SHORT COMMUNICATION
Cytokines regulate the ability of human LAK-cells to kill human tumour cells in vitro

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When human peripheral blood mononuclear cells (PBM) are exposed to the cytokine interleukin-2 (IL-2) in vitro, they develop a potent cytotoxic ability referred to as lymphokine-activated killer cell (LAK-cell) activity. LAK-cell activity is characterised by the ability to kill fresh tumour cells in a non-MHC-restricted manner, while leaving normal cells (e.g. lymphocytes) unaffected (Rosenberg & Lotze, 1986). For this reason, LAK-cells have attracted much attention as potential tools in the treatment of advanced human malignant disease and have been used clinically. There have been notable successes, particularly in the case of melanoma and renal cell carcinoma; nevertheless, the general efficacy of LAK-cell therapy has not been as great as originally envisaged (Rosenberg et al., 1985; Rosenberg, 1988).

Several cell-types appear to contribute to the development of LAK-cell activity under the influence of IL-2, particularly T-cells, natural killer (NK) cells and large granular lymphocytes (Damle et al., 1986; Kalland et al., 1987). However, LAK-cells differ from NK-cells in being able to kill a range of targets known to be resistant to NK activity (Grimm et al., 1982).

As a rule, LAK-cells cannot be isolated ex vivo. Although the reasons for this are unclear, it is known that the induction of human LAK-cells in vitro can be inhibited by interleukin-4 (IL-4), which is present during the developing immune response (Brookes & Rees, 1988; Gallagher et al., 1988). Our previous report described the effect of IL-3 and IL-4 on the ability of human LAK-cells to kill the ovarian cancer cell line OWmM1. The study reported here extends these observations in a more clinical direction by increasing the range of cytokines tested and by using primary human tumour cells as targets, as a step towards examining the role of cytokines on in vivo human LAK-cell function.

LAK-cells were generated from blood donated by healthy adult volunteers selected from the laboratory staff. PBM were isolated from these samples by density centrifugation over Ficol-Hypaque (Pharmacia) and stimulated with 200 units ml−1 recombinant human IL-2 (Koch-Light Ltd) for 4 days. The culture medium employed was Ham’s F10, supplemented with 10% (v/v) fetal calf serum and containing a final concentration of 4 mM glutamine (all media components were obtained from Flow Laboratories Rickmansworth, UK); cells were maintained at 37°C in a 5% CO2 humidified atmosphere.

The target cells used were either the ovarian cancer cell-line OWmM1, which have been previously shown to be antigenically representative of mucinous cystadenocarcinoma (Al-Azzawi et al., 1987) or primary material obtained during standard cytoreductive surgery; primary samples were confirmed as being malignant and cells were taken from non-necrotic tissue. Ov-1 and Ov-2 were derived from ovarian cancer patients at Stobhill Hospital, Glasgow while Ov-3 and Bw-1 were from ovarian and bowel cancer patients, respectively, from Addenbrookes Hospital, Cambridge. The killing assay was conducted over a period of 18 h. This prolonged assay was deliberately chosen over the more usual 4 h chromium-release assay in an attempt to mimic in vivo conditions more closely, where LAK-cells are expected to remain active for prolonged periods. In addition, the adherent nature of the target cells allowed us to measure the remaining live cells directly with the aid of the reducible dye MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide]. The percentage viability of the target cells was established following their exposure to the LAK-cells (Mosmann, 1983; Gallagher et al., 1988).

Thus, target cells were exposed to LAK-cells at an effector-to-target (E:T) cell ratio of 5:1 for 18 h in 96-well, flat-bottom plates (Sterilin). It was usual for $5 \times 10^4$ target cells to be placed in each well. At the end of the assay period, LAK-cells and dead target cells were removed by gentle but thorough washing and the culture fluid replaced with complete medium containing 0.5 mg ml$^{-1}$ MTT. Four hours later, all the medium was carefully removed from each well and replaced with 200 μl of dimethylsulphoxide. Following gentle agitation, the colour density was established as $A_{570}$. A calibration curve (cell number × $A_{570}$) was established for each target cell type and used to determine the number of viable cells remaining in each experimental well. The percentage killing achieved was then calculated by direct comparison of the test wells with control wells, which contained target cells but no LAK-cells. Six replicate wells were established per test point.

Recombinant human cytokines were obtained from Koch-Light Ltd (Haverhill, UK). A range of concentrations of each material was employed, in order to straddle that required for optimal activity in the biological system normally used to characterise particular cytokines (for example, the thymocyte co-stimulator assay for IL-1), as described by the manufacturers. The concentrations reported here represent the highest tested in our experiments: IL-1, 200 units ml$^{-1}$; IL-2, 200 units ml$^{-1}$; IL-3, 1,000 units ml$^{-1}$; IL-4, 1,000 units ml$^{-1}$; IL-6, 1,000 units ml$^{-1}$ and GM-CSF, 1,000 units ml$^{-1}$. These concentrations were found to be the most effective here and in our previous study (Gallagher et al., 1988). It should be pointed out that the units described here are meaningless in terms of their biological activity on LAK-cells, since such effects are poorly defined. The nomenclature is retained to provide a reference to the amount of material present.

We first examined the ability of the cytokines themselves to affect the growth of the tumour cells, in the absence of LAK-cells. The experimental results are shown in Table I. The target cells were exposed to the cytokines for the full 18 h of the assay period, then their viability was assessed by the MTT method. The results clearly show that the cytokines were not able to cause the death of the established cell-line, or the primary ovarian or bowel tumour material. As a prelude to investigating the ability of cytokines to regulate LAK-cell function, we first demonstrated that the cytokines did not increase the resistance or susceptibility of the target cells to LAK-cell lysis (data not shown). This was...
achieved by pre-incubating the target cells with the appropriate cytokine for 6 h then washing carefully before the 18 h killing period. The ability of these cytokines to affect the killing of the target cells by the LAK-cells was then investigated. A different LAK-cell donor was used for each target cell type, for reasons of availability, and the cytokines were again present for the whole of the assay period. The experimental results are described in Table 1. The cytokines fell into three clear groups. The first group (IL-1, IL-6 and GM-CSF) did not affect the ability of LAK-cells to kill tumour targets. The second group (IL-3 and IL-4) greatly reduced the observed killing. This inhibition was effective in the case of IL-4 and the Ovan-2 target, but substantial reductions in killing were observed whenever IL-3 or IL-4 was present with the LAK-cells. Finally, the presence of IL-2 greatly enhanced the ability of LAK-cells to kill human tumour-cell targets. For all target cell types examined, the results obtained from the LAK-cells+IL-2, LAK-cells+IL-3 and IL-4 experiments were significantly different (greater killing with IL-2, less with IL-3 and IL-4; Student’s t test, P < 0.05) from the appropriate ‘LAK-cells alone’ experiment.

Table 1 Cytokines’ influence on human LAK-cell function

| Cytokines | Percentage killing |
|-----------|--------------------|
|            | OWmM1  | OVAN-1 | OVAN-2 | OVAN-3 | BWL-1 |
| Medium alone | 0      | 0      | 0      | 0      | 0      |
| IL-1       | 3      | n.d.   | 7      | 0      | 2      |
| IL-2       | 0      | 8      | 5      | 4      | 9      |
| IL-3       | 0      | n.d.   | n.d.   | 2      | 0      |
| IL-4       | 0      | -2     | 0      | -3     | n.d.   |
| IL-6       | 0      | n.d.   | -4     | 0      | n.d.   |
| GM-CSF     | 6      | 4      | 0      | n.d.   | 8      |
| LAK-cells alone | 74±8   | 83±12  | 28±7   | 41±14  | 68±20  |
| LAK-cells+IL-1 | 79±12  | n.d.   | 32±6   | 41±2   | 77±3   |
| LAK-cells+IL-6 | 79±13  | n.d.   | 34±7   | 33±13  | n.d.   |
| LAK-cells+GM-CSF | 75±10  | 88±4   | n.d.   | 21±4   | n.d.   |
| LAK-cells+IL-3 | 21±2   | 52±3   | n.d.   | n.d.   | 11±1   |
| LAK-cells+IL-4 | 42±11  | 30±11  | 2±1    | 37±12  | n.d.   |
| LAK-cells+IL-2 | >99*   | >99*   | 52±11  | 78±4   | 81±14  |

Cytokines were tested for their ability to kill tumour cells and to influence the ability of LAK-cells to kill these tumour cells. Cytokines were added for the full 18 h of the assay period at the following concentrations: IL-1 and IL-2, 200 U/ml; IL-3, IL-4, IL-6 and GM-CSF, 1,000 U/ml. The percentage killing was calculated as described in the text; the mean ± standard deviation of killing achieved in six replicate wells is shown.

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