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CSA production in murine long term bone marrow cultures after in vitro treatment with ranopterins[1]

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The presence of colony stimulating activities (CSAs) in the supernatants of murine long term bone marrow cultures (MLTBMCs) was analysed after treatment with two pteridine fractions (ranopterins — Rpts) — pterin-6-carboxyllic acid and pterin. CSAs were measured by the production of colonies and clusters from macrophage-granulocytic (MG-) cell lines in a semi-solid medium (murine bone marrow agar cultures). The results show a well expressed macrophage-granulocytic colony stimulating activity (MG-CSA) in the supernatants, after the treatment with the pterin-6-carboxyllic acid. This finding supports the hypothesis [1] that ranopterins induce GM-CSA not only directly — in agar cultures, but also indirectly — via CSAs production by stromal macrophages in the MLTBMCs.

Key words: mouse bone marrow,long term cultures, ranopterins, pterin, pterin-6-carboxyllic acid, CSA, MG-CSF.

We have previously reported that "in vitro" treatment with some ranopterins stimulate the macrophageal-granulocytic colony formation (MG-CFU) in murine bone marrow agar cultures [1, 2].

In order to determine whether the haemopoietic stimulation of macrophage-granulocyte (MG-) cell line induced with ranopterines was mediated only directly — by the effect of the biologically active substances on earlier progenitors [1], or indirectly — by the release of colony stimulating factors (CSFs), we analysed the presence of colony stimulating activities (CSAs) in the supernatants of long term bone marrow cultures (LTBMCs) after treatment by different doses of ranopterins.

[1] Part of the work was presented in the International Congress:
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Material and methods

LTBMCs were stabilized according to the technique described by C o u t i n h o et al. [3]. Two weeks after the stabilization, different doses of ranopterins — pterin and pterin 6-carboxyllic acid (10, 25 and 50 µg/ml culture) were added, and seven days later the supernatants were collected.

Measurements of CSAs in the supernatants were performed by assessing of MG-CFU colony formation where murine bone marrow cells were cultured in semi-solid media (agar cultures), in the presence of different concentrations of supernatans. As a positive control for the colony stimulating activity the conditioned medium from the 3T3 cell line was used [4]. As a negative control the conditioned medium from LTBMCs without addition of ranopterins was applied.

The supernatants of LTBMCs treated with two ranopterins, induced the formation of macrophageal-granulocytes (MG-) and/ or macrophageal (M-) colonies in the murine bone marrow agar cultures, cytologically characterized after in situ staining with methylene blue and fast green [5], by fluorochromation in situ for basic cytoplasmic proteins [6] as well as by the methods of SEM and TEM electron microscopy (applied for the cells present in colonies).

Results and discussion

The results from the study show a well-expressed macrophageal-granulocyte colony-stimulating effect after the treatment with one of the ranopterin fractions — Pterin 6-carboxyllic acid. The number of CFU-MG depends also on the concentration of the supernatant added, as well as on the dose of the ranopterins administered to the LTBMC. The CSA production is higher when the higher dose (50 µg/ml LTBMC) of Pterin 6-carboxyllic acid was added. In contrast, no MG-CFU formation occurs when

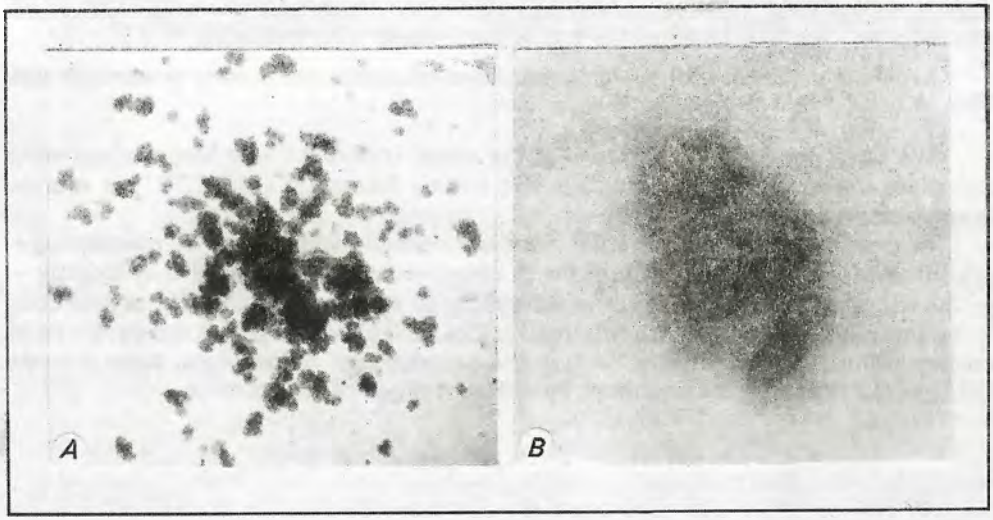

Fig. 1. Macrophageal-granulocyte (MG-) colony from murine bone marrow agar culture, after a 7 day culturing

A — MG-colony, × 350; *B* — monocytes and macrophages from the same colony, × 1000, Immersion

supernatants from control LTBMCs were added to agar cultures, even when concentrated supernatants were applied.

The cytological and cytochemical study of different cell groups and the cell types included in colonies and clusters shows a different degree of macrophageal maturation with the prevalence of immature cells and small macrophages in some of them (Fig. 1). The latest are probably "active" in the synthesis and secretion of a great variety of compounds and biological activities, the tumour-necrotic factors (TNF) including, which has been confirmed for some similar small- and medium-sized macrophageal populations of multiple location [7].

The results showed that some ranopterins induce, in a dose-dependent manner, the production of CSA in LTBMCs which supports our hypothesis that some ranopterins induce hemopoietic (MG-colony stimulating activity) not only directly, but also via CSF-s production in LTBMCs. The results of our experiments in vitro correlate also with data [8], establishing that some ranopterins increase the activity of hemopoietic organs in vivo.

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