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PHYTOHEMAGGLUTININ STIMULATED LYMPHOCYTE GROWTH IN MALIGNANT MELANOMA

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Abstract — Lymphocytes from peripheral blood of patients with malignant melanoma were cultivated for 7 days using two-layered cultivation method in semisolid medium. The ability of phytohemagglutinin (PHA) stimulated peripheral blood lymphocytes to form colonies was tested in 36 malignant melanoma patients and in 16 healthy donors. In patients with malignant melanoma a mean number of colonies (1017 ± 437) was lower than in healthy donors (1711 ± 249), and the difference was significant ($p < 0.05$). The mean number of colonies in 12 patients with the progression of disease was significantly lower than in 13 patients with complete response (745 ± 442 vs. 1588 ± 401, $p < 0.05$). Similar difference was found when the mean number of colonies in 11 patients receiving interferon therapy (641 ± 424) was compared with the mean number of colonies in healthy donors and in patients with complete remission.

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Introduction — Peripheral blood lymphocytes (PBL) from patients with advanced malignant disease may exhibit impaired immune responsiveness to antigenic and mitogenic stimuli. One of the assays developed for studying cell mediated immunity in cancer patients is based on the ability of PBL to form colonies in semisolid medium (1, 2). There are reports on the depression of T-lymphocite levels in melanoma patients (3) and the number of T-cell colonies may reflect the extent of the disease (1, 2).

The aim of our study was to determine the ability of PBL from malignant melonoma patients to form colonies in semisolid medium. The values obtained were compared with the mean number of colonies calculated for the group of healthy donors.

Material and methods — The T-colony assay was performed according to Claësson et al. (4). Heparinized blood was obtained by venipuncture from 36 patients with malignant melanoma and 16 healthy donors. The blood was mixed with equal volume of Hank's balanced salt solution (HBSS), layered on a 3 ml of Ficoll-Hypaque gradient (Pharmacia Fine Chemicals, Uppsala, Sweden) and centrifuged for 30 minutes at 450 g. The mononuclear band was removed, washed twice with HBSS, and cell number was determined. The cell number was adjusted to 1×10^6 cells/ml in Eagle MEM medium supplemented with 10 % fetal calf serum (FCS) (Gibco, Paisley, Scotland) and 5 μl/ml PHA (Torlak, Beograd, Yugoslavia).

Cells were stimulated overnight in suspension culture. Afterwards, fifty thousand cells were resuspended in EMEM with 10 % FCS containing 0.3 % of agar (Difco Laboratories, Detroit, USA); the suspension was layered over a bottom layer of a 0.5 % agar in the medium containing 5 μl PHA. On the day 7 the colonies were counted. A clump containing more than 50 cells was considered as a colony. The patients were divided into three groups according to the extent of the diasease at the time when blood samples were collected. Eleven patients were treated with human leukocyte interferon (crude extract, Imunološki zavod, Zagreb, Yugoslavia) for 6 months in cumulative dose of 60 million units. Common statistical parameters were calculated from the data obtained, while the differences were tested by the use of Student t test.

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Results — The results of colony count are presented in Table 1. In the group of 16 healthy donors the mean number of colonies was 1711, while in the group of 36 patients it was 1017. There was significant difference between both groups ($p < 0.05$). Afterwards, the patients were divided into three groups according to the extent of disease (Fig. 1). The first bar represents the mean number of colonies in the control group with the corresponding standard deviation. The second bar represents the relevant value for a group of 12 patients with the progression of disease. In this gorup a significant decrease in the number of colonies was noted ($p < 0.05$). The third bar represents the results obtained in 11 patients receiving interferon therapy. The mean number of colonies was similar as in the previous groups of patients. The fourth bar represents the mean number of colonies in 13 patients without activity of the disease. The mean number was similar as in the control groups and the difference is significant when compared with the second and third group (1588 ± 401, 745 ± 442, 641 ± 424, respectively: $p < 0.05$).

Group	No. of patients	Colony count AM ± 1SD	Range
Healthy volunteers	13	1711 ± 249	1310—2130
All patients	36	1017 ± 437	142—2146
Progression of disease	12	745 ± 442	142—1660
IFN treatment	11	641 ± 424	162—1485
Complete remission	13	1588 ± 401	927—2146

Table 1 — The mean number of colonies of PHA stimulated lymphocytes in malignant melanoma patients and healthy donors.

Discussion — The results of this study reveal that the number of PHA stimulated colonies in tumor bearing patients is significantly decreased when compared with healthy donors. The number of colonies seems to be in correlation with the tumor load, since the mean number of colonies was significantly lower in patients with progression of the disease in comparison with patients without active disease. Depressed PHA responses in the IFN treated patients is probably partly due to the presence of tumor mass and to the effect of treatment with IFN. Similar data were observed in the study by Harris et al. (5) in patients with renal carcinoma. In few patients the number of colonies returned within the range of the control group when response to the treatment had been achieved. It is suggested that the time course of colony assay may be of interest.

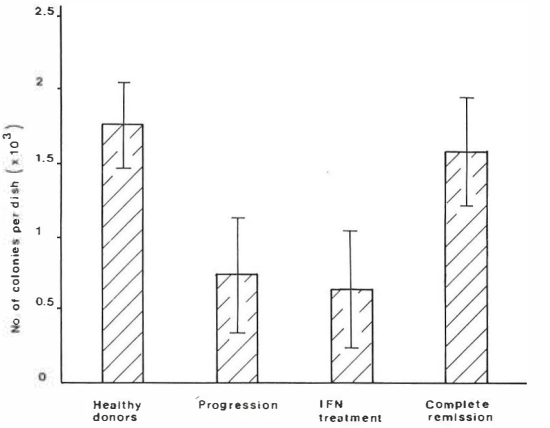

Fig. 1 — Colony-count of PHA stimulated lymphocytes in malignant melanoma patients according to the extent of the disease, and treatment.

Izvleček

RAST KOLONIJ PHA-STIMULIRANIH LEVKOCITOV IZ PERIFERNE KRVI BOLNIKOV Z MALIGNIM MELANOMOM

Limfocite iz periferne krvi bolnikov z malignim melanomom smo 7 dni gojili v dvoplastnem polmehkem agarju. Sposobnost limfocitov, da ob stimulaciji s fitohemaglutininom (PHA) tvorijo kolonije, smo testirali pri 36 bolnikih z malignim melanomom in pri 16 zdravih krvodajalcih. Pri bolnikih z malignim melanomom je bilo povprečno število kolonij nižje (1017 ± 437) kot pri zdravih dajalcih (1711 ± 249); razlika je bila statistično značilna ($p < 0.05$). Povprečno število kolonij je bilo pri 12 bolnikh z napredovalo boleznijo značilno nižje kot pri 13 bolnikih s popolnim odgovorom na zdravljenje (745 ± 422 vs. 1588 ± 401; $p < 0.05$). Podobno razliko smo ugotovili, ko smo primerjali povprečno število kolonij pri 11 bolnikih zdravljenih z interferonom (641 ± 424) s povprečnim številom kolonij pri zdravih dajalcih in bolnikih v popolni remisiji.

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