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LETTER TO THE EDITOR

Dear Sir,

In vitro and *in vivo* activation of CD4$^+$ lymphocytes by autologous tumor cells

During the last 10 years, attempts have been made to isolate tumor-specific lymphocytes. Thus far, most of the so-called tumor-specific cytotoxic T-lymphocyte (CTL) responses observed were directed against melanoma cells (Anichini et al., 1987; Hersey and Bolhuis, 1987). Only occasionally were such responses observed against larynx and bladder carcinoma (Cozzolino et al., 1987) or ovarian carcinoma (Ferrini et al., 1985). For most types of spontaneous tumor it remains uncertain whether they are able to provoke an immune response. Various causes may underlie low anti-tumor responses. First, the host may be immunodeficient (Wheelock et al., 1983). Second, the tumor cells may induce immunosuppressive mechanisms, such as lowering or abrogation of the activity of tumor-directed CTL (Mukherji et al., 1986). Third, tumor cells can be non-immunogenic when they lack expression of major histocompatibility complex (MHC) or tumor-specific or -associated antigens (Barlett, 1972).

Various lymphocyte sources, activation protocols and cloning strategies have been used to clone tumor-specific lymphocytes. The results, however, have been conflicting. For instance, Whiteside et al. (1986) found a lower frequency of proliferating T lymphocytes among tumor-infiltrating lymphocytes (TIL) than among peripheral blood lymphocytes (PBL) of the same patients. They measured frequencies of proliferating T lymphocytes by limiting dilution in a microculture system containing interleukin-2 (IL-2), phytohemagglutinin (PHA) and allogeneic feeder cells.

In contrast, Vose (1982) reported that TIL had a higher frequency of proliferating T lymphocytes than PBL from the same patient. He measured frequencies of proliferating T lymphocytes by limiting dilution in the presence of optimal concentrations of lectin-free medium containing IL-2 and autologous PBL as feeder cells. As yet, it is not clear whether such putative tumor-specific CTL comprise CD4$^+$ (helper/inducer) or CD8$^+$ (suppressor/cytotoxic) cells.

In this study we tested whether autologous tumor cells could induce responses of CD4$^+$ and/or CD8$^+$ lymphocytes. To this purpose PBL were cultured in a mixed lymphocyte-tumor-culture (MLTC), i.e. with or without autologous tumor cells (isolated from ascites or pleura) for 1 week, followed by another week of culture in the presence of IL-2 to select for tumor-activated lymphocytes. Finally, the tumor-activated lymphocytes were expanded in a lymphocyte-specific culture system containing PHA, IL-2 and allogeneic feeder cells (van de Griend et al., 1985), in order to obtain sufficient cell numbers to perform functional and phenotypic analysis. After 2 weeks of culture, activation markers such as proliferative activity, the presence of CD25 receptors and cytotoxic activity of PBL stimulated with autologous tumor cells in vitro were examined.

Cytotoxicity by MLTC-activated PBL of cancer patients was found against fresh autologous and/or allogeneic tumor cells derived from lung, ovarian, mammary and endometrial carcinomas. However, in the absence of PHA, cytotoxic activities

TABLE I – ELEVATED PERCENTAGES OF CD25$^+$ LYMPHOCYTES IN FRESH PBL OF CANCER PATIENTS

Patient number	Type of tumor	Malignancy grade	Isolated from	Chemotherapy	% CD25 lymph.
1	Endometrial	Pap V[2]	Ascites	Yes	17
2	Endometrial[1]	Pap V	Ascites	No	12
3	Ovarian	Pap V	Ascites	No	29
4	Ovarian	Pap V	Ascites	Yes	0
5	Ovarian	Pap V	Pleura	Yes	10
6	Ovarian	Pap V	Ascites	Yes	7
7	Ovarian	Pap V	Ascites	Yes	3
8	Ovarian	Pap V	Ascites	Yes	22
9	Ovarian	Pap V	Ascites	No	24
10	Ovarian	Pap V	Ascites	Yes	6
11	Mammary	Pap III	Ascites	Yes	8
12	Mammary	Pap V	Ascites	Yes	2
13	Mammary	Pap V	Pleura	No	14
14	Mammary	Pap V	Pleura	Yes	0
15	Mammary	Pap III	Pleura	Yes	22
16	Mammary	Pap V	Pleura	No	0
17	Mesothelioma	Pap V	Ascites	No	6
18	Gall-bladder	Pap III	Ascites	No	7
19	N.D.[4]	Pap II	Ascites	Yes	0
20	N.D.	Pap I	Pleura	No	6
21	N.D.	Pap II	Ascites	Yes	11
22	N.D.	Pap II	Ascites	Yes	11
Healthy donors (n = 21)					0–10[3]

[1]Including sarcomatous components.–[2]Pap I and II = non-malignant cells were found in pleura or ascites; Pap III = observed cells were suspected of malignancy; Pap IV and V = malignant cells were found in pleura or ascites.–[3]Median = 2.–[4]N.D. = not determined.

TABLE II – *IN VITRO* ACTIVATION OF CD4+-ENRICHED LYMPHOCYTES BY AUTOLOGOUS TUMOR CELLS

Days of culture after MLTC/IL-2[2]	Tumor cells	Fresh PBL percentage of positive cells				
		CD3	CD4	CD8	CD16	CD25
0	–	50	33	17	8	0
		Absolute number of positive cells[1] × 10^4				
14	–	207	263	4	21	263
14	+	402	391	5	57	397

[1]Absolute number of positive cells were calculated: total number of cells per ml culture × percentage of positive cells.–[2]*CD4+-enriched* lymphocytes were obtained by labelling PBL with anti-CD8 MAbs. Positive and negative fractions were separated by a fluorescence-activated cell sorter. The negative fraction was used as *CD4+-enriched* lymphocytes. *CD4+-enriched* lymphocytes were stimulated in a MLTC and cultured for 1 week in IL-2, then for 2 more weeks in a lymphocyte-expansion system.

decreased to very low levels (results not shown). Ferrini et al. (1985) reported having obtained cytotoxic tumor-specific cloned lymphocytes. However, these clones were cultured in the presence of PHA and results obtained in the absence of PHA were not reported.

Although cytotoxicity could not be induced in PBL by autologous tumor cells, our additional results indicate that the tumors used in this study were immunogenic. Fresh PBL of 9 out of 22 cancer patients showed augmented levels of CD25+ lymphocytes (11–29%), while fresh PBL from healthy individuals comprised 0–10% CD25+ lymphocytes (Table I). This suggests that they were activated in vivo (possibly by the tumor cells). PBL from 4 of these cancer patients (3 with normal and 1 with an elevated level of CD25+ lymphocytes among fresh PBL) were cultured with and without autologous tumor cells, and the proliferative activity of CD4+ and CD8+ lymphocytes was subsequently determined. In all 4 cases, CD4+ lymphocytes responded to autologous tumor cells, as measured by enhanced proliferation in comparison to PBL cultured without autologous tumor cells, although the responses were of different strengths (data not shown). CD8+ lymphocytes were stimulated much less, or not at all. Moreover, when CD4+-enriched *lymphocytes were stimulated with autologous tumor cells, an increase in absolute numbers of CD4+ as well as CD25+ lymphocytes was observed in comparison to the* CD4+-enriched *cells cultured without autologous tumor cells (Table II). In contrast,* CD8+-enriched *lymphocytes, stimulated and cultured under the same conditions as* CD4+-enriched *cells, did not proliferate. The absence of CD4+ lymphocytes during the first week of MLTC could explain the failure of CD8+ lymphocytes to proliferate.*

Yours sincerely,

C. Gravekamp, M. Bontenbal, C.P.M. Ronteltap, D. Van Duyvenbode and R.L.H. Bolhuis

Department of Immunology, Dr Daniel den Hoed Cancer Center, Groene Hilledijk 301, 3075 EA Rotterdam, The Netherlands.

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