Comparative Evaluation of Particulate and Bacterial Opsonization by Plasma of Normal and Neoplastic Individuals[1] (37240)

J. C. PISANO, J. P. JACKSON, AND N. R. DI LUZIO
Tulane University School of Medicine, New Orleans, Louisiana 70112

Previous studies from this laboratory have shown that patients with carcinoma manifest a profound depletion in activity of plasma opsonic, or recognition, factors (1–4). These plasma factors are generally responsible for the promotion of phagocytosis of a variety of foreign particulates including bacteria. Decreased recognition factor activity in plasma derived from patients with cancer was initially observed by evaluating the phagocytic function of rat liver slices incubated in either normal human plasma or plasma from cancer patients and an ^{131}I-triolein labeled gelatinized RE test lipid emulsion (1). Patients with diseases other than cancer had essentially normal opsonic activity (1). Additional studies have demonstrated that the opsonic deficiency could be demonstrated in patients with neoplasia using human macrophages with human cancer patient serum (4). The decreased recognition factor activity was found to be more extensive in patients with metastatic carcinoma (2) than in those with nonmetastatic tumors.

In essential agreement with the observations of Hektoen (5) on the specificity of opsonins in phagocytosis of particulates, Vaughn (6) and Saba and Di Luzio (7) demonstrated that opsonic factors are particle specific. Pisano *et al.* (4) demonstrated that, in addition to a loss of opsonic activity to the gelatinized lipid emulsion, plasma from patients with carcinoma had decreased opsonic activity for radioactive colloidal gold and colloidal technetium sulfide.

The loss of opsonic activity to lipid emulsion, colloidal gold, and colloidal technetium sulfide suggested the possibility that the presence of neoplasia resulted in a generalized loss of opsonic factors. However, although each particulate is chemically distinct, all three of these particulates are gelatin-stabilized and it is conceivable that the loss of opsonic activity is specific for gelatin. Therefore, the present study was designed to study the opsonic activity of plasma from carcinoma patients to promote the phagocytosis of heat-killed bacteria, a nongelatin-stabilized particulate. Additionally, human leukocytes obtained from normal individuals and cancer patients, were comparatively evaluated for the ability to phagocytize bacteria in order to delineate any possible derangement in leukocyte cell function.

Materials and Methods. Liver slices were prepared from 250 g Sprague–Dawley rats as previously described (1–4). Phagocytosis of the lipid emulsion by Kupffer cells of liver slices was assessed by adding an approximately 250 mg liver slice to 1.5 ml of Krebs–Ringer phosphate buffer (pH 7.5) and 1.5 ml of either control or experimental plasma containing 100 units of heparin and 2 mg of gelatinized ^{131}I-triolein labeled RE test lipid emulsion (8, 9). Following a 30 min incubation period at 37°, the liver slices were removed from the media and washed three times with 0.9% saline. The uptake of the radiolabeled lipid emulsion by the liver slices was determined with a Nuclear-Chicago Autogamma scintillation system.

Blood was obtained by venipuncture from six healthy individuals and six patients with various carcinomas (Table I). The blood samples were obtained prior to the institution of therapy in view of previous observations that therapeutic procedures result in restoration of opsonic activity (2). Heparin was added to the blood and the erythrocytes

[1] Supported in part by U.S. Public Health Service Grant CA13746, the American Cancer Society, and the Edward Schleider Foundation.

TABLE I. Diagnostic Information of Patients with Carcinoma Serving as Donors of Plasma.

Patient	Age	Sex	Established diagnosis
M. D.	60	M	Recurrent carcinoma, base of tongue
J. T.	28	M	Recurrent carcinoma, esophagus
I. T.	60	F	Adenocarcinoma of stomach with liver metastasis
J. B.	41	M	Bronchogenic carcinoma
M. L.	71	F	Squamous cell carcinoma of chest wall
S. F.	60	F	Carcinoma of left breast

were allowed to sediment for 3 hr at room temperature. The supernatant, containing plasma and leukocytes, was collected and the leukocytes were removed by low speed (60 *g*) centrifugation. The leukocytes remained viable throughout these procedures, as determined by trypan blue exclusion and colloidal carbon phagocytosis.

The leukocytes were washed three times with tissue culture medium (TC 199, Difco) and then adjusted to contain 1.0×10^6 leukocytes/ml of TC 199. To 1.0 ml of cellular suspension was added 1.0 ml of plasma, from either a normal individual or a carcinoma patient, and 0.1 ml of a thrice-washed, heat-killed, 18 hr culture of *Staphylococcus albus* containing 1.0×10^7 bacteria/ml of saline. Leukocytes were also incubated with 2 mg of gelatinized ^{131}I-triolein labeled RE test lipid emulsion in place of the bacteria. The leukocyte–bacteria mixtures were incubated at 37°, with constant agitation, for 1 hr. Following incubation the cellular suspensions were rapidly cooled to 4° and centrifuged at low speeds (30*g*) for 10 min. The cellular pellet was resuspended in TC 199 and washed three times, then spread on a glass microscopic slide and allowed to air-dry. The slides were stained with Wright's stain and the percentage of 300 monocytes containing one or more bacteria was determined. The leukocyte–lipid emulsion mixture was evaluated for phagocytic activity by gamma scintillation counting of the cellular pellet following three washes in TC 199.

Results. In agreement with previous studies (1–4) the addition of human plasma promoted a greater than 40-fold increase in phagocytosis of the lipid emulsion by rat liver slices compared to buffer controls (Table II). In addition, the control plasma enhanced the uptake of lipid emulsion by leukocytes 8-fold compared to buffer controls. Similarly normal human plasma induced a 4-fold increase in monocyte phagocytosis of *S. albus*.

Plasma obtained from patients with carcinoma was not able to support the phagocytosis of gelatinized lipid emulsion by either rat liver slices or leukocytes derived from normal subjects (Table II). In comparison to the results obtained with normal human plasma (Table II), plasma derived from cancer patients manifested an 80% reduction in the ability to support lipid emulsion phagocytosis by rat liver slices and a 64% reduction when human blood leukocytes were employed.

In contrast to the decreased opsonic activity obtained when the lipid emulsion was employed, plasma derived from cancer patients exhibited normal opsonic activity for the promotion of *S. albus* phagocytosis

TABLE II. Ability of Normal Human Plasma to Enhance the Phagocytosis of Gelatinized "RE Test" Lipid Emulsion and *S. albus*.

	Phagocytic uptake		
	Lipid emulsion		*S. albus*
Media	Rat liver slices (% ID/100 mg)[b]	Human leukocytes[a] (% ID/10^6 cells)[b]	human leukocytes[a] (% with bacteria)[b]
Buffer	0.6 ± 0.2	3.2 ± 0.7	22 ± 1
Normal human plasma	26.2 ± 2.1	24.9 ± 1.5	86 ± 7

[a] Leukocytes obtained from the plasma donors.

[b] Values are means ± SEM of 6 determinations.

TABLE III. Opsonic Activity[a] of Plasma Obtained from Patients with Carcinoma for Gelatinized RE Test Lipid Emulsion and *S. albus*.

Subject	Phagocytic uptake: Gelatinized RE test lipid emulsion — Rat liver slice (% of control)[b]	Phagocytic uptake: Gelatinized RE test lipid emulsion — Human leukocytes (% of control)[b]	Phagocytic uptake: *S. albus* — human leukocytes (% of control)[b]
M. D.	19.5	34.6	101.0
J. T.	15.3	31.2	102.1
I. T.	163	24.2	101.4
J. B.	26.6	30.9	92.6
M. L.	21.2	36.5	99.4
S. F.	19.4	41.2	95.4
Mean	19.7	33.2	98.7

[a] Opsonic activity is assayed by the ability of plasma to support the phagocytosis of the gelatinized reticuloendothelial lipid emulsion and heat-killed *S. albus* by human leukocytes or Kupffer cells of rat liver slices.

[b] Control values for these experiments, which were obtained from simultaneously run experiments, were those reported in Table II; control values were set at 100%.

by monocytes (Table III). Similarly, leukocytes obtained from cancer patients manifested normal bacterial phagocytosis when incubated in either normal serum or serum obtained from cancer patients (Table IV).

Discussion. These results demonstrated that plasma from patients with carcinoma was not able to support the phagocytosis of gelatinized RE test lipid emulsion by either rat liver slices or human leukocytes, but manifests normal opsonic activity for *S. albus*. The finding of decreased opsonic activity for the lipid emulsion is in essential agreement with previous studies from this laboratory employing a variety of *in vitro* systems (1–4).

TABLE IV. Comparative Phagocytosis of *S. albus* by Leukocytes Obtained from Normal Subjects or Individuals with Carcinoma.[a]

Leukocyte source	No.	% Leukocytes containing bacteria[b]; incubation media: Buffer	Plasma: Normal	Plasma: Cancer
Normal	6	7.3 ± 1.3	78.0 ± 4.2	78.0 ± 3.1
Carcinoma	6	6.8 ± 0.9	78.2 ± 2.1	79.3 ± 2.2

[a] Blood leukocytes were obtained and incubated with heat-killed *S. albus* in either buffer, normal human plasma, or plasma from tumor-bearing subjects.

[b] Uptake of bacteria by leukocytes was assayed after 1 hr incubation; results are expressed as the mean ± SEM.

Previous studies (4) had demonstrated that the opsonic defect of cancer patients could be observed with three different gelatin-stabilized colloids. Since no opsonic defect was observed when *S. albus* was employed as the phagocytizable particle it would appear that the opsonic deficiency is not a general one, but manifests some degree of specificity towards gelatin.

Previous studies have demonstrated that the intravenous administration of particulate materials effectively depletes plasma of recognition factor to that agent (10). Additionally, studies have demonstrated that the administration of leukemic cells to normal rats also results in the loss of plasma recognition factor activity when tested with the gelatinized RE test lipid emulsion (12). Since the depletion of humoral recognition factor activity following the intravenous administration of leukemic leukocytes was not seen when normal leukocytes were administered, it has been suggested that plasma recognition factor activity complexes with the leukemic cell (12). Thus, it appears that leukemic cells share common features to that of the gelatin preparation employed as judged by their equivalent ability to deplete recognition factor activity.

Reduction in recognition factor activity

observed in patients with various neoplastic diseases (1–4) as well as in animals with leukemia (13) suggests that the human tumor cells, as well as the rat leukemic cell, may share a common surface chemical characteristic which results in recognition factor depletion (13). Likewise, it has been proposed (13) that if recognition factor activity is essential for phagocytosis or macrophage induced contact lysis of tumor cells (14), the restoration of recognition factor activity could possibly serve as a potential means of protecting the host from the proliferation of abnormal cells.

The normal opsonic activity for bacteria and a normal degree of phagocytosis by blood leukocytes would indicate that the decreased resistance to infection observed in cancer patients is probably not explained by decreased bacterial phagocytosis. Thus, the increased incidence of infection in the cancerous individual must reside in some area of the host-defense system other than phagocytosis.

The decreased opsonic activity for lipid emulsion has now been observed with the rat liver slice system, rat peritoneal macrophages, human pulmonary macrophages, and human leukocytes (1–4). The consistency of this observation suggests that it may be of possible diagnostic and, perhaps, prognostic value in regard to carcinoma.

Summary. Plasma obtained from six healthy individuals was capable of promoting the phagocytosis of *Staphylococcus albus* and gelatinized radiolabeled lipid emulsion by human blood leukocytes. Experimental plasma derived from six patients with carcinoma manifested normal ability to opsonize *S. albus* when compared to the control plasma. In contrast, plasma from these patients was unable to support the phagocytosis of a gelatinized RE test lipid emulsion by either rat liver slices or human leukocytes. In comparison with normal plasma, the experimental plasma manifested an 80 and 64% reduction in lipid emulsion phagocytosis by rat liver slices and human leukocytes, respectively. These results suggest that the loss of opsonizing ability previously observed in patients with carcinoma is not a general phenomena but may be restricted to gelatin-stabilized colloids.

1. Pisano, J. C., Di Luzio, N. R., and Salky, N. S., Nature (London) **226**, 1049 (1970).

2. Pisano, J. C., Di Luzio, N. R., and Salky, N. S., J. Lab. Clin. Med. **76**, 141 (1970).

3. Pisano, J. C., and Di Luzio, N. R., RES J. Reticuloendothel. Soc. **1**, 659 (1970).

4. Pisano, J. C., Jackson, J. P., Di Luzio, N. R., and Ichinose, H., Cancer Res. **32**, 11 (1972).

5. Hecktoen, L., J. Infec. Dis. **2**, 128 (1908).

6. Vaughn, R. B., Brit. J. Exp. Pathol. **46**, 71 (1965).

7. Saba, T. M., and Di Luzio, N. R., RES J. Reticuloendothel. Soc. **2**, 437 (1965).

8. Di Luzio, N. R., and Riggi, S. J., RES J. Reticuloendothel. Soc. **1**, 136 (1964).

9. Filkins, J. P., and DiLuzio, N. R., RES J. Reticuloendothel. Soc. **3**, 471 (1966).

10. Murray, I. M., Amer. J. Physiol. **204**, 655 (1963).

11. Saba, T. M., and Di Luzio, N. R., J. Physiol. (London) **216**, 197 (1969).

12. Di Luzio, N. R., Pisano, J. C., and Salky, N. K., Advan. Exp. Med. Biol. **15**, 373 (1971).

13. Di Luzio, N. R., McNamee, R., Miller, E. F., and Pisano, J. C., RES J. Reticuloendothel. Soc. **12**, 314 (1972).

14. Amos, D. B., Ann. N. Y. Acad. Sci. **87**, 273 (1960).

Received Jan. 21, 1973. P.S.E.B.M., 1973, Vol. 142.