ONCOLOGY

Bivalphor: a Myelopeptide Possessing Antitumor Activity

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It is shown that the bone marrow-derived hexapeptide bivalphor restores the functions of human T lymphocytes inhibited by products of HL-60 leukemia cells.

Key Words: *bivalphor; T lymphocytes; HL-60 tumor cells; proliferative response*

It has been established that tumor cells, regardless of their origin, release substances inhibiting T lymphocytes, the main component of antitumor resistance [4,5]. A characteristic feature of this inhibition is the loss of T-lymphocyte capacity for a proliferative response to the mitogens [4,5]. It has been shown, for instance, that the level of T-lymphocyte response to phytohemagglutinin (PHA) drastically drops in the presence of leukemia cell-released products [2,4]. This tumor cell-induced dysfunction of T lymphocytes is accompanied by a drop of lymphokine activity, in particular of interleukin-2 (IL-2) [4]. At the same time, it has been shown that a complex preparation of bone marrow-derived peptides (myelopeptides, MP) [6] can restore the leukemia blast-induced response of human T lymphocytes to PHA [2].

The goal of the present study was to investigate the ability of an individual MP, namely, bivalphor, to abolish human tumor cell-induced suppression of T lymphocytes. This peptide has the following structure: Leu-Val-Val-Tyr-Pro-Trp; it was earlier isolated from a total MP preparation [3]. For the purposes of the present investigation it was formylated by the N-terminal amino group.

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MATERIALS AND METHODS

The MP in question was obtained from medium conditioned by a 20-hour culture of swine bone marrow cells [6], determined as described earlier [3], and formylated by the N-terminus according to the conventional method [1]. The amino acid-formylated MP (bivalphor) was also subjected to further modifications (acetylation, "shortening") as described [1].

The growth of myeloid human leukemia HL-60 cells was maintained in RPMI-1640 medium supplemented with 15% fetal calf serum, 20 mM HEPES, 2 mM L-glutamine, and 50 μg/ml gentamicin. The tumor-conditioned medium, referred to below as TCM HL-60, was harvested in the logarithmic phase of growth (3rd day).

T lymphocytes were obtained from the peripheral blood of healthy donors by Ficoll-Paque (Pharmacia) density gradient centrifugation, purified, and stimulated with mitogen according to the method of Chiao *et al.* [4], namely, T lymphocytes in a concentration of 1 mln/ml were incubated with 3 μg/ml PHA (Flow Labs.) in the above-mentioned medium in 24-well plates (Nun-

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Table 1. Ability of Bivalphor to Restore Leukemia Cell Product–Induced Inhibition of the Human T–Lymphocyte Proliferative Response

Agent	^{3}H-thymidine incorporation in T-cell DNA	
	cpm	% of control
–	500	1.5
PHA (control)	32 000	100.0
10% TCM HL–60	15 400	48.1
Total MP preparation, 50 μg/ml	31 400	98.2
Bivalphor, μg/ml:		
100	28 600	89.4
50	24 000	75.0
5	18 200	56.8
Peptide 2, μg/ml:		
100	8 000	25.0
50	12 000	37.5
5	14 200	44.4

Note. A typical result of one of 4 experiments is presented; each value is the mean of 3 parallel assays (scatter±400 cpm).

clon) for 3 days. The level of PHA-induced T-lymphocyte proliferation was measured by ^{3}H-thymidine incorporation in the cellular DNA. ^{3}H-thymidine (2 μCi/ml, 19 Ci/mmol) was added to the wells 4 hours before the completion of incubation. The radioactive labeling of DNA (cpm) was recorded on a β-counter (Beckman) using a toluene-based scintillation fluid.

RESULTS

It is known that both tumor blast cells from patients with acute myeloid leukemia (AML) and the AML-derived HL-60 cell line produce a protein that inhibits T-lymphocyte functioning [4]. The inhibition is expressed, notably, in a significant reduction of the T-lymphocyte proliferative response to PHA [2,4]. It was found earlier that the addition of 10% TCM HL-60 to the incubation mixture at the start of stimulation results in a 50% inhibition of the T-lymphocyte proliferative response to PHA as compared to the control (100%; incubation with PHA in the absence of TCM). The inhibition could be abolished by the addition of IL-2 or the total MP preparation [2,4].

Table 2. Loss of Ability of Bivalphor to Restore T–Lymphocyte Functions after its Chemical Modifications

Agent	T-cell proliferative response, % of control level
PHA (control)	100.0
10% TCM HL–60	50.4±8.2
Bivalphor, 100 μg/ml	86.2±4.5
Modifications:	
"shortened" at C–terminus	48.5±5.2
"shortened" at N–terminus	52.1±3.4
N–acetyl	58.2±3.9

Note. The mean result of 3 experiments is presented. Absolute ^{3}H–DNA content in the control of experiments Nos. 1, 2, and 3 is 21,000±360 cpm, 18,000±260 cpm, and 30,000±410 cpm, respectively.

We established (Table 1) that bivalphor, like the total MP preparation, can restore the PHA-induced response to the normal level. As a control we used a peptide of another structure (Phe-Leu-Gly-Phe-Pro-Thr; further named peptide 2) that was also isolated from the total MP preparation [3]. In contrast to bivalphor, peptide 2 augmented the suppressive effect of TCM on T lymphocytes (Table 1). Four experiments using both bivalphor and peptide 2 were conducted, the typical results of one of them being presented in Table 1. The results were of an analogous trend, i.e., in all cases bivalphor, unlike peptide 2, raised the PHA-induced T-lymphocyte proliferative response. The effect of bivalphor was reproducible and comparable to that of the total MP preparation. According to our preliminary data, the effect of bivalphor on human T lymphocytes was accompanied by a normalization of the production of IL-2, which, as was noted above, also restores the T-lymphocyte proliferative response inhibited by the products of tumor cells.

Thus, bivalphor can abolish the described suppression of T-lymphocyte responsiveness, i.e., it is a prospective candidate for use in oncology. In view of this, it seemed important to ascertain whether it loses its activity after chemical modification. It was found that "shortening" of the peptide by the removal of one or two amino acids from the N- or C-terminus leads to the loss of

its ability to restore the T-lymphocyte response to PHA (Table 2). The hexamyelopeptide formylated at the N-terminal amino group and acetylated at this group (N-acetyl) also loses the capacity to restore T-lymphocyte functions suppressed by the products of leukemia cells (Table 2). At the same time, N-terminus-formylated bivalphor doubtless acquires resistance to aminopeptidases. This should prolong its effect in the organism and therefore make it possible to lower the therapeutic doses of the preparation.

In order to find out whether bivalphor restores or protects the T-lymphocyte functions from the inhibitory action of tumor cells, we compared the response to PHA under experimental conditions of adding the peptide at various times after the start of incubation with TCM, i.e., when T lymphocytes had already been exposed to the influence of HL-60 cell products. In all experiments bivalphor was added in a dose that ensured the normal response to PHA under the usual conditions (simultaneous addition of TCM and peptide). The addition of peptide one hour after the start of the experiment (Table 3) resulted in a reproducible elevation of the T-lymphocyte response the control level (114% on average). However, the addition of peptide 24 hours after the start of the experiment did not completely restore the T-lymphocyte response (80.3% of the control level), although the level of response did exceed (by 30%) that obtained in the total absence of the peptide (Table 3). These results show that bivalphor restores T-lymphocyte functions inhibited by leukemia cell-derived product(s) rather than protects T lymphocytes from their effect, as was supposed earlier.

The data presented demonstrate that an individual MP (bivalphor) can abolish tumor cell-induced suppression of T lymphocytes. Among the distinctive features of this agent in comparison with clinically used preparations (e.g., IL-2) are worth noting its low molecular weight and the simplicity of synthesizing it. These properties are encouraging for the use of bivalphor in the treatment of malignancies and, perhaps, of other disorders associated with T-lymphocyte dysfunction.

Table 3. Bivalphor Restores T-Lymphocyte Functions but Does Not Protect Them from the Suppressive Effect of Tumor Cells

Experimental conditions	T-cell proliferative response, % of control level
PHA (control)	100.0
10% TCM HL-60	51.3±6.8
Bivalphor, 100 μg/ml:	
added simultaneously with 10% TCM HL-60	87.1±4.3
added after incubation of T cells with 10% TCM HL-60:	
1 hour	114.3±6.5
24 hours	80.3±4.8

Note. The mean results of 3 experiments are presented. Absolute ^{3}H-DNA content in the control of experiments Nos. 1, 2, and 3 is 20,500±340 cpm, 16,000±210 cpm, and 22,000±380 cpm, respectively.

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