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Increased Monocyte Phagocytosis in Cancer Patients

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Abstract—*Phagocytosis of IgG opsonized sheep erythrocytes (EA) by peripheral blood monocytes was evaluated in* 73 *individuals:* 29 *patients with malignant neoplastic diseases,* 24 *patients with non malignant diseases and* 20 *normal donors. In a* 30-min *assay, phagocytosis values observed in* 16 *of* 29 *cancer patients and in* 3 *of* 24 *control patients were above the upper limit of the* 2 S.D. *interval for the normal donor population. The enhanced EA phagocytosis was dependent on a higher percentage of phagocytic cells as well as on an increased phagocytic rate. Mean values of phagocytosis obtained for cancer patients were statistically different* ($P < 0.01$) *from those obtained for normal donors and control patients. According to our still limited number of observations, no association could be demonstrated between increased monocyte phagocytosis and histological type, anatomical site and clinical stage of the tumors.*

INTRODUCTION

THE MONONUCLEAR phagocyte system is markedly affected by neoplastic disease. The frequent occurrence in cancer patients of cutaneous anergy to a variety of antigens and skin-sensitizing agents [1, 2] as well as the decreased *in vitro* chemotactic response of monocytes from 50% of cancer patients [3] strongly suggests that an impairment of the inflammatory response is often associated with the neoplastic disease. This conclusion is further supported by evidence that surgical removal of the tumor is often followed by restoration of monocyte function [4].

The exact mechanisms by which neoplastic disease affects monocytes are still unknown; however, tumor products able to inhibit *in vitro* monocyte chemotactic response and *in vivo* inflammatory reactions have been recently described [5].

An abnormal phagocytic response has also been observed in cancer patients. Phagocytosis was found to be increased in patients with localized malignancies [6] and Hodgkin's disease [7]. Blood clearance of aggregated albumin was increased in cancer patients as compared to patients with other non neoplastic chronic diseases [6]; the stimulatory status of the mononuclear phagocyte system was directly related to the mass of the tumor tissue but not to the extent of the disease.

Dysfunctions of the mononuclear phagocyte system similar to those described in cancer patients have been also observed in tumor bearing mice. These alterations include impairment of inflammatory responses [8], decreased bactericidal activity [9], decreased macrophage chemotactic response [10] and increased macrophage phagocytosis [11].

In the present investigation we have evaluated the capacity of peripheral blood monocytes from cancer patients to phagocytize IgG coated sheep erythrocytes (EA).

Our results, which show an increased monocyte phagocytic activity in about 50% of cancer patients, confirm and extend previous observations based on the blood clearance of radiolabelled aggregates. Moreover, EA monocyte phagocytosis is a simple and reliable assay which does not involve patient manipulation.

MATERIALS AND METHODS

Cell preparations

Mononuclear cells were isolated from peripheral venous blood. Twenty ml of hepari-

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nized venous blood was diluted with 20 ml of RPMI 1640 Medium and layered onto 30 ml of Lymphoprep (Nyegaard, Oslo, Norway) in disposable polypropylene tubes (No. 2074, Falcon Plastics). After centrifugation at 400 ***g*** for 40 min at room temperature the interface cell layer was removed and washed twice at 4°C with RPMI 1640 Medium containing 10% foetal calf serum. The cell suspension contained 75–80% lymphocytes, 15–20% monocytes, 1–2% basophils and 1–3% granulocytes. Cell concentration was adjusted at 10×10^6 cells/ml. One ml of the final cell suspension, containing about $1.5-2 \times 10^6$ monocytes, was added to 35 mm Petri dishes (No. 3001, Falcon Plastics) and incubated for 2 hr at 37°C in 5% CO_2 in moist air. After incubation, adherent cells were mostly monocytes as estimated by peroxidase staining. In a series of preliminary experiments we have been able to establish that normal donors, control patients and cancer patients exhibited similar numbers of adherent cells per microscope field as well as a similar degree of variation in terms of lymphocyte contamination as estimated by percentage of adherent peroxidase negative cells.

EA phagocytosis

Sheep erythrocytes (E) were obtained from Istituto Sieroterapico Sclavo, Siena, Italy. One ml of E (1×10^9E/ml PBS) was incubated with 0.01 ml of IgG rich fraction of rabbit anti-Forssman antibody (A), prepared by Sephadex G-200 gel chromatography of whole antiserum, in a 37°C water bath for 30 min. EA were washed three times in PBS and resuspended with 10 ml RPMI 1640 Medium containing 10% FCS. One ml of EA (1×10^8EA/ml) was added to 35 mm Petri dishes containing monocyte monolayers ($1-2 \times 10^6$ monocytes/dish) and incubated for 30 min at 37°C in 5% CO_2 in moist air unless differently specified. At the end of incubation, monocyte–EA cultures were washed twice with PBS and once with Tris buffered NH_4Cl lysing solution to remove non-phagocytized EA. Cultures were than fixed with methanol for 2 min. Monocyte monolayers were observed with a dark phase microscope at 400 ×. Percentage of EA phagocytic cells (one EA or more inside the cell) was evaluated by counting at least 200 cells. Average number of EA/monocyte was evaluated by counting the number of EA contained in 100 phagocytic cells. EA phagocytosis by 100 monocytes represents the total number of antibody coated erythrocytes (EA) ingested by 100 monocytes. Samples were numbered and read blind.

Cancer patients

Twenty-nine patients were selected. They were admitted to the Surgery Branch of the University of Rome, between April and November 1978 for treatment of primary malignant diseases. They were not on chemotherapy nor had they received radiation therapy. No attempts were made to exclude patients with local infections or those treated for non-neoplastic diseases. Terminal or debilitated patients were excluded. The spectrum of tumor types tested in this study (Table 1) is different from that of a general hospital and reflects the status of the Surgery Branch as a referral centre for lung cancer and colon cancer. Twenty-four males and five females were studied; the mean age was 58 ± 9.8 yr and ranged from 36 to 85 yr.

Table 1. Histology, site of origin of cancer and EA monocyte phagocytosis

Cancer	No. of cases	EA Phagocytosis* Normal	Increased
Epidermoid carcinoma			
Lung	14	6	8
Esophagus	2	2	0
Adenocarcinoma			
Lung	2	1	1
Colon	5	1	4
Stomach	2	1	1
Breast	1	0	1
Ovary	1	1	0
Pancreas	1	0	1
Thymoma	1	1	0
Total	29	13	16

*Values exceeding the upper limit of the 2 S.D. interval for the normal donor population were considered as increased.

Control patients

Patients admitted to the Surgery Branch in the same period for treatment of non-malignant neoplastic diseases or non-neoplastic diseases were studied to control for factors such as hospitalization, medications and the psychologic stress associated with serious diseases (Table 2).

The mean age of 13 male and 11 female control patients was 50 ± 18 yr and ranged from 18 to 81 yr.

Table 2. EA monocyte phagocytosis in control patients

Disease	No. of cases	EA Phagocytosis* Normal	Increased
Peptic ulcer	3	3	0
Kidney stones	3	3	0
Acute aspecific lymphadenitis	2	2	0
Traumatic lesions	5	4	1
Breast fibroadenoma	3	3	0
Breast fibrocystic dis.	1	1	0
Mediastinum fibrolipoma	1	1	0
Prostate nodular hyper.	1	1	0
Goitre	2	1	1
Hiatus hernia	2	1	1
Colon diverticulitis	1	1	0
Total	24	21	3

*Values exceeding the upper limit of the 2 S.D. interval for the normal donor population were considered as increased.

Normal donors

Fifteen healthy male and five female individuals with a mean age of 33.2 ± 11 yr range from 18 to 63 yr, were also studied to provide a control group for medications and the stress of hospitalization. Those individuals were not taking any medications regularly.

RESULTS

An example of individual variation among normal subjects in EA phagocytosis by peripheral blood monocytes is represented in Fig. 1. Monocyte monolayers from four healthy individuals were incubated with EA for 15–30–60–120 min; at the end of incubation percentage of phagocytic cells (panel a), average number of EA per phagocytic cell (panel b), and total number of EA phagocytized by 100 adherent cells (panel c) were determined by dark phase microscopy.

EA phagocytosis by monocytes is time dependent. Between 15 and 120 min there is a progressive increase in the percentage of phagocytic monocytes, in average number of EA per phagocytic cell and in number of EA phagocytized by 100 adherent cells. Phagocytosis values obtained for donors LR, MP and AP were similar, whereas monocytes from donor PR expressed a higher degree of phagocytic activity; it is noteworthy that the enhanced response of donor PR could be demonstrated at each time point we have tested.

Results obtained in experiments performed on different days were highly reproducible. Phagocytosis values obtained when monocytes from the same healthy donors were tested in different experiments were always within 2 S.D. interval of the normal donor population (unpublished observations). In Fig. 2 is shown the EA phagocytosis expressed by peripheral blood monocytes obtained from 20 normal subjects, from 24 patients with non-malignant diseases, and from 29 patients with malignant diseases. The shaded area shown in

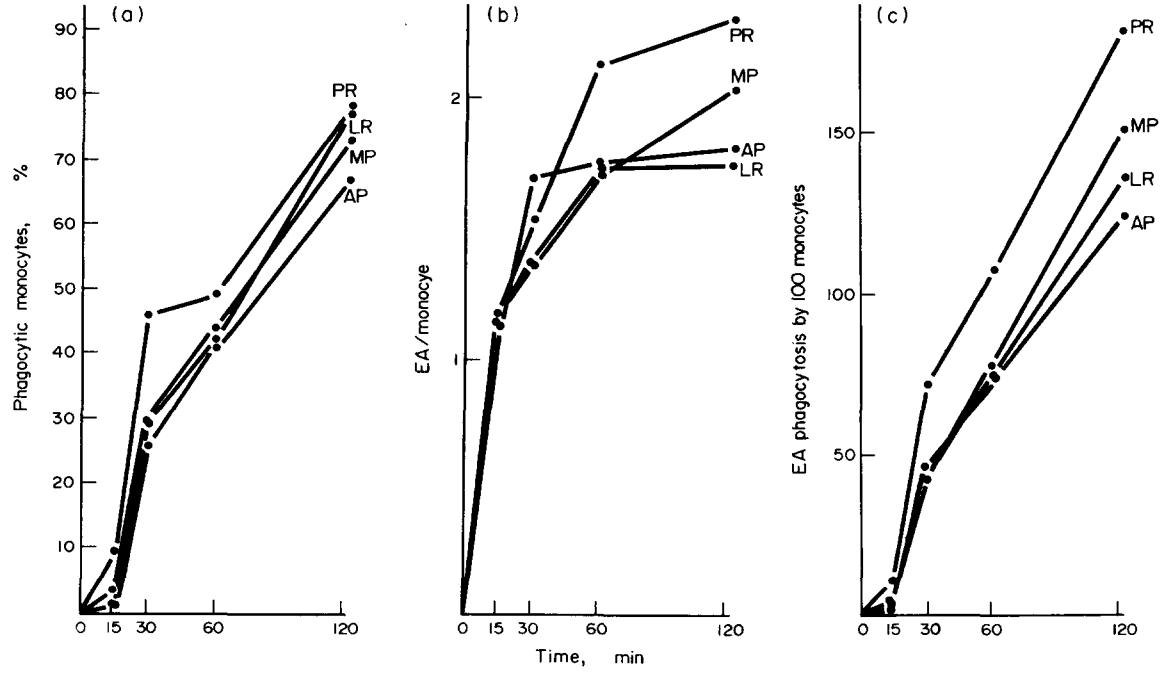

Fig. 1. Time course of EA phagocytosis by peripheral blood monocytes obtained from four normal donors. (a) Percentage of phagocytic cells. (b) Average number of EA per phagocytic cell. (c) Total number of EA phagocytized by 100 monocytes.

Fig. 2 represents the mean value (71.7) ± 2 S.D. of EA phagocytized by 100 adherent cells from healthy donors. This area should include 95% of the experimental values obtained from a normal donor population. Our results indicate that phagocytosis values observed in 3 of 24 control patients (12.5%) and in 16 of 29 neoplastic patients (55%) exceeded the upper limit of the 2 S.D. interval. The mean value for control patients was 81.45 whereas for neoplastic patients it was 116.66. The mean value for neoplastic patients was significantly different from those for normal donors and control patients (in both instances $P<0.01$), whereas the difference between normal donors and control patients was not statistically significant. Both percentage of phagocytic cells and phagocytic rate were found to be increased in monocytes from cancer patients. In Fig. 3 we have reported the percentages of phagocytic cells and the average numbers of EA per phagocytic cell observed in the same monocyte populations. Adherent cells from 11 of 29 neoplastic patients (38%) and from 2 of 24 control patients (8%) exhibited a percentage of phagocytic cells above the mean ± 2 S.D. of the normal donor population. The phagocytic rate was found to be increased in 14 of 29 cancer patients (48%) but only in 2 of 24 control patients (8%). Mean values for cancer patients were statistically different ($P<0.01$) from those for normal donors and control patients, whereas the differences between control patients and normal donors were not statistically significant. At present we cannot offer any explanation for the enhanced response observed in the three control patients. The increased phagocytic response was not correlated to age, sex, ABO group, Rh status, number of circulating lymphocytes, monocytes and granulocytes. Moreover, in our limited number of cases, we could not observe any relationship between increased monocyte phagocytosis and histological type, anatomical site or clinical stage of the tumor.

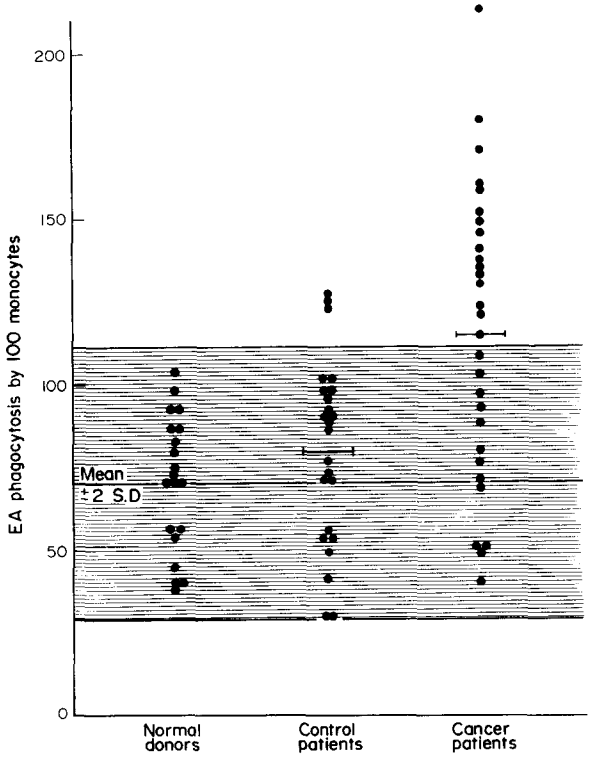

Fig. 2. EA monocyte phagocytosis in 20 normal donors, in 24 control patients and in 29 cancer patients. The shaded area represents the mean value ± 2 S.D. of the normal donor population. The mean value for cancer patients is significantly different (P<0.01) from those for normal donors and control patients. The difference between normal donors and control patients is not statistically significant (Student's t-test).

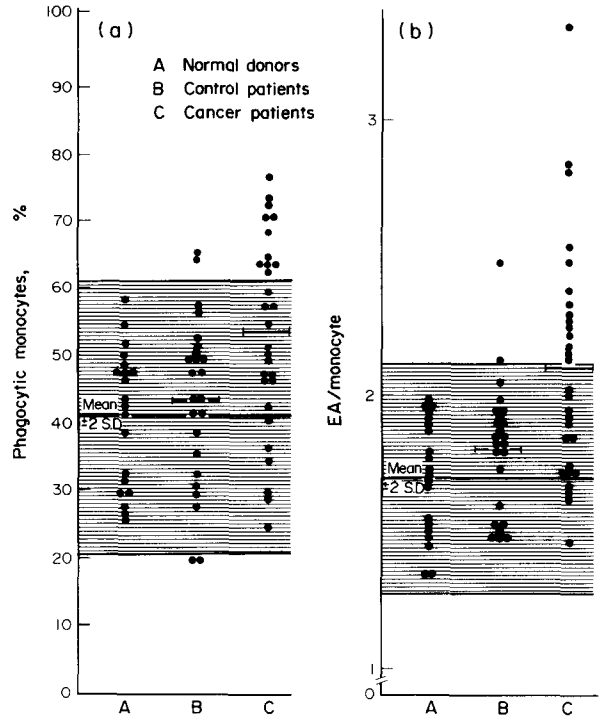

Fig. 3. Percentage of phagocytic monocytes (a) and average number of EA per phagocytic monocyte (b) in 20 normal donors, in 24 control patients and in 29 cancer patients. The shaded areas represent the mean value $\pm$ S.D. of the normal donor population. In both instances the mean values for cancer patients are significantly different (P<0.01) from those for normal donors and control patients. The differences between normal donors and control patients are not statistically significant (Student's t-test).

DISCUSSION

In the present paper we provide evidence that monocytes from cancer patients display an increased phagocytic activity for opsonized erythrocytes. Both percentage of phagocytic cells and average number of EA per phago-

cytic cell were increased in about 50% of cancer patients independently of the histological type, the anatomical site and the clinical stage of the tumor.

In a recent report [12] it has been shown that peripheral blood monocytes from cancer patients have an increased number of surface Fc receptors. Since EA phagocytosis is critically dependent on the degree of opsonization of the particle as well as on the number of Fc receptors present on the monocyte cell membrane, it may be reasonably postulated that the increased phagocytic activity observed in monocytes from cancer patients is directly related to the higher density of Fc receptors present on their cell membrane.

Either intrinsic differences in the monocyte–macrophage population or serum factors able to modify the monocyte function may be alternatively or concomitantly responsible for the higher density of Fc receptors, and consequently, for the increased phagocytic activity observed in monocytes from cancer patients. In this regard, it has been recently shown that soluble factors present in the serum of cancer patients induced increased numbers of Fc receptors on human monocytes [13].

The existence in the monocyte–macrophage population of a functional heterogeneity mostly related to maturational events is now well documented [14–16]. Monocytes can be differentiated from mature macrophages by lysosome content, IgG receptors, phagocytic activity, proliferative response and ability to interact with lymphocytes [17]. It can be argued that every event, ageing included, able to influence monocyte maturation may also alter the cell function. In this regard, an abnormal monocytopoiesis might be responsible for the increased phagocytic activity of monocytes from cancer patients through an alteration of their half-time in the peripheral blood. Although no information is at present available for humans, an abnormal production of macrophage colonies has been observed in the bone marrow of tumor bearing mice [18]. Macrophage phagocytosis is an energy dependent multistage process that can be stimulated by several agents both *in vivo* [19] and *in vitro* [20]. Soluble factors able to enhance macrophage phagocytosis *in vivo* have been demonstrated in the serum of tumor bearing mice [11]. On the basis of these observations it can be postulated that soluble factors, either released or induced by the tumor, may be involved in the enhancement of monocyte phagocytosis in cancer patients.

Finally, another monocyte function has been found to be abnormal in cancer patients. Peripheral blood monocytes from 50% of patients with malignant disease display a decreased *in vitro* chemotactic response [3]. Increased phagocytosis and decreased chemotactic response may both be related to the same monocyte dysfunction or, alternatively, may represent two unrelated phenomena. The observation that peritoneal macrophages from tumor bearing mice display both the abnormalities is suggestive for the former interpretation [11].

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