26. Statistical Consultants, Inc. PCNONLIN and NONLIN 84: Software for the statistical analysis of non-linear models. *Am Stat* 1986, **40**, 52.
27. Egorin MJ, Van Echo DA, Olman EA, Whiteacre MY, Forrest A, Aisner J. Prospective validation of a pharmacologically based dosing scheme for the *cis*-diamminedichloroplatinum(II) analogue diammine-cyclobutanedicarboxylatoplatinum. *Cancer Res* 1985, **45**, 6502–6506.
28. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976, **16**, 31.
29. Calvert AH, Harland SJ, Newell DR, *et al.* Early clinical studies with cis-diammine-1,1-cyclobutane dicarboxylate platinum II. *Cancer Chemother Pharmacol* 1982, **9**, 140–147.
30. Joss RA, Kaplan S, Goldhirsch A, Sessa C, Brunner KW, Cavalli F. A phase I trial of *cis*-diammine-1,1 cyclobutane dicarboxylate platinum II (carboplatin, CBDCA, JM-8)with a single dose every five week-schedule. *Invest New Drugs* 1984, **2**, 297–304.

Eur J Cancer, Vol. 29A, No. 8, pp. 1127–1132, 1993.
Printed in Great Britain

0964–1947/93 $6.00 + 0.00

In vivo Biological Results of the Association Between Interleukin-2 and Interleukin-3 in the Immunotherapy of Cancer

P. Lissoni, S. Barni, E. Tisi, F. Rovelli, S. Pittalis, R. Rescaldani, L. Vigoré, A. Biondi, A. Ardizzoia and G. Tancini

The concomitant generation of macrophage-mediated suppressive events, as documented by the increase in neopterin and soluble interleukin-2 (IL-2) receptor (SIL-2R), and the enhanced production of cortisol, would represent the most investigated phenomena responsible for the reduced anticancer efficacy of IL-2 immunotherapy in humans. Based on our preliminary experimental studies suggesting a modulatory role of IL-3 on immune and endocrine effects induced by IL-2, a study was performed to evaluate the influence of IL-3 on biological effects of IL-2 cancer immunotherapy. We have evaluated 12 immunotherapeutic courses with IL-3 plus IL-2, which were performed in 6 patients with metastatic non-small cell lung cancer. The results were compared to those seen in 22 courses with IL-2 alone, carried out in 12 patients with metastatic non-small cell lung cancer. IL-3 was given intravenously at a daily dose of 1 μg/kg/b.w. at 6 p.m. for 14 consecutive days, starting 7 days before IL-2. IL-2 was given subcutaneously at a dose of 3 million IU twice/daily for 5 days/week for 3 weeks. The increase in serum levels of the specific macrophage marker neopterin, induced by IL-2, was completely blocked by IL-3. The IL-2-induced SIL-2R rise was significantly lower during IL-3 plus IL-2 than under IL-2 alone. The increase in cortisol levels in response to IL-2 was neutralised by IL-3. The increase in lymphocyte, T lymphocyte, natural killer (NK) cell, activated T lymphocyte and eosinophil mean number was significantly higher during IL-3 plus IL-2 than during IL-2 alone. Episodes of fever, asthenia, anorexia, vomiting, anaemia and thrombocytopenia were significantly more frequent in patients receiving IL-2 alone than in those treated with IL-3 and IL-2. This preliminary study would suggest that IL-3 may improve the tolerability of IL-2 immunotherapy and enhance the biological antitumour properties of IL-2 by neutralising cortisol increase and macrophage-mediated suppressive events, with a following potential amplification of Il-2 anticancer efficacy.
Eur J Cancer, Vol. 29A, No. 8, pp. 1127–1132, 1993.

INTRODUCTION

IT IS known that lymphokine-activated killer (LAK) cells, generated by interleukin-2 (IL-2) from natural killer (NK) cells and T lymphocyte precursors may destroy *in vitro* almost all tumour histotypes [1]. On the other hand, only few tumour histotypes seem to respond *in vivo* to IL-2, mainly renal adenocarcinoma and malignant melanoma [2–4]. It has to be considered that IL-2 administration induces not only immune effects, but also metabolic [5, 6], endocrine [7], neuroendocrine [8, 9] and cardiovascular effects [10]. The causes responsible for the low *in vivo* anticancer efficacy of IL-2 could be related to a greaty variety of immunosuppressive events [11, 12], mediated either by the endocrine system or by the immune system. Furthermore, the following suppressive events have been described during IL-2 cancer immunotherapy: (1) stimulation of cortisol secretion [7–9], which plays an inhibitory effect on IL-2-dependent immune functions [13]; (2) abnormal increase in soluble IL-2 receptors (SIL-2R) [12,14], which bind IL-2 by

Correspondence to P. Lissoni.
P. Lissoni, S. Barni, F. Rovelli, S. Pittalis, A. Ardizzoia and G. Tancini are at the Division of Radiation Oncology; E. Tisi is at the Division of Thoracic Surgery; R. Rescaldani and L. Vigoré are at the Laboratory of Microbiology, San Gerardo Hospital, Monza, Milan, Italy; and A. Biondi is at the Mario Negri Institute, Milan, Italy.
Revised 3 Sep. 1992; accpeted 19 Oct. 1992.

competing for IL-2 with IL-2 cell surface receptor on immune cells [15]; (3) abnormal increase in serum levels of neopterin [16], which has been proven to be associated with macrophage-mediated immuno-suppressive events during IL-2 immunotherapy [16]; (4) interference with pineal function [9], which exerts a relevant immunostimulatory role through the circadian release of its main hormone melatonin (MLT) [17].

At present, no immunotherapeutic strategy [18], including bacillus (BCG), interferons and IL-2 itself, has appeared to be able to induce an antitumour immune activation without determining a concomitant generation of suppressor cells, mainly consisting of the monocyte–macrophage system. Macrophages may inhibit IL-2 dependent anticancer immune responses through several mechanisms, including an inhibitory effect on NK cell proliferation [19], a production of prostaglandin E_2 [20], a stimulation of SIL-2R release [21] and a production of tumour growth factors [22]. In fact, macrophage infiltration into tumour tissue has been shown to stimulate cancer cell proliferation [23]. As far as macrophage behaviour during IL-2 immunotherapy is concerned, our previous clinical studies have suggested that the increase in SIL-2R and neopterin in response to IL-2 injection is related to the occurrence of macrophage-mediated suppressive events [16] and it is associated with a reduced clinical efficacy of IL-2 [24].

No evaluated drug has appeared to be able to block macrophage-mediated suppressive events during IL-2 immunotherapy [11, 12], or during previous immunotherapies of cancer [18]. Our previous experimental investigations have shown that interleukin-3 (IL-3) may modulate some macrophage-mediated suppressive events induced by IL-2 [25]. On these bases, we have designed an immunotherapeutic combination with IL-2 and IL-3, in an attempt to investigate in humans the influence of IL-3 on IL-2-induced immunobiological effects.

MATERIALS AND METHODS

We have analysed 12 immunotherapeutic courses with IL-3 and IL-2, which were performed in 6 patients with metastatic non-small cell lung cancer (NSCLC) (M/F:5/1; median age 61 years, range 52–74). Tumour histotypes were, as follows: adenocarcinoma, 4; large cell carcinoma, 1; epidermoid carcinoma, 1. All patients were untreated for their metastatic disease and they showed visceral lesions as dominant metastasis sites. Human recombinant IL-2 was supplied by Euro-Cetus (Amsterdam, Holland) and human recombinant IL-3 was supplied by Sandoz Pharma Ltd (Basel, Switzerland). Each course consisted of IL-2 at a dose of 3×10^6 IU twice/day (8.00 a.m. and 8.00 p.m.) for 5 days/week for 3 consecutive weeks, and of IL-3 at a dose of 1 μg/kg b.w./day for 14 consecutive days, starting 7 days before the first IL-2 injection. IL-2 was subcutaneously given into different parts of the abdominal wall. IL-3 was given intravenously in 50 ml of saline over 60 min at 6.00 p.m. The immunotherapeutic courses with IL-3 plus IL-2 were repeated after a 14-day rest period. Each patient received two immunotherapeutic courses. The daily dose of IL-2 was comparable to that described by other authors, who used the subcutaneous route of injection [26]. The dose of IL-3 and its schedule of administration were established according to our previous experimental studies in animals [25], showing that low doses of IL-3 are enough to modulate neopterin release from macrophages in responses to IL-2.

The results were compared to those observed during 22 immunotherapeutic courses with IL-2 alone, which were administered to 12 patients affected by metastatic NSCLC (adenocarcinoma, 7; epidermoid carcinoma, 5). Control patients were comparable with patients treated with IL-3 plus IL-2 for all main clinical variables, including age, performance status, tumour load, sites of disease, previous anticancer therapies and supportive care. They were chosen within a historical control group of 16 patients; we considered those patients in whom a complete immune and endocrine investigation was available. 10 patients received two immunotherapeutic courses, while the other 2 cases received only one course of therapy, because they refused the second one for personal reasons. Control patients received IL-2 as therapy of their neoplastic disease, because they refused chemotherapy or were unable to tolerate the conventional chemotherapies for important medical illnesses other than cancer. The experimental protocol of immunotherapy was explained to each patient or control, and informed consent was obtained.

For immune detections, venous blood samples were collected during the morning 7 days before the onset of IL-2 injection, at days 0, 7, 14 and 21 of IL-2 administration, and 7 days after IL-2 interruption (day 28). In each blood sample, we have measured the absolute number of lymphocytes, T lymphocytes (CD3), NK cells (CD16), activated T lymphocytes (CD25). Eosinophil number was also determined. Finally, serum levels of neopterin and of SIL-2R were determined at the same intervals. Lymphocyte subsets were detected with a flow cytometric analysis by fluorescence activated cell sorter (FACS) and monoclonal antibodies supplied by Becton-Dickinson (Milan, Italy). Serum levels of neopterin were measured with the radioimmunoassay (RIA) method, using commercial kits (Henning, Berlin, Germany). Serum levels of SIL-2R were measured with an enzyme immunoassay and commercially available kits (T Cell Sciences, Cambridge, Massachusetts, U.S.A.).

Moreover, to evaluate the influence on cortisol circadian secretion, venous blood samples were drawn at 8.00 a.m., 12.00 a.m., 6.00 p.m. and 12.00 p.m., by performing the study on the first day of IL-2 injection. Serum levels of cortisol were measured by RIA and commercial kits (Sclavo, Siena, Italy). Both immune and endocrine investigations were performed during the first immunotherapeutic course. Moreover, patients receiving a successive immunotherapeutic course were also immunologically monitored during the successive course of therapy.

Results were reported as mean (± S.D./S.E.). Data were statistically analysed by the Student's t-test, analysis of variance and χ^2 test, as appropriate.

RESULTS

Changes in SIL-2R and in neopterin serum concentrations observed in patients treated with IL-3 plus IL-2 or with IL-2 alone are illustrated in Fig. 1. According to analysis of variance, mean levels of both SIL-2R and neopterin were significantly higher in patients treated with IL-2 alone than in those receiving IL-3 plus IL-2.

Total lymphocytes and T lymphocytes significantly increased in both groups of patients ($P < 0.01$ vs. pretreatment values), as evaluated by analysis of variance and illustrated in Fig. 2. However, according to the Student's t-test, peak mean values of both types of cells were significantly higher in patients treated with IL-3 plus IL-2 than in those treated with IL-2 alone ($P < 0.05$).

Mean number of eosinophils observed in response to IL-2 or to IL-3 plus IL-2 was significantly higher in respect to the values seen before therapy ($P < 0.001$), according to analysis

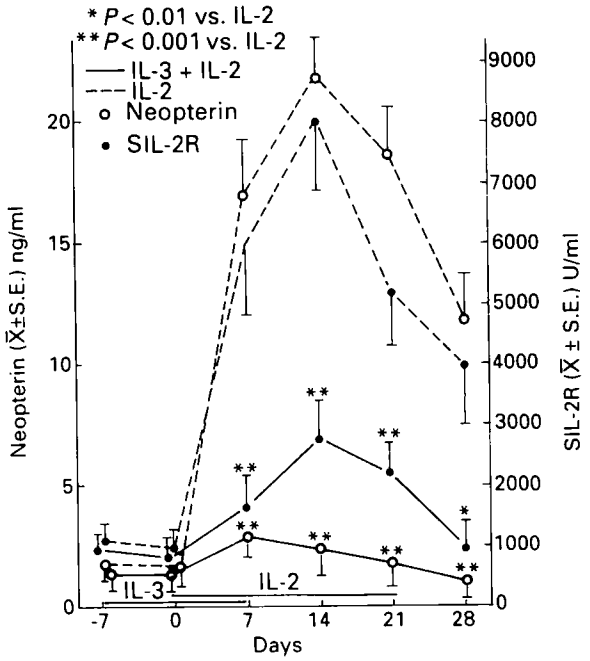

Fig. 1. Mean serum levels of neopterin and soluble IL-2 receptor (SIL-2R) during cancer immunotherapy with IL-2 or IL-3 plus IL-2.

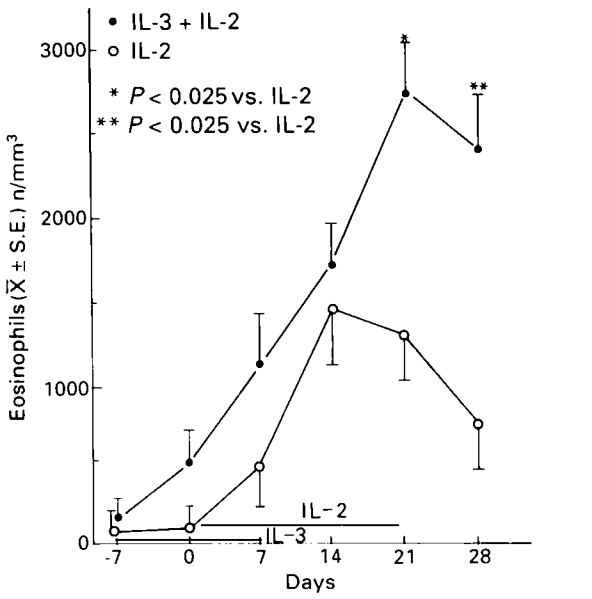

Fig. 3. Effects of cancer immunotherapy with IL-2 or IL-3 plus IL-2 on eosinophil mean number.

of variance and the Student's t-test, as illustrated in Fig. 3. Moreover, eosinophil mean maximum increase was significantly higher in patients treated with IL-3 plus IL-2 than in those receiving IL-2 alone ($P < 0.025$).

A significant increase in NK cell and CD25-positive T lymphocyte mean number was achieved in both groups of patients ($P < 0.05$), as documented by the analysis of variance and illustrated in Fig. 4, but their mean values found at the end of the immunotherapeutic course and at day 7 of the rest period were significantly higher in patients treated with IL-3 plus IL-2 than in those receiving IL-2 alone.

The mean number of lymphocytes, activated T lymphocytes and NK cells observed during the second immunotherapeutic course in both groups of patients in relation to the values seen during the first course are reported in Table 1. The analysis of variance and the Student's t-test showed no significant differences in the mean values of cells between the first and the second course of immunotherapy.

Finally, changes in cortisol circadian secretion, observed during the first day of IL-2 injection and evaluated by the analysis of variance, are illustrated in Fig. 5. IL-2 alone abrogated the physiological daily rhythm of cortisol, because of the evidence

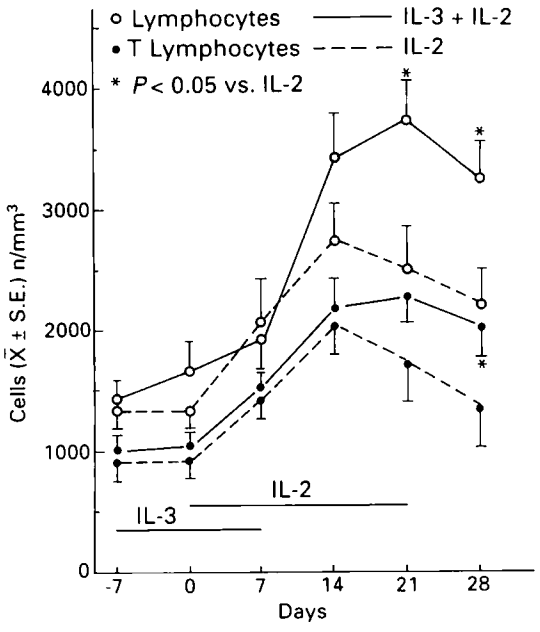

Fig. 2. Changes in lymphocyte and T-lymphocyte mean number during cancer immunotherapy with IL-2 or IL-3 plus IL-2.

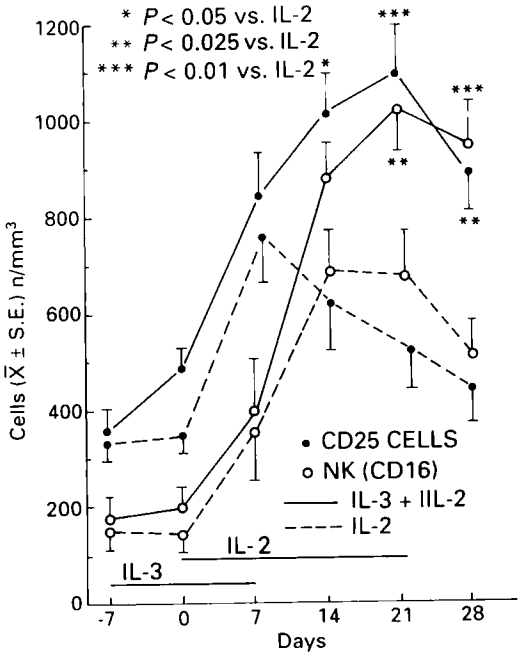

Fig. 4. Mean number of NK (CD16) and activated T lymphocytes (CD25) during cancer immunotherapy with IL-2 or IL-3 plus IL-2.

Table 1. Absolute number (n/mm³, means ± S.D.) of lymphocytes, activated T lymphocytes (CD25), NK cells (CD16) during the 21-day course of IL-2 injection in cancer patients receiving two successive courses of IL-2 alone (n = 12) or IL-2 plus IL-3 (n = 6)

	IL-2					IL-2 + IL-3				
Days	0	7	14	21	28	0	7	14	21	28
1st course										
Lymphocytes	1432 ± 334	2026 ± 567	2776 ± 509	2524 ± 608	2211 ± 478	1518 ± 461	1882 ± 435	3466 ± 489	3783 ± 433*	3292 ± 341*
CD25-cells	366 ± 118	758 ± 307	591 ± 267	524 ± 179	441 ± 108	438 ± 107	833 ± 255	1046 ± 249*	1088 ± 282‡	894 ± 226†
NK cells	158 ± 74	341 ± 218	682 ± 289	631 ± 270	512 ± 192	187 ± 86	384 ± 206	894 ± 269	1022 ± 258*	935 ± 288†
2nd course										
Lymphocytes	1648 ± 301	2214 ± 481	2845 ± 516	2667 ± 546	2304 ± 445	1781 ± 409	2024 ± 399	3515 ± 501	3893 ± 411*	3339 ± 386*
CD25 cells	385 ± 109	804 ± 371	608 ± 263	561 ± 205	483 ± 127	409 ± 141	918 ± 231	103 ± 273	1112 ± 264‡	956 ± 198†
NK cells	171 ± 88	401 ± 235	669 ± 208	644 ± 231	539 ± 204	218 ± 106	445 ± 156	948 ± 234	1198 ± 254*	1087 ± 255†

*$P < 0.05$ vs. IL-2; †$P < 0.025$ vs. IL-2; ‡$P < 0.01$ vs. IL-2.

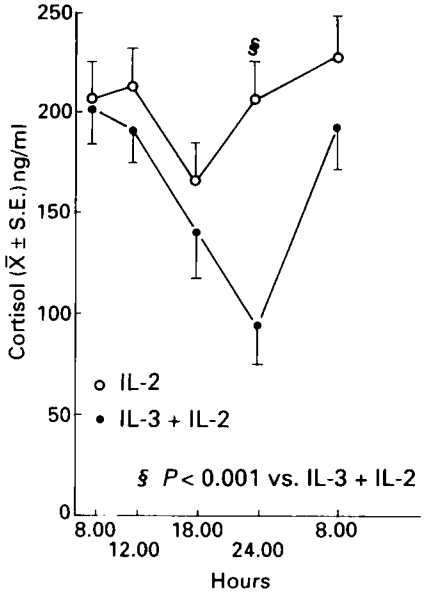

Fig. 5. Effect of cancer immunotherapy with IL-2 or IL-3 plus IL-2 on circadian secretion of cortisol serum levels.

of an increase in its concentrations during the night. On the contrary, the physiological night decrease in the levels of cortisol was maintained in all patients treated with IL-3 plus IL-2, and mean levels of cortisol found at midnight in patients treated with IL-3 plus IL-2 were significantly lower than those seen in patients who received IL-2 alone.

The toxicity was more pronounced during the immunotherapy consisting of IL-2 alone. In particular, as evaluated by the χ^2 test, the frequency of episodes of fever greater than 38°C, asthenia, anorexia, vomiting and depressive symptoms was significantly higher in patients treated with IL-2 alone than in those who received IL-3 plus IL-2. Anaemia and thrombocytopenia occurred in 6/22 and in 5/22 courses with IL-2 alone, respectively, and in none of patients receiving IL-3 plus IL-2. These differences were statistically significant according to χ^2 test ($P < 0.05$). The control of side-effects obtained with IL-3 was not limited to the period of IL-3 injection, but persisted throughout the whole period of IL-2 administration. The main toxicities observed during the immunotherapeutic courses are reported in Table 2.

As far as the clinical response is concerned, according to WHO criteria, a partial response was achieved in 1/6 patients treated with IL-3 plus IL-2 (duration 7+ months); he was affected by lung metastases due to lung adenocarcinoma. 4 other patients had stable disease (median duration 5 months, range 3–7+) and 1 progressed. Within the group treated with IL-2 alone, 5 patients with stable disease (median duration 4 months, range 2–7) and 7 patients with progressive disease were seen.

DISCUSSION

The present results would demonstrate that a pretreatment with IL-3 may neutralise the most well-characterised immunosuppressive events which occur during the immunotherapy of human cancer with IL-2. In particular, this study shows that the increase in neopterin and SIL-2R, which are of immunosuppressive significance during IL-2 immunotherapy [16], can be blocked by concomitant administration of IL-3, as previously demonstrated in experimental conditions [25]. Since the gener-

Table 2. Main toxicities observed during 12 immunotherapeutic courses with IL-3 plus IL-2 and during 22 courses with IL-2 alone, performed in 6 and in 12 metastatic NSCLC patients, respectively

Side-effects	Number of courses		Significance (*P*)
	IL-3 + IL-2 (*n* = 12)	IL-2 (*n* = 22)	
Fever > 38°C	3/12	15/22	<0.001
Anorexia	1/12	16/22	<0.001
Nausea/vomiting	0/12	7/22	<0.01
Asthenia	0/12	6/22	<0.01
Pruritus	4/12	7/22	NS
Depressive symptoms	0/12	4/22	<0.05
Hypotension	0/12	0/22	NS
Cardiac complications	0/12	1/22	NS
Anaemia (< 10 g/100 ml)	0/12	6/22	<0.05
Thrombocytopenia (< 100 000/mm^3)	0/12	5/22	<0.05
Transaminase increase	5/12	22/22	<0.01
Local induration	4/12	7/22	NS

NS = not significant.

ation of suppressive events, mainly mediated by macrophages [16], seems to represent the main factor responsible for the reduced antitumour efficacy of cancer immunotherapies investigated up to now [16, 18, 24], the neutralisation of these suppressive mechanisms by IL-3 could determine an amplification of host immune defences against cancer. In fact, these preliminary results show a more prolonged lymphocyte proliferation and activation in response to IL-2 in patients concomitantly treated with IL-3, while in patients treated with IL-2 alone, lymphocyte number tends to decrease following the first few weeks of treatment, perhaps as a consequence of macrophage activation, whose response to IL-2 is generally delayed in respect to that showed by lymphocytes [27]. However, it will be required to establish further studies if the more evident increase in lymphocyte number during IL-3 plus IL-2 in comparison to IL-2 alone is not due to a block upon homing from cells to tissues. In the same way, further studies will be needed to demonstrate that the lower increase in SIL-2R levels during IL-3 plus IL-2 is not the result of decreased lymphocyte stimulation at tissue sites.

Several cytokines have been investigated in association with IL-2 [28], mainly tumour necrosis factor-alpha (TNFα) and interferons, however, without any real enhancement of IL-2 antitumour efficacy. Neverthless, it has to be remarked that all cytokines investigated in association with IL-2 have been given in an attempt to increase host cytotoxic response against tumour, whereas no strategy has been proposed to counteract the concomitant generation of suppressive events related to IL-2 immunotherapy. Therefore, this study shows for the first time the possibility to selectively activate lymphocyte-mediated immune response without simultaneously generating immunosuppressive events related to macrophage activation [18] by a concomitant administration of IL-3. This finding is not surprising since previous studies had already demonstrated that IL-3 may modulate macrophage function [29] and regulate IL-2 receptor expression on some haemopoietic cells [30]. Therefore, IL-3 pretreatment could make macrophages less responsive to the successive injection to IL-2. This hypothesis could explain the lower production of neopterin, a specific marker of macrophage activation [16], in response to IL-2 in patients concomitantly treated with IL-3. On the other hand, the inhibitory effect of IL-3 on IL-2 induced SIL-2R secretion might depend on a direct modulatory action of IL-3 on IL-2 receptor expression on the lymphocyte cell surface, but also, at least in part, on the regulation of macrophages themselves, which play a stimulatory role on SIL-2R release from activated lymphocytes [21]. However, further investigations will be required to better define the interactions between IL-3 and macrophage response to cancer growth. The influence of macrophages on tumour cell proliferation could change with time, and a down-regulation of functions of the monocyte–macrophage system might also result in adverse long term effects for cancer patients. In any case, the investigation of monocyte–macrophage cytotoxicity in response to IL-3 will be required to further establish which is the role of IL-3 in regulating the influence of macrophages on cancer development.

Another potential suppressive event occurring during IL-2 immunotherapy, such as IL-2-induced cortisol rise, seems to be also blocked by IL-3. Because of the central action of cytokines [31], the inhibition of IL-2-induced cortisol secretion by IL-3 might depend on a direct endocrine effect on the hypothalamic–pituitary axis, rather than on the adrenal gland. Moreover, since most cytokines have appeared to stimulate cortisol release, which is related to stress-events, this finding would suggest that IL-3 may be considered as an anti-stress cytokine and that it may play a physiological role in modulating the interactions existing between endocrine and immune systems. In addition, since cortisol inhibits eosinophil production and lymphocyte proliferation [13], the block of IL-2-induced cortisol rise by IL-3 could also contribute to determine a more prolonged lymphocytosis and eosinophilia in patients concomitantly treated with IL-3.

In addition to the apparent amplification of host antitumour biological response, this study would suggest that IL-3 may also reduce IL-2 toxicity, improve its subjective and objective tolerability, and abrogate its haematological side-effects, including anaemia and thrombocytopenia. This evidence could suggest that IL-2 toxicity may be mediated at least in part by macrophages. Moreover, since IL-3 stimulates the proliferation of all haemopoietic cell lines [32], the prevention of IL-2-induced anaemia and thrombocytopenia by IL-3 may be due to a direct differentiating action on stem cells. IL-3 has been proven to be produced during IL-2 immunotherapy [33], but the amounts of IL-3 released in response to IL-2 are probably not sufficient to counteract the fall in red cell and platelet number during IL-2 therapy, which would be due, at least in part, to the activation of macrophages, responsible for the destruction of both types of haemopoietic cells. Finally, it has to be remarked that IL-3 actions described in the present study, including the inhibition of SIL-2R rise, neopterin secretion and cortisol release, have been obtained at a dose which is lower in respect to that generally used when IL-3 is given to stimulate bone marrow proliferation [32]. Further studies, however, will be needed to better define the optimal dose and schedule of administration of IL-3 to realise a complete antagonism of IL-2-induced suppressive events and to enhance host anticancer reactions.

In conclusion, this clinical study shows that a concomitant administration of IL-3 may neutralise the suppressive events responsible for the reduced *in vivo* efficacy of IL-2 including cortisol secretion, SIL-2R and neopterin release, with an apparent increased proliferation of antitumour activated lymphocytes. However, randomised studies with IL-3 plus IL-2 versus IL-2 alone will be needed to confirm the synergistic properties of IL-

3 and to establish whether the inhibition of IL-2-induced suppressive events by IL-3 may really determine an increase in objective tumour regression rate during IL-2 immunotherapy of human neoplastic diseases.

1. Grimm EA, Mazumder A, Zhang HZ, Rosenberg SA. Lymphokine-activated killer cell phenomenon: lysis of natural killer-resistant fresh solid tumor cells by interleukin 2-activated autologous human peripheral blood lymphocytes. *J Exp Med* 1982, **155**, 1823–1841.
2. Rosenberg SA, Lotze MT, Muul LM, *et al.* A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer and interleukin-2 or high-dose interleukin-2 alone. *N Engl J Med* 1987, **316**, 889–897.
3. West WH, Tauer KW, Yannelli JR, *et al.* Constant-infusion recombinant interleukin-2 in adoptive immunotherapy of advanced cancer. *N Engl J Med* 1987, **316**, 898–905.
4. Mitchell MS, Kempf RA, Harel W, *et al.* Effectiveness and tolerability of low-dose cyclophosphamide and low-dose intravenous interleukin-2 in disseminated melanoma. *J Clin Oncol* 1988, **6**, 409–424.
5. Wilson DE, Birchfield GR, Hejazi JS, Ward JH, Samlowski WE. Hypocholesterolemia in patients treated with recombinant interleukin-2: appearance of remnant-like lipoproteins. *J Clin Oncol* 1989, **7**, 1573–1577.
6. Lissoni P, Brivio F, Pittalis S, *et al.* Decrease in cholesterol levels during the immunotherapy of cancer with interleukin-2. *Br J Cancer* 1991, **64**, 956–958.
7. Atkins MB, Gould JA, Allegretta M, et al. Phase I evaluation of recombinant interleukin-2 in patients with advanced malignant disease. *J Clin Oncol* 1986, **4**, 1380–1391.
8. Denicoff KD, Durkin TM, Lotze MT, *et al.* The neuroendocrine effets of interleukin-2 treatment. *J Clin Endocrinol Metab* 1989, **69**, 402–410.
9. Lissoni P, Barni S, Archili C, *et al.* Endocrine effects of a 24-hour intravenous infusion of interleukin-2 in the immunotherapy of cancer. *Anticancer Res* 1990, **10**, 753–758.
10. Lissoni P, Barni S, Cattaneo G, *et al.* Evaluation of the cardiovascular toxicity related to cancer immunotherapy with interleukin-2 by monitoring atrial natriuretic peptide secretion: a case report. *Tumori* 1990, **76**, 603–605.
11. Wiebke EA, Rosenberg SA, Lotze MT. Acute immunologic effects in interleukin-2 therapy in cancer patients: decreased delayed type hypersensitivity response and decreased proliferative response to soluble antigens. *J Clin Oncol* 1988, **6**, 1440–1449.
12. Lissoni P, Barni S, Rovelli F, *et al.* Correlation of serum interleukin-2 levels, soluble interleukin-2 receptors and T lymphocyte subsets in cancer patients. *Tumori* 1990, **76**, 14–17.
13. Fauci AS, Dale DC. The effect of hydrocortisone on the kinetics of normal human lymphocytes. *Blood* 1975, **46**, 235–243.
14. Lotze MT, Custer MC, Sharrow SO, Rubin LA, Nelson DL, Rosenberg SA. *In vivo* administration of purified interleukin-2 to patients with cancer: development of interleukin-2 receptors following interleukin-2 administration. *Cancer Res* 1987, **47**, 2188–2195.
15. Rubin LA, Jay G, Nelson DL. The released interleukin-2 receptor binds interleukin-2 efficiently. *J Immunol* 1986, **137**, 3841–3845.
16. Lissoni P, Tisi E, Brivio F, *et al.* Increase in soluble interleukin-2 receptor and neopterin serum levels during immunotherapy of cancer with interleukin-2. *Eur J Cancer* 1991, **27**, 1014–1016.
17. Maestroni GJM, Conti A, Pierpaoli W. Melatonin regulates immunity via an opiatergic mechanism. *Clin Neuropharmacol* 1986, **9** (Suppl 4), 479–481.
18. Broder S, Muul L, Waidmann TA. Suppressor cells in neoplastic disease. *J Natl Cancer Inst* 1978, **61**, 5–11.
19. Bordignon C, Villa F, Allavena P, *et al.* Inhibition of natural killer activity by human bronchoalveolar macrophages. *J Immunol* 1982, **129**, 587–591.
20. Chouaib S, Fradelizi D. The mechanism of inhibition of human IL 2 production. *J Immunol* 1982, **129**, 2463–2467.
21. Nelson DL, Rubin LA, Kurman CC, Fritz ME, Boutin B. An analysis of the cellular requirements for the production of soluble interleukin-2 receptors *in vitro*. *J Clin Innumol* 1986, **6**, 114–120.
22. Sandru G, Veraguth P, Stadler BM. Stimulation of tumor cell growth in humans by a mononuclear cell-derived factor. *Cancer Res* 1988, **48**, 5411–5416.
23. Farinas MC, Rodriguez-Valverde V, Zarrabeitia MT, Parra-Blando JA, Sanz-Ortiz J. Contribution of monocytes to the decreased lymphoproliferative response to phytohemagglutinin in patients with lung cancer. *Cancer* 1991, **68**, 1279–1284.
24. Lissoni P, Barni S, Ardizzoia A, *et al.* Second line therapy with low-dose subcutaneous interleukin-2 alone in advanced renal cancer patients resistant to interferon-alpha. *Eur J Cancer* 1992, **28A**, 92–96.
25. Lissoni P, Ardizzoia A, Barni S, *et al.* Biological ratio of IL-3 use in IL-2 immunotherapy. *Eur J Cancer* 1991, **27**(Suppl 3), S18.
26. Atzpodien J, Körfer A, Franks CR, Poliwoda H, Kirchner H. Home therapy with recombinant interleukin-2 and interferon-α2b in advanced human malignancies. *Lancet* 1990, **1**, 1509–1512.
27. Higashi N, Nishimura Y, Higuchi M, Osawa T. Human monocytes in a long-term culture with interleukin-2 show high tumoricidal activity against various tumor cells. *J Immunother* 1991, **10**, 247–255.
28. Atzpodien J, Kirchner H. Cancer, cytokines and cytotoxic cells: interleukin-2 in the immunotherapy of human neoplasms. *Klin Wochenschr* 1990, **68**, 1–11.
29. Frendl G, Beller DI. Regulation of macrophage activation by IL-3. *J Immunol* 1990, **144**, 3392–3399.
30. Riedel D, Lindemann A, Brach M, Mertelsmann R, Herrmann F. Granulocyte–macrophage colony-stimulating factor and interleukin-3 induce surface expression of interleukin-2 receptor p55-chain and CD4 by human eosinophils. *Immunology* 1990, **70**, 258–261.
31. Del Rey A, Besedovsky HO, Sorkin E, Dinarello CA. Interleukin-1 and glucocorticoid hormones integrate an immunoregulatory feedback circuit. *Ann NY Acad Sci* 1987, **496**, 85–92.
32. Devereux S, Linch DC. Clinical significance of the haemopoietic growth factors. *Br J Cancer* 1989, **61**, 2–5.
33. Heslop HE, Duncombe AS, Reittie JE, *et al.* Interleukin 2 infusion induces haemopoietic growth factors and modifies marrow regeneration after chemotherapy or autologous marrow transplantation. *Br J Haematol* 1991, **77**, 237–244.