Short Communication

CYTOTOXICITY OF LYMPHOCYTES FROM BLOOD, TUMOUR AND REGIONAL LYMPH NODES AGAINST K562 CELLS AND AUTOPLASTIC COLORECTAL TUMOUR CELLS

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It has been reported from this laboratory that repeated washing increases the cytotoxicity of lymphocytes, extracted from human colorectal carcinomas, against the autoplastic cancer cells (Hutchinson et al. 1981a). The identity of the effector cells remains unclear, but possible candidates are either a thymus-derived lymphocyte (T cell) or a natural killer cell (NK cell). Different effector-cell populations appear to mediate the cytotoxicity of peripheral blood lymphocytes (PBL), tumour-intrinsic lymphocytes (TIL) and lymph-node cells (LNC). Only PBL are usually effective against the NK-sensitive erythroleukaemia cell line K562 (Moore & Vose, 1981; Vose et al., 1981), whereas similar proportions of all 3 lymphocyte populations react against autoplastic colorectal tumour cells (Werkmeister et al., 1979; Hutchinson et al., 1981a; Vose et al., 1981).

NK cells, therefore, might plausibly be the effectors in PBL and T cells in TIL and LNC. Against this hypothesis Vose et al. (1981) found, using PBL, that a T-cell-enriched population of effector cells showed the greatest autoplastic tumour kill, whereas the greatest anti-K562 activity was present in the corresponding non-T-cell population. By contrast, we have found that PBL were more reactive against allogeneic than autoplastic colorectal tumour cells and such reactivity might be due to the action of NK cells (Hutchinson et al., 1981a).

The 3 objectives of the present study were chosen in an attempt to clarify these uncertainties regarding the nature of the effector cells. We have first compared the reactivity of PBL, TIL and LNC against K562 and autoplastic tumour-cell targets. The cytotoxicities of PBL and TIL* were assessed before and after treatment with NH₄Cl, which is a reversible inhibitor of NK-cell activity (Kay et al., 1977). Lastly the responses of unwashed and washed TIL have been compared against both K562 cells and autoplastic tumour cells.

Sixteen primary colorectal carcinomas were obtained from patients undergoing surgical resection of their tumour. The ages of these patients ranged from 41 to 78 years and 5 were female and 11 male.

The methods used to separate tumour cells and TIL from disaggregated carcinomas and to prepare PBL and LNC suspensions have previously been described in detail (Symes & Riddell, 1973; Hutchinson et al., 1981a). Tumour cells were labelled with 100 µCi ⁵¹Cr for 2 h at 37°C. Effector and target cells were cocultured for 2 h in the case of tumour cells and for 4 h in the case of K562 cells (Potter & Moore, 1979), at three E/T ratios—5:1, 10:1 and 20:1. There were 3 cultures at each ratio and the % release

*In the experiments involving TIL, the cell populations treated with NH₄Cl were those detailed in Hutchinson et al. (1981a). The untreated TIL were obtained from a second series of patients.
of $^{51}$Cr was determined as before (Hutchinson et al., 1981a). Spontaneous release (always <25%) was obtained from cultures containing tumour cells alone and maximum release from cultures of tumour cells to which a 1:50 dilution of Triton X-100 was added.

The % cytotoxicity was calculated using the formula:

$$\% \text{ cytotoxicity} = \frac{\% \text{ }^{51}\text{Cr release (test)} - \% \text{ spontaneous release}}{\% \text{ maximum release} - \% \text{ spontaneous release}} \times 100$$

Effector populations of PBL and TIL, where appropriate, were treated with Tris-buffered NH$_4$Cl by the method of Boyle (1968). The control of PBL or TIL populations not treated with NH$_4$Cl was suspended in distilled H$_2$O for 10 sec to lyse contaminating erythrocytes.

The cytological characteristics of the autoplastic tumour-cell PBL and TIL populations have been previously described (Hutchinson et al., 1981a).

As determined by 0-165% w/v trypan-blue exclusion, cell viability was $91.4 \pm 3.8\%$ (s.d.) ($N=18$) for K562 cells, $57.7 \pm 4.6$ ($N=9$) for colorectal tumour cells, $98.0 \pm 1.4$ ($N=14$) for PBL, $71.0 \pm 6.8$ ($N=18$) for TIL and $91.4 \pm 5.5$ ($N=12$) for LNC.

There was no significant difference for PBL between their levels of reactivity...

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**Fig. 1.**—The comparative cytotoxicity of peripheral-blood lymphocytes (PBL), tumour-intrinsic lymphocytes (TIL) and lymph-node cells (LNC) against $^{51}$Cr-labelled K562 cells and autoplastic colorectal-carcinoma cells. The cytotoxicity of PBL was similar against both targets, but at the E/T ratio (20:1 TIL ($P<0.05$) and LNC ($P=0.025$) showed greater reactivity against colorectal tumour cells.
against K562 cells and colorectal tumour cells, using a rank sum test, whereas TIL and LNC both showed greater reactivity against colorectal tumour cells (Fig. 1). These findings support the hypothesis that different effectors mediate anti-tumour cytotoxicity in PBL as opposed to TIL and LNC. Therefore the effect of treatment with NH₄Cl on the reactivity of PBL and TIL was compared. Exposure to NH₄Cl reduced PBL cytotoxicity to a variable extent against colorectal tumour cells at an E/T ratio of 20:1, but a similar effect was not seen for TIL (Fig. 2). Also, treatment with NH₄Cl reduces, but does not totally ablate cytotoxic cell activity against allogeneic colorectal tumour cells (Hutchinson et al., 1981a) and in the present study not all PBL preparations were equally susceptible to the action of NH₄Cl (Fig. 2). In summary, the cytotoxicity of PBL toward autologous targets is primarily (but not exclusively) mediated by NK cells.

That NK cells are not involved in TIL cytotoxicity is demonstrated by the effect of washing the cells a further ×6 in Medium 199. Washing did not affect TIL cytotoxicity against K562 cells but it did substantially increase their reactivity against autologous tumour cells. Percentage increments in cytotoxicity after washing ranged from 0 to 4·0% against K562 cells and from 2·7 to 22·0% against tumour cells (P < 0·001) (Fig. 3).

We have previously found that incubating washed TIL in the patient's own plasma reduced their cytotoxicity against autologous tumour cells to the level of unwashed TIL (Hutchinson et al., 1981b). Perhaps a plasma-blocking factor was removed by repeated washing. In support Fig. 4 shows that LNC from nodes containing metastases, wherein the LNC

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Fig. 2.—The effect of treatment with NH₄Cl on the cytotoxicity of PBL and washed TIL against autologous tumour cells. The activity of PBL was significantly reduced (P < 0·05) but that of TIL was unchanged.
might be especially exposed to blocking factors, were less reactive than the corresponding LNC from tumour-free nodes. It could be argued that tumour cells within a lymph node could reduce LNC cytotoxicity by means of "cold" target inhibition. However, if LNC can react against embolic tumour cells, it is difficult to explain the development of a metastasis in the first place.
The cytotoxicity of LNC from tumour-free nodes was directly proportional to the proximity of the nodes to the primary tumour (Fig. 4). Although this finding is at variance with the data of Vose et al. (1981), it accords with the concept that LNC reactivity is an antigen-specific T-cell-mediated phenomenon, since LNC are not reactive against K562 cells.

In summary, PBL reactivity against autologous tumour cells is probably mainly NK-cell-mediated. The corresponding cytotoxicity of TIL and LNC is probably T-cell-mediated. Further experiments using T-cell-enriched effectors (Vose et al. 1981) or monoclonal antibodies against subclasses of T cell (Janossy, 1981) might confirm these suggestions.

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