of treatment persisted at 1 month without evidence of worsening and without the appearance of new marked deficits. This result is surprising because the neuropsychological side effects of cytokine therapy have been shown to be related to the duration of therapy (14). However, the 50% reduction in the doses of IL-2 administered after the first week of treatment may explain the absence of worsening in the patients' performance at later stages of treatment. In the same way, the cessation of administration of IL-2 during the second week of treatment in IL-2+IFN-α patients might explain the absence of change in the performance of these patients at 1 month. In contrast to what has been observed by others (16), IFN-α treatment was not associated with marked cognitive disturbances. This might be due to the short time period during which this study was conducted, and it is conceivable that several months of IFN-α treatment would induce further cognitive alterations.

In conclusion, the results of the present investigation demonstrate that IL-2 and IFN-α therapies are associated with early subtle neuropsychological changes depending on the administered cytokines, suggesting distinct mechanisms of action of these two cytokines in the brain. These changes are not associated with other toxic effects of cytokine immunotherapy, including sleep disturbances. In addition, these results provide new hypotheses concerning the mechanisms of neurotoxicity of cytokine immunotherapy that can be tested in future studies using, for instance, brain imaging techniques. The assessment of the exact impact of these cognitive alterations on functionality of patients in daily tasks also requires further investigation.

This study was supported by Ligue Nationale contre le Cancer, Fondation pour la Recherche Médicale and Association pour la Recherche sur le Cancer. The authors thank the patients for their participation in the study, the hospital staff for their collaboration, and Dr. J. Gumnick for her assistance.

REFERENCES

1. Dantzer R. How do cytokines say hello to the brain? Neural versus humoral mediation. Eur Cyt Netw 1994;5:271–3.
2. Kluger MJ. Fever: role of pyrogens and cryogens. Physiol Rev 1991;71:93–127.
3. Besedovsky HO, Del Rey AE, Sorkin E. Immune-neuroendocrine interactions. J Immunol 1985;135(Suppl 2):750S–754S.
4. Dantzer R, Wollman EE, Yirmiya R. Cytokines, stress and depression. New York: Kluwer Academic/Plenum; 1999.
5. Bluthé RM, Beaudu C, Kelley KW, Dantzer R. Differential effects of IL-1ra on sickness behavior and weight loss induced by IL-1 in rats. Brain Res 1995;677:171–6.
6. Yirmiya R. Endotoxin produces a depressive-like episode in rats. Brain Res 1996;711:163–74.
7. Connor T, Song C, Leonard BE, Merali Z, Anisman H. An assessment of the effects of central interleukin-1β, -2, -6 and tumor necrosis factor-α administration on some behavioural, neurochemical, endocrine, and immune parameters in the rat. Neuroscience 1998;84:923–33.
8. Smith A, Tyrrell D, Coyle K, Willman JS. Selective effects of minor illnesses on human performance. Br J Psychol 1987;78: 183–8.
9. Smith A, Tyrrell D, Coyle K, Higgins P. Effects of interferon-alpha on performance in man: a preliminary report. Psychopharmacology 1988;96:414–6.
10. Capuron L, Lamarque D, Dantzer R, Goodall G. Attentional and mnemonic deficits associated with infectious disease in humans. Psychol Med 1999;29:291–7.
11. Anisman H, Merali Z. Anhedonic and anxiogenic effects of cytokine exposure. In: Dantzer R, Wollman EE, Yirmiya R, edi-

tors. Cytokines, stress and depression. New York: Kluwer Academic/Plenum; 1999. p. 199–233.
12. Aubert A, Vega C, Dantzer R, Goodall G. Pyrogens specifically disrupt the acquisition of a task involving cognitive processing in the rat. Brain Behav Immun 1995;9:129–48.
13. Gibertini M, Newton C, Friedman H, Klein TW. Spatial learning impairment in mice infected with *Legionella pneumophila* or administered exogenous interleukin-1β. Brain Behav Immun 1995;9:113–28.
14. Denicoff KD, Rubinow DR, Papa MZ, Simpson C, Seipp CA, Lotze MT, Chang AE, Rosenstein D, Rosenberg SA. The neuropsychiatric effects of treatment with interleukin-2 and lymphokine-activated killer cells. Ann Intern Med 1987;107:293–300.
15. Pavol MA, Meyers CA, Rexer JL, Valentine AD, Mattis PJ, Talpaz M. Pattern of the neurobehavioral deficits associated with interferon-alfa therapy for leukemia. Neurology 1995;45:947–950.
16. Meyers CA. Mood and cognitive disorders in cancer patients receiving cytokine therapy. In: Dantzer R, Wollman EE, Yirmiya R, editors. Cytokine, stress and depression. New York: Kluwer Academic/Plenum; 1999. p. 75–81.
17. Walker LG, Wesnes KP, Heys SD, Walker MB, Lolley J, Eremin O. The cognitive effects of recombinant interleukin-2 (rIL-2) therapy: a controlled clinical trial using computerised assessments. Eur J Cancer 1996;32A:2275–83.
18. Walker LG, Walker MB, Heys SD, Lolley J, Wesnes K, Eremin O. The psychological and psychiatric effects of rIL-2 therapy: a controlled clinical trial. Psychooncology 1997;6:290–301.
19. Atzpodien J, Kirchner H. The out-patient use of recombinant interleukin-2 and interferon-alfa-2b in advanced malignancies. Eur J Cancer 1991;27(Suppl 4):S88–91.
20. Capuron L, Ravaud A, Dantzer R. Early depressive symptoms in cancer patients receiving interleukin-2 and interferon-alpha-26 therapy. J Clin Oncol 2000;18:2143–51.
21. Robbins TW, James M, Owen AM, Sahakian BJ, McInnes L, Rabbitt PM. Cambridge Neuropsychological Test Automated Battery (CANTAB): a factor analytic study of a large sample of normal elderly volunteers. Dementia 1994;5:266–81.
22. Robbins TW, James M, Owen AM, Sahakian BJ, Lawrence AD, McInnes L, Rabbitt PM. A study of performance on tests from the CANTAB battery sensitive to frontal lobe dysfunction in a large sample of normal volunteers: implications for theories of executive functioning and cognitive aging. J Int Neuropsychol Soc 1998;4:474–90.
23. Sleijfer DT, Janssen RA, Buter J, De Vries EG, Willemse PH, Mulder NH. Phase II study of subcutaneous interleukin-2 in unselected patients with advanced renal cell cancer on an outpatient basis. J Clin Oncol 1992;10:1119–23.
24. Négrier S, Ravaud A, Delva R, Chevreau C, Douillard JY, Fargeot P, Drevon M, Gomez F. Combination of cytokines in metastatic renal cell carcinoma: is the subcutaneous route less active than the intravenous route? [abstract]. Proc Am Soc Clin Oncol 1999; 18:331A.
25. Kirkwood JM, Strawderman MH, Ernstoff MS, Smith TJ, Borden EC, Blum RH. Interferon alfa-2b adjuvant therapy of high-risk resected cutaneous melanoma: the Eastern Cooperative Oncology Group Trial EST 1684. J Clin Oncol 1996;14:7–17.
26. Fray PJ, Robbins TW, Sahakian BJ. Neuropsychiatric applications of CANTAB. Int J Geriatr Psychiatry 1996;11:329–36.
27. Robbins TW, Sahakian BJ. Computer methods of assessment of cognitive function. In: Copeland JRM, Abou-Saleh MT, Blazer DG, editors. Principles and practice of geriatric psychiatry. Chichester (UK): John Wiley & Sons; 1994. p. 205–9.
28. Owen AM, Sahakian BJ, Semple J, Polkey CE, Robbins TW. Visuo-spatial short-term recognition memory and learning after temporal lobe excisions, frontal lobe excisions or amygdalo-hippocampectomy in man. Neuropsychologia 1995;33:1–24.
29. Robbins TW. Dissociating executive functions of the prefrontal cortex. Philos Trans R Soc Lond B Biol Sci 1996;351:1463–71.
30. Owen AM, James M, Leigh PN, Summers BA, Maesden CD, Quinn NP, Lange KW, Robbins TW. Fronto-striatal cognitive deficits at different stages of Parkinson disease. Brain 1992;115: 1727–51.
31. Owen AM, Downes JJ, Sahakian BJ, Polkey CE, Robbins TW. Planning and spatial working memory following frontal lobe lesions in man. Neuropsychologia 1990;28:1021–34.
32. Owen AM, Doyon J, Petrides M, Evans AC. Planning and spatial working memory: a positron emission tomography study in humans. Eur J Neurosci 1996;8:353–64.
33. Baker SC, Rogers RD, Owen AM, Frith CD, Dolan RJ, Frakowiak RSJ, Robbins TW. Neural systems engaged by planning: a PET study of the Tower of London task. Neuropsychologia 1996;34: 515–26.
34. World Health Organization. WHO handbook for reporting results of cancer treatment. Geneva: World Health Organization; 1979. WHO offset publication no. 48.
35. Karnofsky DA, Burchenal JH. The clinical evaluation of chemotherapeutic agents in cancer. In: MacLeod CM, editor. Evaluation of chemotherapeutic agents in cancer. New York: Columbia University Press; 1949. p. 191–205.
36. Montgomery SA, Asberg A. A new depression scale designed to be sensitive to change. Br J Psychiatry 1979;134:382–9.
37. Monk MM, Leng VC, Folkard S, Weitzman ED. Circadian rhythms in subjective alertness and core body temperature. Chronobiologia 1983;10:49–55.
38. Lowe C, Rabbitt P. Test/re-test reliability of the CANTAB and ISPOCD neuropsychological batteries: theoretical and practical issues. Neuropsychologia 1998;36:915–23.
39. Pearson K. On a criterion that a given system of deviations from the probable in the case of a correlated system of variables is such that it can reasonably be supposed to have arisen in random sampling. Philos Magazine 1900;50:157–75.
40. Pace A, Pietrangeli A, Bove L, Rosselli M, Lopez M, Jandolo B. Neurotoxicity of antitumoral IL-2: evoked cognitive potentials and brain mapping. Ital J Neurol Sci 1994;15:341–6.
41. Caraceni A, Martini C, Belli F, Mascheroni L, Rivoltini L, Arienti F, Cascinelli N. Neuropsychological and neurophysiological assessment of the central effects of interleukin-2 administration. Eur J Cancer 1993;29A:1266–9.
42. Karp B, Yang JC, Khorsand M, Wood R, Merigan TC. Multiple cerebral lesions complicating therapy with interleukin-2. Neurology 1996;47:417–24.
43. Drevets WC, Videen TO, Price JL, Preskorn SH, Carmichael ST, Raichle ME. A functional anatomical study of unipolar depression. J Neurosci 1992;12:3628–41.
44. Araujo DM, Lapchak PA, Collier B, Quirion R. Localization of interleukin-2 immunoreactivity and interleukin-2 receptors in the rat brain: interaction with the cholinergic system. Brain Res 1989;498:257–266.
45. Lapchak PA, Araujo DM, Quirion R, Beaudet A. Immunoautoradiographic localization of interleukin-2-like immunoreactivity and interleukin-2 receptors (Tac antigen-like-immunoreactivity) in the rat brain. Neuroscience 1991;44:173–84.
46. Hanisch UK, Quirion R. Interleukin-2 as a neuroregulatory cytokine. Brain Res Rev 1995;21:246–84.
47. Hanisch UK, Seto D, Quirion R. Modulation of hippocampal acetylcholine release: a potent central action of interleukin-2. J Neurosci 1993;13:3368–74.
48. Dantzer R, Aubert A, Bluthe RM, Gheusi G, Cremona S, Laye S,

Konsman JP, Parnet P, Kelley KW. Mechanisms of the behavioural effects of cytokines. In: Dantzer R, Wollman EE, Yirmiya R, editors. Cytokine, stress and depression. New York: Kluwer Academic/Plenum; 1999. p. 83–105.
49. Hull M, Fiebich BL, Lieb K, Strauss S, Berger SS, Volk B, Bauer J. Interleukin-6-associated inflammatory processes in Alzheimer's disease: new therapeutic options. Neurobiol Aging 1996; 17:795–800.
50. Rogers J, Webster S, Lue LF, Brachova L, Civin WH, Emmerling M, Shivers B, Walker D, McGeer P. Inflammation and Alzheimer's disease pathogenesis. Neurobiol Aging 1996;17:681–6.
51. Dunn AL, Crnic LS. Repeated injections of interferon-alpha A/D in Balb/c mice: behavioral effects. Brain Behav Immun 1993;7: 104–11.
52. Shuto H, Kataoka Y, Horikawa T, Fujihara N, Oishi R. Repeated interferon-alpha administration inhibits dopaminergic neural activity in the mouse brain. Brain Res 1997;747: 348–51.