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Pinocytic Activity and CSF Production of Macrophages During the Growth of the Lewis Lung Carcinoma in Mice

H. VON MELCHNER and P. HILGARD*†

Westdeutches Tumorzentrum, Innere Universitätsklinik (Tumorforschung), Hufelandstr. 55, D-4300 Essen 1, Federal Republic of Germany

Abstract—^{198}Au *colloidal gold was used for recording the pinocytic activity of peritoneal macrophages derived from Lewis lung carcinoma bearing C57BL mice. While depressed in an early stage of tumour growth, macrophage function becomes highly activated in a late stage of disease. A correlation between pinocytosis of macrophages* in vitro *and* in vivo *was found. High sensitivity of depressed macrophages to thioglycolate stimulation in an early phase of tumour growth was observed. The sensitivity to inflammatory stimulants decreased in parallel to macrophage activation during disease progression. In addition, a correlation between pinocytosis and CSF production by the same macrophages was found.*

INTRODUCTION

SEVERAL investigations suggested that macrophages played a central role in controlling tumour growth and tumour spread [1, 2]. The influence of malignant growth on the host's macrophage function is however still controversial. Macrophage stimulation as well as macrophage inhibition during the course of tumour growth has been reported [3, 4]. Recently, depressed RES activity was described in early and late stages of the growth of the Lewis lung carcinoma [5]. Since this experimental tumour is widely used in many laboratories for the investigation of tumour and host factors, we want to report our results on additional approaches to the evaluation of macrophage function in this experimental tumour system.

MATERIALS AND METHODS

Mice

C57BL mice were used throughout the experiments. The colony stimulating factor (CSF) assay was carried out using bone marrow cells from C3H mice. Lewis lung carcinoma was maintained and transplanted as previously described [6]. Colloidal gold (^{198}Au) with a particle size between 5 and 20 nm was obtained from the Radiochemical Centre, Amersham (U.K.).

Experimental design

The macrophage pinocytic activity was assayed *in vitro* and *in vivo*. For the *in vitro* assay the following groups, each consisting of 6–9 mice, were formed: non-stimulated controls, stimulated controls, non-stimulated tumour bearing mice and stimulated tumour bearing mice. Macrophage stimulation was carried out by intraperitoneal injection of a 2.5% thioglycolate medium 3 days prior to the harvest of the cells. Peritoneal cells were harvested by peritoneal lavage with HBSS containing 1000 i.u. heparin/l. After pooling the cells from individual animals in each group they were thoroughly washed in HBSS. After resuspension in Eagles MEM (supplemented with 10% fetal calf serum) 2×10^6 cells were introduced into glass cover slip containing 35 mm Falcon plastic culture dishes. Three parallel dishes were prepared from each cell pool. After 2 hr incubation at 37°C in a humid and 10% CO_2 containing atmosphere, the dishes were again washed three times in

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*Present address: Department of Haematology, Royal Postgraduate Medical School, Hammersmith Hospital, London, W12 0HS, U.K.
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HBSS to remove non-adherent cells. Colloidal gold, 0.05 μCi, was added to each dish and the adherent cells were re-incubated for another 4 hr. Non-cellbound radioactivity was washed off with HBSS and the remaining activity on the cover slip was determined in a conventional gamma scintillation counter. Control cover slips were prepared in a similar fashion without the addition of cells.

The ratio of gold uptake by thioglycolate stimulated and non-stimulated normal peritoneal macrophages had been established in previous experiments. As can be inferred from Fig. 1, this was a constant ratio and was not dependent on the gold particle concentration in the culture medium, although pinocytosis of normal and stimulated macrophages was strictly concentration dependent. The CSF assay was performed as described by Metcalf [7]: 0.1 ml of macrophage conditioned medium (for 4 days in RPMI 1640 supplemented with 5% fetal calf serum) was assayed for its colony stimulating activity in semi-solid agar cultures. Thirteen and sixteen days after transplantation of the Lewis lung carcinoma, groups of 10 control and 10 tumour bearing mice were injected each with 5 μCi ^{198}Au intravenously. Twenty-four hours later blood samples were collected by heart puncture and, after sacrifice of the animals, the spleens were removed, and radioactivity was measured in a gamma scintillation counter. The results were expressed as CPM/ml of blood and CPM/total spleen. The results obtained in tumour bearing animals were compared to the corresponding control animals and the statistical significance was evaluated by the U-Test for two random variables according to Wilcoxon, Mann and Whitney.

RESULTS

The radioactivity taken up by peritoneal macrophages harvested 3, 10 and 16 days after tumour transplantation is demonstrated in Table 1. Figure 2 gives the mean values of CPM/10^5 adherent cells as a percentage of the corresponding controls. It is evident that 3 days after the tumour transplantation a suppression of gold uptake was found. The pinocytic response to thioglycolate stimulation resulted in an increase of activity exceeding that of normal controls by a factor of 1.5. Ten days after tumour transplantation macrophage gold uptake was still suppressed and the response to stimulation became poor. During the final stage of tumour growth (day 16) peritoneal macrophages appeared to be maximally stimulated since no additional uptake could be recorded after thioglycolate injection.

Figure 3 illustrates the blood and spleen activity resulting from intravenous injection of gold particles on days 13 and 16 after tumour transplantation. The increased blood clea-

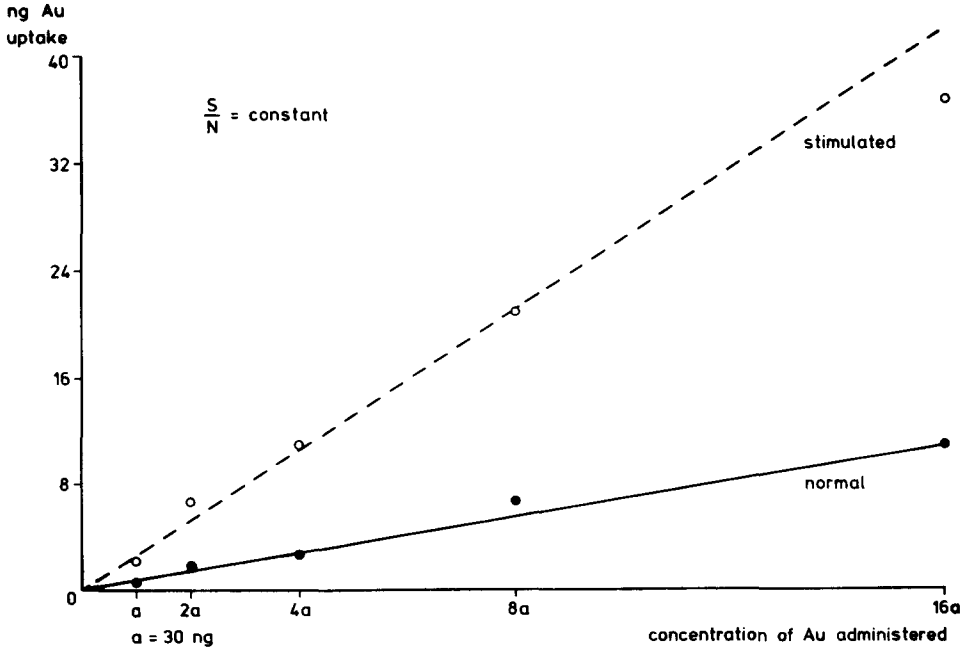

Fig. 1. Dose-response curves of gold uptake of normal and stimulated macrophages.

Table 1. *Mean values with standard deviations of* 3–4 *parallel assays in CPM/*10^5 *adherent cells from control and tumour-bearing mice, C = non-stimulated control macrophages, TU = non-stimulated tumour macrophages, SC = stimulated control macrophages, STU = stimulated tumour macrophages*

	CPM/10^5 cells		
	Day 3	Day 10	Day 16
C	$\bar{x}$ = 113.2 s = 20.4	$\bar{x}$ = 104.6 s = 8.5	$\bar{x}$ = 90.5 s = 16.9
TU	$\bar{x}$ = 83.6 s = 26.9	$\bar{x}$ = 66.6 s = 3.4	$\bar{x}$ = 195.1 s = 12.5
SC	$\bar{x}$ = 198.1 s = 47.2	$\bar{x}$ = 121.8 s = 12.6	$\bar{x}$ = 171.1 s = 16.9
STU	$\bar{x}$ = 240.2 s = 66.7	$\bar{x}$ = 98.2 s = 9.2	$\bar{x}$ = 160.0 s = 25.1

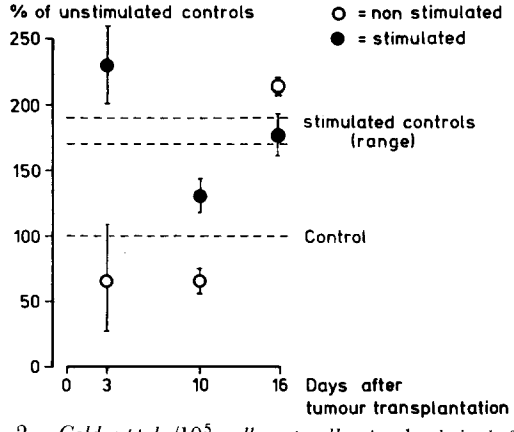

Fig. 2. *Gold uptake/*10^5 *adherent cells* ±s.d. *derived from the peritoneal cavity of non-stimulated and stimulated tumour bearing mice in percent of the normal control animals.*

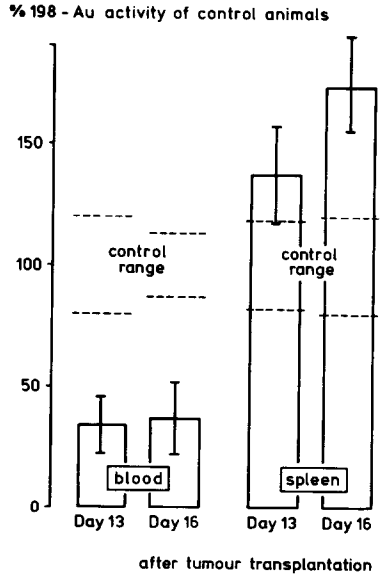

Fig. 3. *Blood clearance and splenic uptake of gold particles (with standard deviations) in tumour-bearing mice expressed as a percentage of control mice.*

rance and splenic uptake of the label by tumour bearing animals correlates well with the *in vitro* finding of macrophage stimulation during the final stage of tumour growth.

The CSF production of macrophages was tested on day 10 after tumour transplantation, when the pinocytic activity was clearly suppressed. The mean number of colonies/2×10^5 bone marrow cells stimulated by conditioned media derived from tumour animals was 51 ±7, as opposed to 94±12 if media from normal animals were used. This difference was highly significant ($P<0.05$). Thioglycolate activated macrophages did not promote colony growth in agar culture. However, when mixed with sera from endotoxin treated mice unimpaired colony growth was noted, indicating the absence of inhibitory activity in the activated macrophages.

DISCUSSION

The uptake of particles by peritoneal macrophages is considered to be an expression of their functional state. The present investigations suggest that macrophage function is depressed during the early stage of tumour growth since peritoneal macrophages derived from animals with an early Lewis lung carcinoma showed a significant decrease of their pinocytic activity. This finding supports the results obtained by other investigators [8] who suggested a humoral macrophage inhibitor produced by malignant cells. Although the spontaneous uptake of gold particles was decreased, non-specific stimulation by thioglycolate resulted in an increase of macrophage activity exceeding that of controls. This high sensitivity of the suppressed macrophages to inflammatory agents however decreased with progression of the disease. The spontaneous activity of macrophages during late stages of tumour growth rendered them unresponsive to further non-specific stimulation. The present data indicate that non-specific immunostimulants might be useful only in early stages of the growth of the Lewis lung tumour. *Corynebacterium parvum* for instance, exerts its antitumour and antimetastatic activity in this tumour model only if given on the day of transplantation or shortly thereafter [9]. It is conceivable that the ineffectiveness of *Corynebacterium parvum* at later stages of tumour growth is related to the unresponsiveness of the animals' macrophages to stimulation. Spontaneous activation of the macrophages during the final stage of tumour growth is surprising but whether this phenomenon is of

Table 2. Mean values and standard deviations of CPM/ml of blood and CPM/total spleen of tumour-bearing and control animals, 13 and 16 days after tumour transplantation

Day 13				Day 16			
Blood		Spleen		Blood		Spleen	
CPM × 10^3/ml		CPM × 10^3/total spleen		CPM × 10^3/ml		CPM × 10^3/total spleen	
Control	Tumour	Control	Tumour	Control	Tumour	Control	Tumour
4.313	1.283	295.201	169.522	3.54	1.44	318.28	131.46
2.711	1.423	236.799	174.409	4.31	1.58	271.08	142.94
4.826	1.340	225.873	95.429	3.15	1.38	343.37	88.88
3.542	1.014	433.343	149.568	3.76	1.11	375.53	95.33
3.109	1.006	296.129	164.043	3.52	1.35	410.66	167.53
4.061	1.192	476.496	226.524	3.84	1.71	59.79	127.59
3.633	1.223	374.985	243.995	3.39	1.04	259.61	106.32
3.156	0.995	306.967	106.630	4.03	1.26	297.63	155.32
2.525	1.263	362.208	127.966	4.50	1.51	323.23	155.58
3.021	—	295.602	—	2.96	—	346.31	—
$\bar{x}=3.490$	$\bar{x}=1.193$	$\bar{x}=330.360$	$\bar{x}=162.010$	$\bar{x}=3.70$	$\bar{x}=1.38$	$\bar{x}=300.55$	$\bar{x}=130.11$
s.d. = 0.732	s.d. = 0.156	s.d. = 80.830	s.d. = 49.889	s.d. = 0.49	s.d. = 0.22	s.d. = 96.04	s.d. = 28.14

any significance to the host or not, remains uncertain.

Macrophage derived CSF is known to be one of the most important stimuli to differentiation of haemopoietic cells [7]. Since earlier investigations had shown that hyperreactive macrophage stem cells appeared during tumour growth [10], macrophage CSF production was tested in the present experimental system at a time when the macrophage pinocytic activity was clearly depressed. The simultaneous depression of CSF production indicates a possible correlation between pinocytic activity and CSF production. Decreased CSF availability from macrophages may be the cause of depressed haemopoietic activity found in some experimental tumours. Furthermore, it is conceivable that decreased CSF production results also in a reduction of stem cell derived cytotoxic macrophages, thus allowing tumour growth to escape from the host's defence mechanisms.

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