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Effects of surgery on the generation of lymphokine-activated killer cells in patients with breast cancer

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Natural killer (NK) cell activity and the capacity to generate lymphokine-activated killer (LAK) cell activity were studied in 43 patients with operable breast cancer before and after surgery. Results were compared with those from ten healthy subjects. Patients with breast cancer had normal LAK and NK cell activity before surgery. A subgroup of patients with stage III disease had depressed LAK cell activity ($P < 0.013$). NK cell activity decreased by over 50 per cent on the first day after surgery and did not return to preoperative levels by day 7 ($P < 0.0005$). Generation of LAK cell activity was unaffected by surgery. The addition of 10 per cent autologous plasma to the culture medium during the induction of LAK cell activity in vitro *did not suppress LAK cell activity in patients with breast cancer. These results suggest that perioperative adjuvant immunotherapy based on interleukin 2 and LAK cells is not ruled out by systemic suppressive effects from either cancer or surgery.*

Immunotherapeutic treatment of malignancy using interleukin (IL) 2 has been studied in some detail since the first clinical trial was reported by Rosenberg *et al.*[1]. Initial enthusiasm was stimulated by the encouraging results of treatment with IL-2 and lymphokine-activated killer (LAK) cells in patients with metastatic renal cancer and melanoma[1–3]. Subsequent reports of the severe potential toxicity of the regimen, the cost and logistical difficulty of providing the treatment, and the low and unpredictable response rate[4–7] diminished this enthusiasm.

A review of the evidence from animal models may be useful in suggesting possible causes for the disappointing results. The toxicity of IL-2 is less in mice and equivalent doses cannot be used clinically[8]. Human subjects frequently have advanced disease whereas animal models have used tumour at an early or occult stage. Many human studies have not used LAK cells with IL-2, despite experimental evidence for synergism between the lymphokine and LAK cells in a tumoricidal capacity[9,10]. There are considerable logistical problems involved in the use of LAK cells but the only substantial comparison of treatment with and without such cells[11] suggests that complete remission is more frequent with LAK cell treatment.

The unique antitumour cytotoxic activity of LAK cells was the basis for early studies of IL-2-based immunotherapy[8]. It is now clear that this direct cytolytic activity is unlikely to play a major role in the tumour regression observed clinically and experimentally. LAK cells do not migrate to tumour tissue[12] and are found there relatively infrequently even in recently treated responding lesions[13]. The exact role of these cells is therefore unclear. LAK cells are active secretors of cytokines, particularly γ interferon and tumour necrosis factor α[14], and may bring about tumour cell destruction indirectly, by affecting the activity of other parts of the immune system. It has been shown, for example, that LAK cells modulate a T cell-mediated immune response in the mouse[15].

Suppression of the generation of LAK cell activity by immunosuppressive substances produced by the tumour has been thought to be a cause of treatment failure, and there is a reduced capacity to generate LAK cells in patients with advanced cancer[16,17]. *In vitro* studies have shown that several tumour cell lines secrete substances capable of inhibiting LAK cell generation[18,19]. The clinical findings may result partly from cancer-associated malnutrition, and the relevance of the LAK cell-inhibiting substances *in vivo* remains uncertain. These findings suggest that LAK cell–IL-2 treatment might be most effective in an adjuvant setting, after surgical debulking of a tumour. Surgery has transient suppressive effects on several components of the immune system, including T cell numbers and natural killer (NK) cell function[20–23], and an adverse effect on LAK cell production in response to IL-2 in some groups of patients[24]. Before considering adjuvant perioperative LAK cell–IL-2 treatments, possible suppressive effects on LAK cell generation of factors from the tumour, and of surgery itself, must be defined. This study defines these effects in a relatively homogeneous group of patients with breast cancer.

Patients and methods

Patients

Patients with operable breast cancer of Union Internacional Contra la Cancrum (UICC) stages I–III were studied in the perioperative period. Control subjects comprised healthy laboratory workers. None of the subjects studied was suffering from clinically evident acute bacterial or viral infection at the time of sampling, and none was taking corticosteroids, azathioprine or cyclosporin.

Samples

Samples (20 ml) of venous blood were collected into lithium heparin tubes and allowed to cool to room temperature. Mononuclear cells obtained by density centrifugation over Histopaque 1077 (Sigma Chemicals, Poole, UK) were washed twice in RPMI 1640 (Gibco, Paisley, UK) medium, resuspended in the same medium with 10 per cent heat-inactivated fetal calf serum (FCS) (Gibco), and numbers and viability assessed. Samples were taken on the morning of surgery, and on the first, seventh and 28th mornings after surgery.

Lymphokine-activated killer cell generation

Peripheral blood mononuclear cells (PBMCs) were resuspended at a density of 10^6 cells ml^{-1} in RPMI 1640 medium containing 10 per cent FCS, 200 units ml^{-1} recombinant human IL-2 (Glaxo, Geneva, Switzerland) and antibiotics (penicillin 50 $\mu g\ ml^{-1}$, streptomycin 50 $\mu g\ ml^{-1}$ and amphotericin 0·125 $\mu g\ ml^{-1}$). Cells were cultured for 72 h at 37°C in an atmosphere containing 5 per cent carbon dioxide. Cultured cells were harvested, washed twice in RPMI, and numbers

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and viability reassessed. They were then used as effector cells in cytotoxicity assays.

Cytotoxicity assays

LAK cell activity was measured in a standard 4-h chromium-release assay[16]. DAUDI target cells labelled with 2 MBq ^{51}Cr per 10^6 cells were exposed to effector cells at three different effector:target ratios, usually 6·25:1, 12·5:1 and 25:1. Results at all ratios tested were concordant.

The mean(s.d.) spontaneous:maximal ^{51}Cr release ratio in the LAK cell assays performed was 15·4(5·5) per cent.

Addition of autologous plasma

Autologous plasma was stored from each blood sample taken. Cytotoxicity assays using effector cells that had been cultured in the presence of 10 per cent autologous plasma were compared with assays using untreated IL-2-stimulated effectors.

Natural killer cell assay

NK cell activity of PBMC preparations was measured immediately after initial separation and washing of the cells. A 4-h chromium-release cytotoxicity assay was used as described above, using the K562 cell line as target.

Nutritional indices

Height and weight were recorded in patients and the body mass index derived. Upper-arm skinfold thickness and serum albumin levels were determined. Malnutrition was defined as a value below the fifth centile of two or more of these three parameters.

Statistical analysis

Comparison between patients with cancer and controls was carried out by the Mann–Whitney *U* test, and of results from the former group at different times by the Wilcoxon signed rank test. LAK cell cytotoxicity in stage subgroups was compared using Student's *t* test.

Results

Patient population

The study population comprised 43 patients with breast cancer and ten normal healthy volunteers. The mean ages of the two groups were 61 and 27 years respectively. Eighteen breast cancers were UICC stage I, 20 stage II and five stage III. Only two patients with cancer were malnourished.

Effects of cancer on lymphokine-activated killer cell generation

The median cytotoxicity at an effector:target ratio of 12·5:1 was 67 per cent for the patient group compared with 64 per cent for the normal volunteers. This difference was not significant ($P < 0·42$, Mann–Whitney *U* test). *Table 1* shows these effects for patients with different stages of disease. Separate analysis of the effects of plasma from patients with stage III tumours on the cytotoxicity assay did not reveal any suppressive activity (median cytotoxicity 54 per cent without plasma, 61 per cent with 10 per cent autologous plasma). NK cell activity was not suppressed before operation in this subgroup.

Table 1 *Effects of cancer on lymphokine-activated killer cell generation*

	No.	Median (range) cytotoxicity at an effector:target ratio of 12·5:1 (%)
Control subjects	10	64 (27–84)*
Patients with breast cancer		
Stage I	18	64 (24–83)†
Stage II	20	70 (36–79)‡
Stage III	5	48 (32–71)
Total	43	67 (24–90)

*$P < 0·42$ (control *versus* breast cancer group, Mann–Whitney *U* test); †$P < 0·05$ (stage I *versus* stage III, $t = 2·09$, 21 d.f.); ‡$P < 0·013$ (stage II *versus* stage III, $t = 2·55$, 23 d.f.)

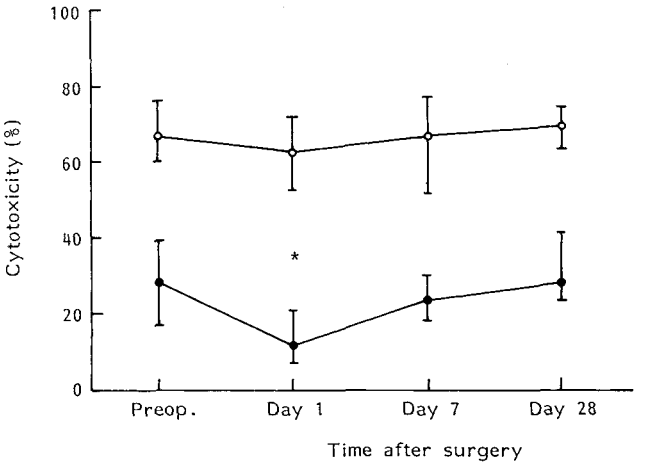

Figure 1 *Effect of surgery on in vitro cytotoxicity of lymphokine-activated killer (○) and natural killer (●) cells. Results of a 4-h ^{51}Cr-release assay at an effector:target ratio of 12·5:1. *P < 0·0005 (Wilcoxon rank sum test)*

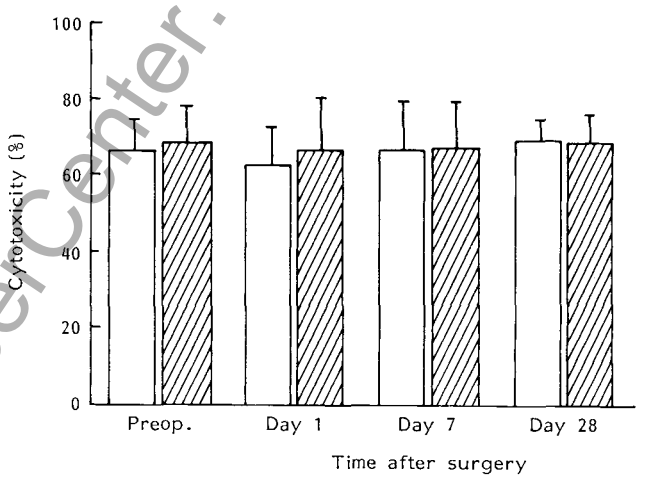

Figure 2 *Effect of autologous plasma on generation of lymphokine-activated killer cell cytotoxicity. Results of a 4-h ^{51}Cr-release assay at an effector:target ratio of 12·5:1. Values are median (semi-interquartile range). □, No plasma; ▨, 10 per cent autologous plasma*

Effects of surgery on lymphokine-activated killer cell generation

LAK cell cytotoxicity against DAUDI target cells was not altered by surgery in these patients, but NK cell activity was suppressed (*Figure 1*). Sixteen patients underwent wide local excision of the tumour and 27 simple mastectomy; all patients had axillary clearance to level II. There was no significant difference in LAK or NK cell activity between these two subgroups at any of the intervals studied.

Effects of autologous plasma

The addition of 10 per cent autologous heparinized plasma during the 3-day incubation period *in vitro* did not affect LAK cell cytotoxicity at any time (*Figure 2*).

Discussion

Previous studies[16,17] have described a decrease in the ability of patients with gastric or colonic cancer to generate LAK cell responses when stimulated with IL-2. The present study, however, found no deficiency in the potential for LAK cell activity generation before surgery. In the small number of patients with locally advanced disease there did appear to be some reduction in cytotoxic capacity; this result is similar to

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the findings of other workers, whose patients had disease of comparable average severity. Nutritional status influences lymphocyte function[25], which may explain the differences found between the present and previous studies. Only two of the present patients were clinically malnourished (one with stage I and one with stage III disease), compared with one-third of patients undergoing surgery for gastrointestinal malignancy[16].

Tumour cell lines cultured *in vitro* can produce soluble factors that cause profound inhibition of LAK cell activity[19,26]. In the present study there was no detectable inhibitory effect from the plasma of patients on the generation of LAK cells. The mechanisms by which LAK cell therapy causes tumour regression *in vivo* are not well defined, and it is possible that the efficacy of LAK cell treatment is inhibited by tumour-derived suppressor molecules acting locally.

Surgery has a suppressive effect on the immune system[19–23] but the changes vary widely in nature, duration and severity, and may be secondary to the neuroendocrine response to physiological stress. Surgery for gastrointestinal cancer suppresses LAK cell generation responses to IL-2 for several days, and such patients already have subnormal responses to IL-2 before surgery[24,27]. NK cell function was depressed after surgery for up to 7 days in the present study, confirming the findings of others[22,24]. This indicates that the surgery performed on these patients was sufficiently major to initiate some of the expected suppressive responses, an important point in view of the subjectively lesser severity of breast *versus* gastrointestinal cancer surgery. LAK cell generation in the patients in the present study was not affected by surgery (mastectomy or lumpectomy). This indicates that the number of functionally normal circulating precursor cells is not reduced, nor is their ability to respond to *in vitro* stimulation with IL-2. It should, therefore, be possible to generate LAK cells from patients' lymphocytes in the perioperative period. This would be important if adjuvant immunotherapy were a useful adjunct to surgery[28]. The information provided from these results is valuable, as it indicates that perioperative LAK cell–IL-2 treatment would not be rendered ineffective either by tumour-related factors or by the immunological effects of surgery.

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