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Peri-operative modulation of cellular immunity in patients with colorectal cancer

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SUMMARY

The peri-operative cellular immune response is depressed in patients with gastrointestinal cancer, a factor which may facilitate malignant dissemination. We have investigated the effects of peri-operative rIL-2 and a combination of rIL-2 and interferon-alpha (IFN-α) on both peripheral blood lymphocyte function and number in patients undergoing surgical resection for colorectal cancer. Fifty-two patients were randomly allocated to either control, rIL-2 or rIL-2 with IFN-α treatment arms. *In vitro* studies were performed pre-operatively and on post-operative days 1, 4, 7 and 10. Natural killer (NK) and lymphokine-activated killer (LAK) cell function were profoundly depressed in control patients ($P < 0{\cdot}001$; $P < 0{\cdot}01$), an effect abrogated in both treatment groups; indeed NK function was augmented in the rIL-2 and IFN-α group on the first post-operative day in association with an increase in the percentage of cells expressing CD16 and CD56 ($P < 0{\cdot}01$). Flow cytometric analysis of lymphocyte subsets in the control group was unremarkable, except for an early post-operative fall in numbers of lymphocytes. Treatment with either rIL-2 or rIL-2 and IFN-α produced an initial profound reduction in T lymphocyte numbers, followed by a 'rebound' lymphocytosis of activated $CD3^+$ T cells, as demonstrated by a significant increase in co-expression of CD25, CD38 and CD45RO. No significant differences were observed between either of the treatment groups. Adjuvant immunotherapy affects peri-operative anti-tumour immune responses, and this may influence long term outcome in patients undergoing surgery for gastrointestinal cancer.

Keywords peri-operative immunotherapy IL-2 interferon-alpha colorectal cancer T cells natural killer cells lymphokine-activated killer cells

INTRODUCTION

Curative treatment for most solid organ malignancies still depends on surgical resection of the bulk of the tumour. Colorectal cancer is no exception, but despite adequate removal of all macroscopic tumour at operation, most patients will still die from recurrent disease [1]. As surgery alone is inadequate, several groups have used adjuvant chemotherapy with modest success, limited primarily to survival benefits only for those patients with Dukes' Stage C colon cancer [2,3]. The treatment regimen, however, is given for a full year after operation and is associated with not insignificant morbidity.

The role of immunotherapy has yet to be established in patients with malignant disease, but recombinant cytokine therapy has shown some promise in the treatment of a number of advanced cancers [4]. We are interested in the potential role of adjunctive immunotherapy given in the peri-operative period. This seems a logical time for adjuvant therapy on a number of grounds. First, the tumour bulk is at its minimum. Second, the cellular anti-tumour immune responses, which are already depressed in patients with gastrointestinal cancer [5], are decreased further by major surgery [6]. Finally, tumour cells are shed into the circulation at operation [7,8] and with host immune defences already attenuated, there is a clear risk of dissemination. This is well demonstrated in animal models [9].

IL-2 is a key molecule in the cellular immune system [10], being necessary for the proliferation and maturation of cytotoxic T lymphocytes [11], and the development of lymphokine-activated killer (LAK) cells from natural killer (NK) cell precursors [12]. LAK cells have the ability to kill adenocarcinoma cells *in vitro*, and when given in combination with rIL-2 have demonstrated activity against solid tumours in patients [13].

The anti-tumour mechanism of interferon-alpha (IFN-α) has yet to be elucidated. It is known to have direct anti-proliferative activity [14], but IFN-α also augments NK cell activity, and up-regulates expression of both class I MHC antigen and tumour-associated antigen in tumours [15]. Although the highest clinical activity for single therapy with

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IFN-α has been with selected haematological malignancies, such as hairy cell leukaemia and chronic myeloid leukaemia [16], significant activity has been demonstrated in solid organ disease when compared with conventional chemotherapy [17].

In previous studies on patients undergoing surgery for gastrointestinal malignancy this group has shown that peri-operative single-cytokine therapy with IFN-α augments NK cell activity, but is associated with marked anti-proliferative effects on peripheral blood lymphocytes (PBL) [6]. When peri-operative rIL-2 is administered, we demonstrated that both NK and LAK cell activity are enhanced together with significant PBL activation [18]. Recent murine work has investigated the effects of combination therapy with IFN-α and IL-2, and has shown enhanced anti-tumour activity when compared with each agent alone [19]. This synergy presumably derives from the different mechanisms of action of these cytokines, and an elegant murine study demonstrated that tumour expression of class I MHC antigen is of fundamental importance to tumour responsiveness to IL-2 therapy [20].

To assess the use of combination peri-operative immunotherapy, we have performed a randomized controlled study comparing single agent rIL-2 and combination rIL-2 and IFN-α on a number of components of the cellular immune system in patients undergoing surgery for colorectal cancer. High dose cytokine therapy is extremely toxic [17] and clearly unsuitable in this vulnerable peri-operative setting, so, as in our previous studies, we have evaluated a low dose subcutaneous regimen. This method of administration provides sustained levels of cytokine over a 24-h period [21] and has previously shown proven anti-tumour capability [22]. The dose of each cytokine was chosen from previous studies [6,18], having shown immunological effect with minimal toxicity.

PATIENTS AND METHODS

Patients

Fifty-two patients undergoing elective surgery for histologically confirmed colorectal cancer were studied. Patients were prospectively randomized into one of three arms:

Arm 1: surgery alone. Control group proceeded through surgery in the normal manner.

Arm 2: surgery+rIL-2. Each received $1{\cdot}8 \times 10^6$ U/m^2 rIL-2 subcutaneously (EuroCetus, Harefield, UK) twice daily for 5 days starting the evening before surgery. Injections were administered at 08.00 h and 20.00 h.

Arm 3: surgery+rIL-2+IFN-α. Each received treatment as Arm 2, with the addition of 2 MU recombinant IFN-α subcutaneously (Roferon-A; Roche Products, Basle, Switzerland) daily at 20.00 h for 5 days starting the evening before surgery.

All patients gave informed and written consent before entry. The study was approved by Leeds Eastern District Clinical Research (Ethics) Committee.

Immunological studies

Baseline assessment of lymphocyte number and function was performed on the pre-operative day and repeated on post-operative days 1, 4, 7 and 10. From each patient a 35-ml sample of peripheral venous blood was drawn between 07.00 h and 08.00 h, before the morning dose of rIL-2, thereby minimizing diurnal variance and immediate effects of cytokine. In each instance 5 ml were collected into EDTA and the remaining 30 ml into bottles containing eight drops of preservative-free heparin.

Lymphocyte separation

Peripheral blood mononuclear cells (PBMC) were separated from heparinised blood by Lymphoprep (Nycomed Pharma, Oslo, Norway) density gradient centrifugation [23]; washed twice in Hanks' balanced salt solution (HBSS) and resuspended in complete medium consisting of RPMI 1640 (GIBCO Ltd, Uxbridge, UK) supplemented with 10% fetal calf serum (FCS; Imperial Laboratories, Andover, UK), gentamicin (200 μg/ml), Fungizone (2·5 μg/ml), non-essential amino acids, mercaptoethanol (50 μM) and sodium pyruvate (5 mM). Cells were counted in a modified Neubauer counting chamber and diluted in complete medium to the cell density required for each experiment.

NK cell cytotoxicity assay

Freshly isolated PBMC were used for determination of NK cell activity using a standard 4-h ^{51}Cr release cytotoxicity assay with the K562 cell line as target [24]. In brief, 5×10^5 effector cells (PBMC) were incubated with 10^4 ^{51}Cr radio-labelled K562 target cells in triplicate wells of round-bottomed microtitre plates in a total reaction volume of 200 μl of complete medium. Serial doubling dilutions of effector cells were performed so that a range of E:T ratios from 50:1 to 6·25:1 were achieved. After incubation at 37°C in a humidified atmosphere containing 5% CO_2 for exactly 4 h, 100 μl of supernatant were removed from each well and its radioactivity measured in a Cobra Gamma counter (Canberra Packard). Spontaneous and maximal ^{51}Cr release was measured by incubating 10^4 target cells with complete medium alone and detergent, respectively. Percentage cytotoxicity of target cells at each E:T ratio was calculated from the standard formula: (experimental release − spontaneous release)/(maximal release − spontaneous release) × 100%. To standardize cytolytic activity, results were derived from the area under the curve (AUC) of the log (effector)/response curve exactly as described by Dye *et al.* [25]. This analysis provides a comparative measure of efficiency independent of kinetics.

Lymphokine-activated killer cell assay

For the generation of LAK cells, $1{\cdot}5 \times 10^7$ fresh PBMC were co-cultured with 1000 U/ml recombinant IL-2 (Roche Products) in 10 ml complete medium. The upstanding tissue culture flasks were incubated for 3 days at 37°C in a humidified atmosphere containing 5% CO_2 with gentle agitation daily. The cells were harvested, washed once in HBSS, resuspended in complete medium and counted for use as effectors in the cytotoxicity assay. This was performed in the same way as for the NK assay above, but using two NK-resistant cell lines as targets; Daudi (a reference target for LAK cell activity) and COLO 205 (a colonic adenocarcinoma cell line).

Lymphocyte proliferation assay

Freshly isolated PBMC were stimulated using the T cell mitogen concanavalin A (Con A; Sigma Chemicals Ltd, Poole, UK) as previously described [26]. Lymphocytes at a concentration of 10^6 cells/ml were co-cultured with 20 μg/ml Con A in a final volume of 200 μl, in flat-bottomed microtitre plates for 40 h at 37°C in a humidified atmosphere containing 5% CO_2. Lymphocytes without mitogen were simultaneously cultured and used to

give background activity. Proliferation was determined by adding 1 μCi of ^{3}H-TdR (Amersham, Little Chalfont, UK) to each well for the final 18 h of culture. Cells were harvested in a Dynatech 2000 cell harvester and the ^{3}H-TdR uptake of cells was measured on a Philips Beta counter. Results are expressed as a stimulation index (SI) derived from the formula: (ct/min ^{3}H-TdR incorporated by stimulated PBMC)/(ct/min ^{3}H-TdR incorporated by unstimulated PBMC).

IL-2 production

IL-2 production was measured from the Con A-stimulated PBMC used in the proliferation assay. At 24 h of incubation, 100-μl aliquots of the supernatant were carefully removed from each well and pooled so that 0·5 ml of supernatant was obtained from each quintuplicate. These samples were stored at −70°C for batch analysis of IL-2 content.

IL-2 estimations

Analysis of the IL-2 content was determined by the ability to support the growth of the murine IL-2-dependent cytotoxic T cell line CTLL-2 [27]. Briefly 100-μl duplicate doubling dilutions of supernatant were placed in the wells of a flat-bottomed microtitre plate, dilutions of standard IL-2 were placed in separate rows of the same plate for subsequent comparison. Washed CTLL-2 cells (100 μl) were added to each well to a final concentration 2×10^4 cells/ml, and the plate incubated at 37°C in a humidified atmosphere containing 5% CO_2 in air. Following 24 h of culture, 1-μCi aliquots of ^{3}H-TdR were added to each well, and culture maintained for a further 6 h. The cells were harvested and ^{3}H-TdR uptake measured as described above. The concentration of IL-2 in each supernatant was then calculated using log-probit analysis and results expressed as U/ml of IL-2.

Cell surface markers

Enumeration of leucocyte subpopulations was performed by flow cytometry using a panel of directly conjugated MoAbs directed against cell surface antigens. EDTA-treated blood was labelled within 4 h of collection, using a whole blood technique. In brief, 100 μl of undiluted whole blood were incubated for 15 min with a mixture of two MoAbs, each in a dilution 1:10. Erythrocytes were lysed using 'FACS Lysing Solution' (Becton Dickinson Immunocytometry Systems, San Jose, CA). The leucocytes were pelleted, washed once in PBS containing 0·1% azide before being stored at 4°C in the dark until analysis. The following MoAbs were obtained from Dako Ltd (High Wycombe, UK): UCHT1 (CD3, total T cells), MT310 (CD4, helper/inducer T cells), DK25 (CD8, cytotoxic/suppressor T cells), ACT-1 (CD25, p55 subunit IL-2 receptor), UCHL1 (CD45RO, T memory cell marker), and mouse IgG1 isotype controls. Anti-Leu-11c (CD16, NK cells), anti-Leu-19 (CD56, NK and LAK cells) and anti-Leu-17 (CD38, T cell activation marker) were obtained from Becton Dickinson Immunocytometry Systems. All antibodies were directly conjugated with either FITC or PE.

The cell preparations were analysed on a Becton Dickinson FACScan analytical flow cytometer. Lymphocytes were gated by low angle forward and right angle light scatter properties. The percentage of cells reactive with each MoAb was determined by comparison of fluorescently labelled cells with cells that had been labelled with isotype controls. Analysis was performed using 'Lysis II' software (Becton Dickinson), and results were determined by four-quadrant analysis having gated for >99% positive cells on isotype controls.

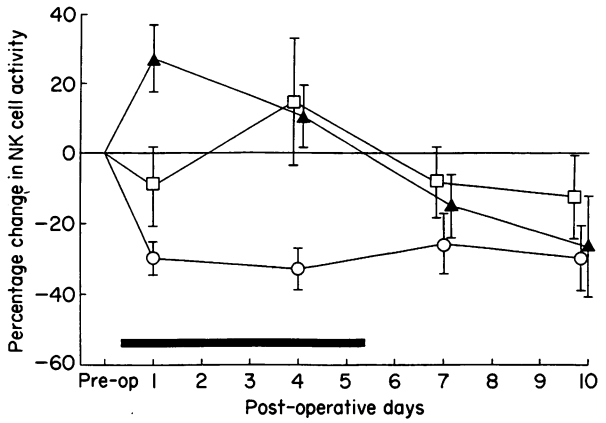

Fig. 1. Effect of peri-operative immunotherapy on natural killer (NK) cell activity of peripheral blood mononuclear cells (PBMC) in patients undergoing surgery for colorectal cancer. The percentage change in cellular cytotoxicity against the cell line K562 using the 4-h ^{51}Cr release assay is shown. Each point represents mean ± s.e.m. The bar indicates the treatment period. ○, Control; □, rIL-2; ▲, rIL-2 + IFN-α.

Total leucocyte counts and differential cell counts were performed on a Technicon H1 analyser in the Pathology Department at St James's University Hospital.

Statistical analysis

Statistical analysis was performed between groups using the *t*-test for unpaired data and within a group using a paired *t*-test. Results are expressed as mean ± s.e.m., with $P < 0{\cdot}05$ regarded as statistically significant.

RESULTS

Toxicity of treatment

Patients in each treatment group tolerated the injections well. There were no significant differences in maximum post-operative pyrexia between any of the groups. However, those patients in both treatment arms had a sustained swinging pyrexia throughout the treatment period. Three patients in the rIL-2 with IFN-α arm did complain of joint aches, controlled easily with paracetamol. No other treatment-related side effects were noted.

Lymphocyte function analysis

Figure 1 demonstrates the percentage change in NK cell function in the three arms of the study. Post-operative NK function was significantly reduced in the control group from 138·9 ± 19·9 pre-operatively to 93·9 ± 10·1 on day 1 ($P < 0{\cdot}001$). Those receiving rIL-2 showed no such fall in activity, and indeed by day 4 the percentage change was significantly different from control ($P < 0{\cdot}01$). Combination therapy with rIL-2 and IFN-α was associated with augmentation of NK activity on the first post-operative day (pre-operatively 115·7 ± 13·6 to 140·6 ± 17·1 on day 1; $P < 0{\cdot}01$), which is significant when compared with both controls ($P < 0{\cdot}0005$) and rIL-2 group ($P < 0{\cdot}05$). This level of enhanced activity over control was still evident on day 4 ($P < 0{\cdot}001$).

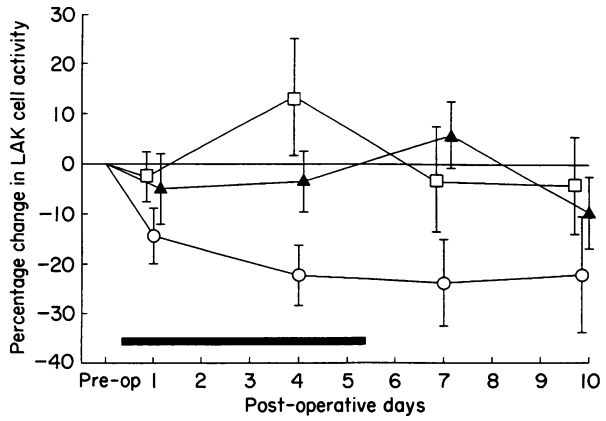

Fig. 2. Effect of peri-operative immunotherapy on lymphokine-activated killer (LAK) cell activity of peripheral blood mononuclear cells (PBMC) in patients undergoing surgery for colorectal cancer. The percentage change in cellular cytotoxicity against the cell line Daudi using the 4-h ^{51}Cr release assay is shown. Each point represents mean ± s.e.m. The bar indicates the treatment period. ○, Control; □, rIL-2; ▲, rIL-2 + IFN-α.

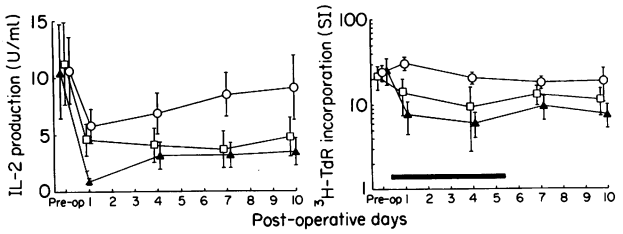

Fig. 3. Effect of peri-operative immunotherapy on the mitogenic response to concanavalin A (Con A) in patients undergoing surgery for colorectal cancer. Proliferation results are expressed as an SI of ^{3}H-TdR incorporation. IL-2 production is expressed as maximal production in U/ml. Each point represents mean ± s.e.m. The bar indicates the treatment period. ○, Control; □, rIL-2; ▲, rIL-2 + IFN-α.

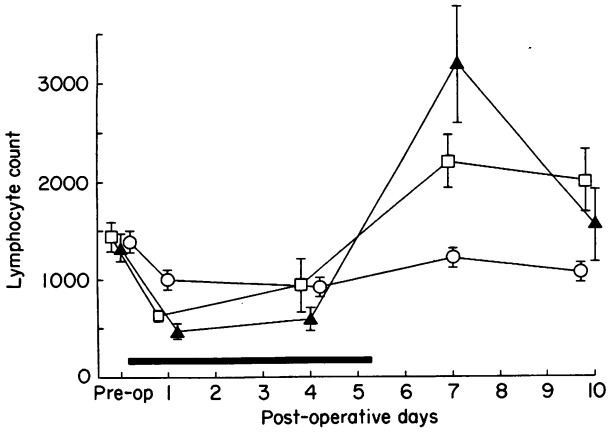

Fig. 4. Effect of peri-operative immunotherapy on total lymphocyte counts in patients undergoing surgery for colorectal cancer. Results are expressed as mean ± s.e.m. of the lymphocyte count × 10^3. The bar indicates the treatment period. ○, Control; □, rIL-2; ▲, rIL-2 + IFN-α.

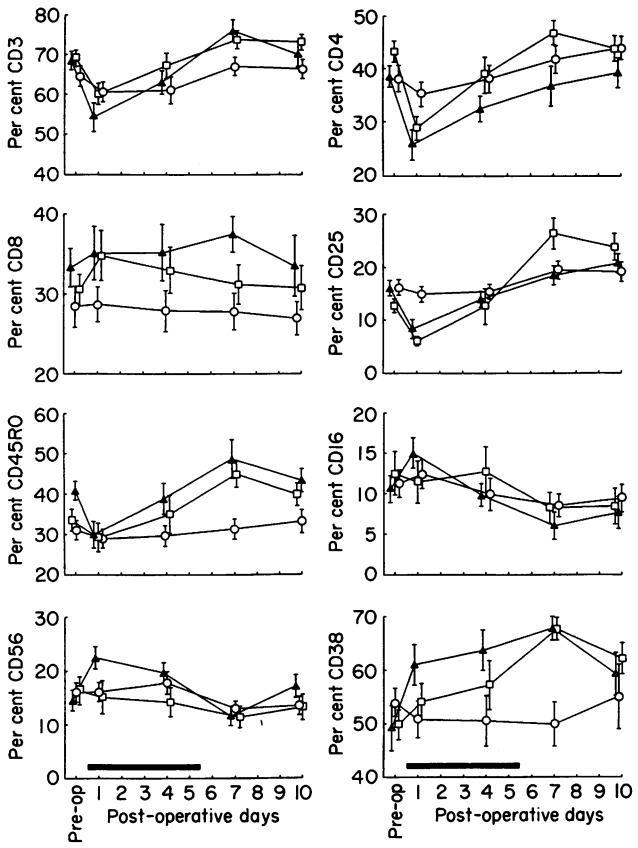

Fig. 5. Effect of peri-operative immunotherapy on percentage expression of a panel of MoAbs (see text for details) in patients undergoing surgery for colorectal cancer. Each point represents mean ± s.e.m. The bar indicates the treatment period. ○, Control; □, rIL-2; ▲, rIL-2 + IFN-α.

The percentage change in LAK cell generation against the Daudi target is shown in Fig. 2. It is seen that treatment with either rIL-2 or rIL-2 and IFN-α overcame the surgically induced reduction in LAK cell activity as demonstrated by the control group (252·9 ± 15·8 pre-operatively to 163·8 ± 24·0 on day 7). Indeed, LAK cell activity in both treatment arms was significantly higher than control on days 4, 7 and 10. The results for COLO205 are very similar (data not shown).

Lymphocyte proliferation in response to Con A in the three groups is shown in Fig. 3, together with maximal lymphocyte IL-2 production. There were no significant post-operative changes in the SI in control patients, although IL-2 production was markedly reduced on days 1 and 4. In the IL-2-treated group, both the SI and IL-2 production were significantly reduced from day 4 ($P < 0·01$). However, it was in those patients receiving combination therapy that the most dramatic fall in both parameters was seen. The IL-2 productive capacity of circulating PBMC was almost abolished on the first post-operative day, and was still low on day 10 ($P < 0·05$). This was in parallel with a highly significant fall in the SI ($P < 0·01$).

Lymphocyte phenotype analysis

Before operation, no significant differences in percentage expression of any of the lymphocyte subset markers were noted between the groups. Immediately after operation, all patients showed an early post-operative lymphopenia (Fig. 4), but this fall was more profound in both treatment groups compared

with control ($P < 0{\cdot}01$ for rIL-2; $P < 0{\cdot}001$ for rIL-2 and IFN-α). Lymphocyte numbers in the control arm remained low throughout the follow-up period, but those on treatment demonstrated a highly significant rebound lymphocytosis on ceasing therapy ($P < 0{\cdot}01$).

Changes in the percentage expression of lymphocyte subset markers are shown in Fig. 5. A number of patterns emerge. The percentage of $CD3^+$ T cells fell significantly in all groups during the early post-operative period ($P < 0{\cdot}01$), but it was in the two treatment arms where the pattern of fall varied. $CD4^+$ T-helper/inducer cells, unchanged in the control group, fell significantly in both treatment groups ($P < 0{\cdot}001$); whereas $CD8^+$ T cytotoxic/suppressor cells, unchanged in Arms 1 and 3, actually rose in the rIL-2 alone group ($P < 0{\cdot}05$). When the CD4:CD8 ratio is examined a fall on day 1 is seen in all the groups, which persisted until day 4 only in the group treated with the combination of cytokines. By day 7, the ratio in the control group was significantly higher than before operation. This was also noted in the combination arm on day 10.

Evidence of lymphocyte activation is reflected by the changes in expression of CD25, CD38 and CD45RO. No significant changes in these markers were observed in the control group. However, parallel changes were seen with both treatment arms. Expression of the inducible β-chain of the IL-2 receptor (CD25) fell markedly during therapy ($P < 0{\cdot}01$), but during the lymphocytotic stage was significantly enhanced ($P < 0{\cdot}01$). The pattern was identical with the CD45RO marker of antigen commitment. CD38 demonstrated a different pattern, with up-regulation of expression throughout the follow-up period ($P < 0{\cdot}01$), in both the treatment arms. Using a double labelling technique, it was demonstrated that the activation marker expression differed between the T cell subsets. CD25 positivity varied specifically within the $CD4^+$ helper T cell subset, whereas changes in both CD38 and CD45RO expression were identifiable on both $CD4^+$ and $CD8^+$ subsets.

Analysing changes in null cell expression (CD16 and CD56, markers of NK and LAK cells), there was a highly significant up-regulation of both CD16 and CD56 in the combination arm on day 1 of treatment ($P < 0{\cdot}01$), and CD56 remained increased on day 4. On ceasing treatment, the values in the treatment arms mirrored those in the control group, with a significant fall demonstrable on day 7 compared with pre-operative values ($P < 0{\cdot}01$ for CD16; $P < 0{\cdot}05$ for CD56).

DISCUSSION

The peri-operative period is a critical time for patients with colorectal cancer. Already in a state of relative immunosuppression [5], such patients are further subjected to the immunocompromising effects of surgery itself [28–31]. Thus, any regimen that can help counteract these cumulative immunodepressant influences is likely to be of direct benefit. Previous studies in our laboratory have investigated the use of cytokines in the peri-operative setting [6,18]. IFN-α was shown to augment significantly NK activity; however, it is not thought to be notably effective as an immunopotentiator, and hence it is not surprising that other parameters of immune function were not significantly altered [6]. In a subsequent study, we found that single therapy with rIL-2 appeared more promising and caused enhancement of both NK and LAK cell activity, associated with T cell activation [18]. This evidence together with the demonstration that in a murine model the anti-tumour activity of combined immunotherapy with rIL-2 and IFN-α is significantly greater than either agent alone [19], led us to compare this treatment against both control and monotherapy with rIL-2.

It is becoming accepted that low dose peri-operative rIL-2 is safe and well tolerated, as demonstrated by ourselves here and previously [18]. In particular we found no evidence of the capillary leak syndrome that characterizes high dose IL-2 toxicity [17]. It was reassuring that the addition of IFN-α to the regimen did not cause any significant side effects.

In contrast to the apparently insignificant clinical effects, treatment profoundly influenced patients' immune status. rIL-2, either alone or in combination with IFN-α, caused long-term *in vivo* activation of T cells, as seen by the increased expression of CD25 and CD38. The percentage of $CD45RO^{high}$ antigen-committed lymphocytes was also significantly increased. As antigen response functions are a feature of the $CD45RO^{high}$ T cell subset, these data can be taken as *prima facie* evidence of increased peripheral immunoreactivity.

Our results are characterized by the prominent initial post-operative lymphopenia previously noted by other authors [13,32]. This fall in lymphocyte numbers was noted in all patients and was attributable to a drop in $CD3^+$ T cells, although the effects on T cell subsets varied from group to group. The drop in $CD4^+$ cells was significantly lower in the treatment groups at day 1, and is thought to be due to sequestration of these highly activated cells into peripheral sites [18,33]. Together with the marked increase in activation, this change in the population of circulating T cells may afford therapeutic benefit, indicating passage of activated lymphocytes to the tumour and lymph node sites. Further support for this hypothesis stems from the observations that the percentages of activated $CD25^+$ $CD38^+$ and $CD45RO^+$ memory cells falls dramatically in the first few days, strongly suggestive of the known tendency of such cells to adhere to accessory and target cell types via interactions mediated by activation-dependent adhesion molecules [34]. Thus, we suggest that the initial effects of rIL-2 are sequestration of the most highly activated cell types. Perhaps as to be expected, the early activation marker CD38 was a more sensitive indicator than CD25, being significantly up-regulated after only one administration of rIL-2.

It is worth noting that both the proliferative responses and IL-2 secretion of mitogen-stimulated PBMC were lower in treated patients than controls. This is most likely to be a function of the reduced percentage of $CD25^+$ T cells in the circulation, and adds further weight to the hypothesis that the most activated cells are being sequestered during the period of cytokine administration, leaving the more anergic and $CD45RO^-$ cells in the circulation. The observation that patients receiving both cytokines showed the most depressed responses is entirely consistent with the known anti-proliferative properties of IFN-α.

Our data clearly show that the drop in NK and LAK cell activity observed in control patients following surgery is due to impaired cellular reactivity, rather than any substantial drop in numbers of either $CD16^+$ (NK cells) or $CD56^+$ (LAK cell precursor) cells [35]. A major effect of either immunotherapy regimen appeared to be an abrogation of the surgery-induced drop in NK and LAK cell activity. Whereas rIL-2 alone was effective in this respect, in combination with IFN-α, an increase

in NK cell function and an increase in the percentage of $CD16^+$ and $CD56^+$ cells were observed.

Although offering the best chance of a cure, surgical resection for colorectal cancer is far from adequate, with survival rates no better than 50% after 5 years [1]. The compelling evidence from experimental models of peri-operative immunotherapy [36] has yet to be replicated in man. This is presumably due to the extreme toxicity observed with high doses of cytokines. Taken together, our data suggest that rIL-2 and IFN-α at modest doses nevertheless significantly modulate the immune status of post-operative cancer patients. Although IFN-α did not appear to augment significantly the effects of rIL-2 on the lymphocyte parameters, it significantly augmented NK/LAK cell precursors. The initial rapid drop in percentages of activated T memory cells argues strongly for egress from the circulation and sequestration in lymph nodes and other tissues. This may have a beneficial effect on potential occult hepatic metastases [37], whereas the elevation in NK cell activity may decrease the numbers of circulating tumour cells liberated during surgery. Another effect of IFN-α, not examined here, is to alter the antigenicity or other properties of any tumour cells remaining after apparently curative surgery, in such a way as to either decrease their proliferative capacity or to render them more vulnerable to attack. Further research is examining the optimal timing of peri-operative immunotherapy, which will pave the way for a large-scale clinical study to investigate survival benefits.

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