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PATIENTS WITH RENAL CANCER HAVE A LARGER PROPORTION OF HIGH-DENSITY BLOOD MONOCYTES WITH INCREASED LUCIGENIN-ENHANCED CHEMILUMINESCENCE[1]

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Abstract—The production of oxygen metabolites is probably important in cancer cell killing. The production of the superoxide anion, O_2^-, can be measured by lucigenin-enhanced chemiluminescence (Cl). Previous studies have shown that whole-blood lucigenin-enhanced Cl is increased in cancer patients and that this increase is related to blood monocyte activity. The present investigation confirmed these findings and showed that whole-blood lucigenin-enhanced Cl was elevated in 17 patients with renal cell adenocarcinoma ($P < 0.001$). The activity of the monocytes was studied more in detail, whereby monocytes were separated into different populations based upon differences in densities, i.e., high-density and low-density monocytes. The cancer patients had a significantly larger proportion of high-density monocytes ($P < 0.05$) than controls. The lucigenin-enhanced Cl of purified high-density monocytes in controls was significantly higher than that of low-density monocytes ($P < 0.01$). The authors conclude that the increase in the lucigenin-enhanced Cl of whole blood observed in cancer patients may partly reflect the increased activity of a larger proportion of high-density monocytes in these patients.

INTRODUCTION

Monocytes are probably involved in the body's defense mechanism directed towards cancer cell growth (1–6). The mechanisms by which these cells kill

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tumor cells are probably analogous to the way microbes are killed, i.e., by cytotoxic proteins and the production of activated oxygen metabolites. The production of such metabolites can be measured by chemiluminescence as a sign of activation of the respiratory burst in phagocytic cells. The initial product of the so-called oxygen radicals, the superoxide anion O_2^-, reacts primarily with lucigenin as an amplifier of the chemiluminescence signal (7). Previous studies have shown that blood monocytes may be separated into different populations based upon differences in densities (8–10). The studies also demonstrated certain differences between the monocyte populations with respect to cytotoxicity, oxidative metabolism, and prostaglandin E_2 production (8, 9, 11, 12). It has been shown previously that the lucigenin-enhanced chemiluminescence (Cl) activity of whole blood is increased in cancer patients (13). This activity has been suggested to be related to the activity of blood monocytes. The aim of the present study was to characterize different monocyte populations with respect to their oxidative activity and to investigate the relationship between whole-blood lucigenin-enhanced Cl and the activity of blood monocytes further in patients with renal cell adenocarcinoma.

MATERIALS AND METHODS

Patients and Controls. The patient population comprised 17 patients (6 women and 11 men) with renal cell adenocarcinoma with an average age of 69 years (range 53–85 years). Eleven patients had clinical metastases: pulmonary (three cases), pulmonary and hepatic (one case), pulmonary and skeletal (one case), the other kidney (two cases), suprarenal (three cases), and lymphatic (one case), and in six patients no metastases had been detected. None of the patients had received any kind of systemic treatment for their malignant disease at the time of blood sampling, and none of the patients had overt signs of infection on the day of blood sampling. The adenocarcinoma had an average diameter of 8.4 cm (range 3–22 cm) ($N = 11$). Blood samples were drawn the day before surgery. Twenty-one healthy laboratory employees served as controls. Their average age was 41 years (range 23–62 years).

Methods. Heparinized venous blood was used. Leukocyte counts, differentials, and hemoglobin concentrations were measured in a Technicon H1 blood cell analyzer.

Preparation of Pure Monocytes. Monocytes were separated on a discontinuous metrizamide gradient, as described previously (13), and modified after the method of Vadas et al. (14). Briefly, stepwise concentrations of 16, 18, 20, and 23% w/v metrizamide (Nyegaard & Co AS, Oslo, Norway) were created. After separation, the cells on top of each metrizamide concentration were harvested and washed in 0.15 mol/liter NaCl and finally suspended in Gey's buffer to a concentration of 1×10^6/ml. Monocytes were found to be distributed on top of the 16%, 18% and 20% concentrations of metrizamide. The purity of the monocytes on top of the 16% metrizamide (low-density monocytes) was 78% ± 14% (*SD*) and that on top of the 18% metrizamide (high-density monocytes) was 32% ± 14% (*SD*), with lymphocytes constituting the only contaminating cells, as estimated by light microscopy and staining for unspecific esterase (15). The monocyte percentage on top of the 20% metrizamide was 11% ± 10% (*SD*) and lymphocytes and granulocytes were the contaminating cells. This study examined the monocytes on top of the 16% and 18% metrizamide gradients.

Chemiluminescence. Whole-blood chemiluminescence was performed according to a pro-

cedure outlined previously (13) using a modification of the method described by Tono-Oka et al. (16). The chemiluminescence of purified monocytes was measured after preincubation of the cells in the measuring vials for 30 min at 37°C (13), after which lucigenin (0.1 g/liter) and serum-opsonized zymosan (4 g/liter) were added. The results are expressed as relative light units (RLUs)/10^6 cells from the peak of the curve. Chemiluminescence was measured in a Biocounter M2010 (Lumac B.V., The Netherlands).

Statistics. For the statistical evaluation of the differences between the groups the Student's *t* test for paired and unpaired samples was used. Calculations were made on a personal computer with the use of the statistical packages, Statgraphics (STSC, Inc., Rockville Maryland) and Instat (San Diego, California).

RESULTS

Chemiluminescence of Whole Blood. In Figure 1 the lucigenin-enhanced Cl of whole-blood from cancer patients and controls is shown. The whole-blood activity in cancer patients was significantly higher than that of the controls ($P < 0.001$).

Differential Cell Counts in Blood. The blood differential cell counts of patients and controls are shown in Table 1. It can be seen that the lymphocytes and eosinophils are significantly lower ($P < 0.05$ and $P < 0.05$, respectively) in cancer patients, whereas neutrophil counts were significantly higher ($P < 0.05$). There were no differences in the blood monocyte counts between the patients and controls.

Chemiluminescence of High-Density and Low-Density Monocytes. The

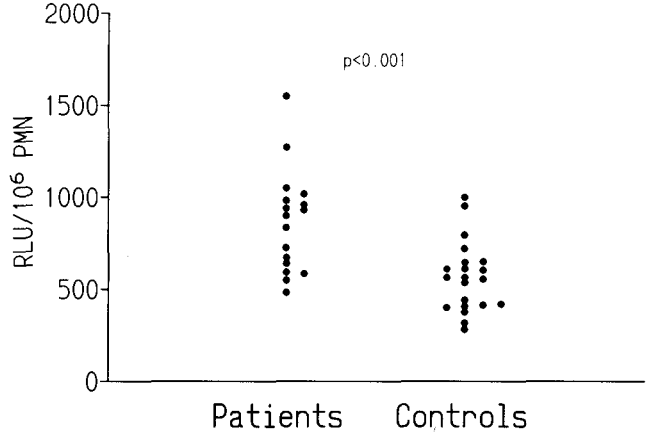

Fig. 1. Lucigenin-enhanced whole blood chemiluminescence. Whole-blood lucigenin-enhanced chemiluminescence in 17 subjects with renal cell adenocarcinoma and 21 controls. The *P* values were evaluated by means of Student's *t* test, $P < 0.001$.

distribution of low-density and high-density blood monocytes is shown in Table 2. The cancer patients had a larger proportion of high-density monocytes in blood ($P < 0.05$, paired t test). In Figure 2 the lucigenin-enhanced Cl of purified high-density and low-density monocytes from controls is shown. A significantly ($P < 0.01$, paired t test) higher activity of high-density monocytes was found, whereas no such difference could be seen in the blood of cancer patients (Figure 3).

DISCUSSION

Our findings suggest that the increased whole-blood lucigenin-enhanced Cl found in cancer patients is partly due to the presence of activated monocytes in the blood of these patients. Thus, it has been shown by others that high-density monocytes are more cytotoxic, (9, 10), have an increased oxidative metabolism (12, 18, 19), and produce more prostaglandin E_2 (8, 9) than low-density monocytes. Compatible with this, an enhanced Cl production by high-density monocytes was observed in our control population. However, this could not be demonstrated in our cancer patients, which may be due to the fact that even in

Table 1. Distribution of Blood Leukocytes in Patients with Renal Cell Adenocarcinoma and Controls[a]

	N	Monocytes (10^9/l)	Lymphocytes (10^9/l)	Neutrophils (10^9/l)	Eosinophils (10^9/l)
Renal cell adeno-carcinoma patients	15	0.44 ± 0.19	1.43 ± 0.59	5.55 ± 3.41	0.10 ± 0.09
Controls	20	0.48 ± 0.18	1.85 ± 0.45	3.43 ± 1.08	0.16 ± 0.07
		NS	$P < 0.05$	$P < 0.05$	$P < 0.05$

[a]Statistical differences between groups were evaluated by means of Student's t test

Table 2. Distribution of Low-Density and High-Density Moncytes in Blood[a]

		Monocytes (%)		
	N	Low-density	High-density	P
Renal cell adeno-carcinoma patients	17	37.5 ± 17	57 ± 18	<0.05
Controls	21	44 ± 23	49 ± 23	NS

[a]The P values in the table were evaluated by means of paired t test.

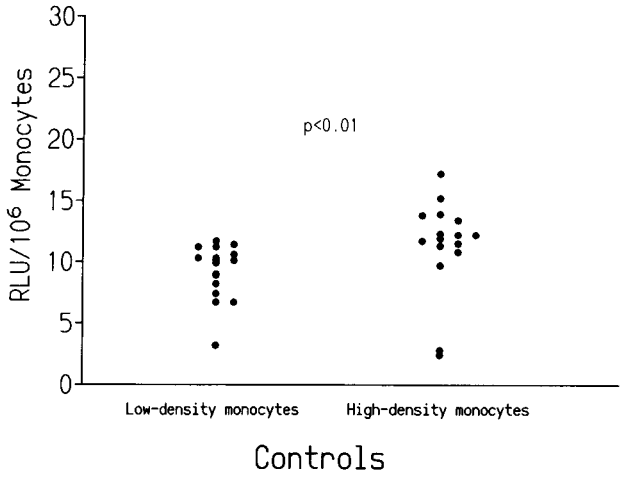

Fig. 2. Lucigenin-enhanced chemiluminescence of purified monocytes from controls. The P values were evaluated from 14 subjects by means of paired t test, $P < 0.01$.

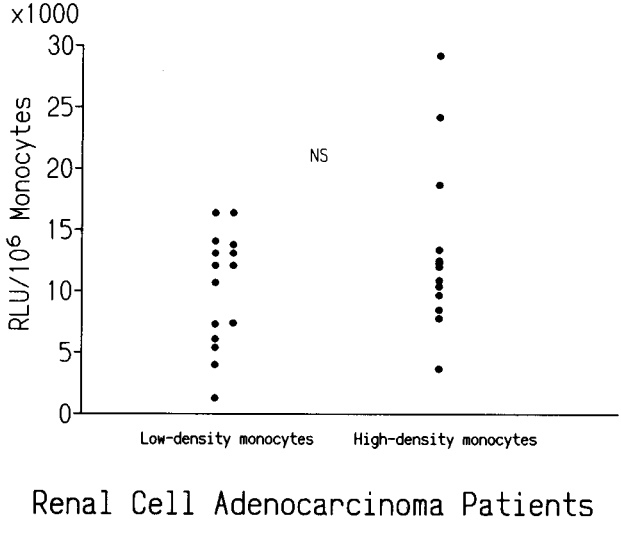

Fig. 3. Lucigenin-enhanced chemiluminescence of purified monocytes from renal cancer patients. The data were evaluated from 12 subjects by means of paired t test, $P = 0.2$.

the low-density cells, almost half of our patients displayed an increased level of activity when compared with the control cells. Therefore, the fact that the patients had both a larger proportion of high-density cells with a higher oxidative activity and a somewhat higher activity present in their low-density monocytes

may explain the increased activity of whole-blood Cl. Our previous study could not uncover any correlation between the extent of the disease and whole-blood chemiluminescence activity; the present study also confirmed the latter observation. However, it is likely that the activation of the monocyte population is caused by molecules produced by the tumor itself (4, 17) or by molecules, presumably cytokines, produced by the cells involved in the body's attack on the tumor, since the activity in patients in whom the cancer has been successfully removed normalizes (to be published). Thus, the activation of the monocyte population seems to be related to the mere presence of the malignancy. The putative cytokines or other molecules involved in the activation of the monocyte population in cancer have not been identified but may include cytokines such as IFN-γ and TNF-α (20), since both these cytokines are potent activators of monocytic oxidative metabolism.

The conclusion of the present study is that patients with renal cancer display an increase in whole-blood lucigenin-enhanced Cl, which may be explained by an increased activation of the blood monocyte population by mechanisms involved in tumoricidal reactions. The activation of the monocyte population was shown by the novel finding of a larger proportion of high-density monocytes in cancer patients when compared with controls and by the fact that almost half of the patients had monocytes with a higher intrinsic activity, irrespective of their density.

Future studies will focus on the possible use of Cl in monitoring disease activity and in the prediction of therapeutic response to biological response modifiers such as interleukin-2 and interferons.

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