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A phagocytosis-enhancing factor in human plasma

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Summary. A phagocytosis-enhancing factor (PEF) with the capacity to stimulate the ingestion of sensitized sheep erythrocytes by human polymorphonuclear and mononuclear leucocytes has been isolated from human plasma by chromatography on DEAE–cellulose and filtration on Sephadex G-150 and Sephadex G-100. PEF is a protein of approximately 70,000 molecular weight which is susceptible to inactivation by heating at 60° or by tryptic digestion. PEF promotes phagocytosis of erythrocytes sensitized with intact 7S antibody or bearing the C3b complement fragment, but not of unsensitized erythrocytes or erythrocytes sensitized with 19S antibody. The specificity of PEF interaction with target erythrocytes and the persistence of its stimulatory effect after the target cells are washed suggest that it promotes phagocytosis by an action on the erythrocytes.

INTRODUCTION

Antibodies of the IgG class, termed opsonins, directed against determinants on the surface of particles, can opsonize these particles so as to increase their rate of ingestion by human neutrophils and mononuclear leucocytes (Quie, Messner and Williams, 1968; Huber and Fudenberg, 1968). The heat-stable opsonic capacity of serum is considered to be due to IgG opsonic antibodies (Quie, 1972). The heat-labile opsonic activity in normal serum has been attributed entirely to proteins of the complement system (Nelson and Lebrum, 1956; Hirsch and Strauss, 1964; Johnston, Klemperer, Alper and Rosen, 1969; Smith and Wood, 1969). Activation of the complement cascade leads to the deposition of the major cleavage fragment of C3, C3b, on the surface of the particles with resultant stimulation of their ingestion (Gigli and Nelson, 1968). Other serum proteins including C5, C3b inactivator, C-reactive protein, and α_2-HS glycoprotein also have been reported to enhance phagocytosis by leucocytes under certain experimental conditions (Shin, Smith and Wood, 1969; Ganrot and Kindmark, 1969; van Oss, Gillman, Bronson and Border, 1974; Stossel, Field, Gitlin, Alper and Rosen, 1975).

A normal human plasma protein apparently distinct from the known opsonic factors was found to enhance human leucocyte phagocytosis of erythrocytes sensitized with antibodies of the IgG class or bearing trace amounts of C3b. This factor has been purified free of chemotactic activity and the characteristics of its enhancement of phagocytosis have been investigated.

MATERIALS AND METHODS

Handling of sheep erythrocytes, human serum and plasma, and guinea-pig serum has been described (Nelson, Jensen, Gigli and Tamura, 1966). Veronal-

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buffered saline, pH 7·5, ionic strength 0·145 M, containing 0·1 per cent gelatin, 0·0015 M Ca^{2+} and 0·0005 M Mg^{2+} (GVB^{2+}); GVB^{2+} containing 5 per cent dextrose ($DGVB^{2+}$); and 0·01 M or 0·05 M disodium ethylenediaminetetra-acetate (EDTA) in veronal-buffered saline (EDTA–GVB) (Nelson *et al.*, 1966) were employed. The methods for preparation of diethylaminoethyl (DEAE) Sephadex A-50, Sephadex G-200 and G-100 (Pharmacia Fine Chemicals, Piscataway, New Jersey), hydroxylapatite (Clarkson Chemical Company, Incorporated, Williamsport, Pennsylvania), carboxymethyl (CM) cellulose (CM-52, Whatman, Maidstone, Kent), and buffers for chromatography were as published (Nilsson and Müller-Eberhard, 1965; Kaplan, Spragg and Austen, 1971). Hexadimethrine bromide (Polybrene) and diisopropyl fluorophosphate (DFP) (Aldrich Chemical Company, Incorporated, Milwaukee, Wisconsin), crystalline pepsin and soybean trypsin inhibitor (SBTI) (Worthington Biochemical Corporation, Freehold, New Jersey), phosphorylcholine (Sigma Chemical Company, St Louis, Missouri) and Hanks's balanced salt solution and Medium-199 (Microbiological Associates, Bethesda, Maryland) were obtained as indicated. Plasma thromboplastin antecedent (PTA)-deficient plasma and Hageman factor (HF)-deficient plasma were obtained from Sera-Tech Biologicals, New Brunswick, New Jersey.

Antibodies against intact sheep erythrocytes (E) were prepared in adult albino rabbits. The animals received a total of 1×10^{12} E in four equal doses during a 2-week period; after another 2 weeks they were boosted with two doses of 1×10^{9} E 1 week apart and bled 7–10 days after the last dose. Anti-sheep E antibodies of the IgG class were isolated by precipitation in 40 per cent ammonium sulphate and chromatography on DEAE–Sephadex A-50 and CM–cellulose (Gigli and Nelson, 1968). The $F(ab')_2$ fragment of IgG was prepared by pepsin digestion of purified IgG followed by Sephadex G-200 gel filtration (Nisonoff, Markus and Wissler, 1961). Rabbit anti-sheep E antibodies of the IgM class were prepared by DEAE–Sephadex A-50 chromatography and Sephadex G-200 gel filtration from sera obtained early in the course of immunization (Metzger, 1970). The protein concentrations of the isolated immunoglobulins and $F(ab')_2$ fragment were established according to their extinction coefficients at $OD_{280\ nm}$ (Miller and Metzger, 1965). Protein concentrations of column fractions were determined by a Folin method (Lowry, Rosebrough, Farr and Randall, 1951). The haemolytic and haemagglutinating activities of the antibodies obtained were measured with standard techniques (Nelson *et al.*, 1966; Gorer and Mikulska, 1954). Human complement components C1, C4, C2 and C3 and the cellular intermediates $EAC\bar{1}$, $EAC\overline{14}$, and $EAC\overline{1423}$ were prepared according to published procedures (Gigli and Nelson, 1968; Ruddy, Klemperer, Rosen, Austen and Kumate, 1970).

Assays of components of the fibrinolytic and kinin-forming systems

Kallikrein activity was determined by incubating 0·05 ml of enzyme source with 0·2 ml of heat-inactivated plasma for 2 min at 37° and measuring the bradykinin generated by its ability to contract the atropinized antihistamine-treated guinea-pig ileum (Kaplan *et al.*, 1971). Plasminogen activator was assayed by its ability to convert plasminogen (20 μg/ml) to plasmin as assayed in fibrin radial diffusion plates (Hyland Division, Travenol Laboratories, Incorporated, Costa Mesa, California) (Kaplan and Austen, 1972). Prekallikrein and plasminogen proactivator activities were measured as above after activation of the precursor source with Hageman factor fragments (Kaplan, Goetzl and Austen, 1973). The shortening of the partial thromboplastin time of PTA-deficient plasma was used to assay PTA (Kaplan and Austen, 1970). A source of PTA (0·05 ml), 0·05 ml cephalin (6 μg/ml) and 0·05 ml PTA-deficient plasma were incubated at 37° for 2 min in plastic tubes. The tubes were tilted each minute and the end point defined as the time required for clot formation.

Phagocytosis by human peripheral leucocytes

Erythrophagocytosis was measured by a modification of a published technique (Gigli and Nelson, 1968). Human blood was collected from normal donors into citrate anticoagulant and 1 per cent dextran (Pharmacia Fine Chemicals, Piscataway, New Jersey) to sediment the erythrocytes (Goetzl and Austen, 1972). The leucocyte-rich supernatants were aspirated, pooled, and centrifuged at 100 *g* for 5 min. The pellet of mixed leucocytes was washed and centrifuged on Ficoll–Hypaque cushions to obtain neutrophils and mononuclear leucocytes (Böyum, 1968). The leucocytes were washed twice with Hanks's solution and resuspended in a standard

phagocytic buffer containing 2 parts of Hanks's solution and 1 part of 0·15 M glucose with 0·5 g/100 ml ovalbumin at a pH adjusted to 7·4 with 2 per cent $NaHCO_3$. Quadruplicate portions of 1 ml of human leucocyte suspension containing $3–4 \times 10^6$ neutrophils/ml were incubated with 1×10^8 sensitized erythrocytes (EA) in 0·2 ml and mixed with a slow rotating motion. After 20 min at 37°, two samples received 2·5 ml of 0·84 per cent NH_4Cl to lyse uningested EA and two received 2·5 ml of 0·15 M NaCl to enable correction for spontaneous release of haemoglobin. Simultaneously, duplicate samples of target EA cells were incubated with phagocytosis buffer alone. One sample received 2·5 ml of 0·84 per cent NH_4Cl to achieve an optical density representing complete lysis of the target cells, and the other received 2·5 ml of 0·15 M NaCl for measurement of their spontaneous lysis in the absence of leucocytes. Samples of leucocytes plus buffer were mixed with 2·5 ml of 0·84 per cent NH_4Cl in order to assess the quantity of haemoglobin released from the few human E contaminating each leucocyte preparation. The samples were centrifuged and the optical density of each reaction mixture was read at 414 nm. The percentage phagocytosis was calculated by dividing the corrected $OD_{414\ nm}$ of the 0·84 per cent NH_4Cl lysate of uningested erythrocytes in each phagocytosis mixture by the $OD_{414\ nm}$ of the lysate of the total available erythrocytes, subtracting this difference from 1 and multiplying by 100 (Gigli and Nelson, 1968). Phagocytosis enhancement was assessed by adding to the phagocytosis mixtures 0·1 ml of the test sample before incubation at 37°; enhancement was expressed as net percentage phagocytosis in the presence of an active factor after subtracting percentage phagocytosis for a control mixture incubated with 0·1 ml of buffer alone.

Human neutrophil chemotaxis

Chemotaxis of human neutrophils was quantitatively assayed by a previously described modification of the Boyden micropore filter technique utilizing 3 μm pore size micropore filters (Millipore Corporation, Bedford, Massachusetts) (Boyden, 1962; Goetzl and Austen, 1972). Neutrophils were obtained from mixed leucocytes of normal donors utilizing methods identical to those employed above in preparing cells for phagocytosis. The cells were washed twice with Hanks's solution and resuspended in Medium 199; the Medium 199 had been made 0·5 per cent in ovalbumin and adjusted to pH 7·4 by dropwise addition of 2 per cent $NaHCO_3$. For each experiment, the initial cell count was adjusted to $2·0 \pm 0·5 \times 10^6$ leucocytes/ml. Chemotactic agents were also diluted in Medium 199, 0·5 per cent ovalbumin, pH 7·4. The interaction of leucocytes and chemotactic factors was carried out at 37° in moist chambers for 2–2½ h so that the mean background neutrophil counts were 2–8 per high power field (hpf) and the stimulated counts were 20–60 per hpf. Each interaction was examined in duplicate chambers and the counts expressed as the mean of ten hpf, five from each of the duplicate filters.

RESULTS

Partial purification of phagocytosis-enhancing factor (PEF)

In preliminary experiments designed to assess the effect of the leucotactic factor plasma kallikrein on erythrophagocytosis by human leucocytes, 100 ml of human plasma was applied to a quaternary ammonium ethyl–Sephadex column equilibrated in 0·0035 M sodium phosphate buffer (pH 7·8). The effluent contained over 90 per cent of the kallikrein activity of the plasma and a portion representing 2 per cent of the effluent enhanced phagocytosis by 38 ± 12 per cent (mean $\pm$ 1 s.d.) in five experiments. Sequential chromatography of such an effluent or of whole plasma on DEAE–Sephadex, Sephadex G-150, and Sephadex G-100 permitted the definition of a distinct plasma phagocytosis-enhancing factor (PEF) unrelated to kallikrein. One hundred ml of plasma separated by centrifugation from normal human blood collected in EDTA and hexadimethrine as previously described (Kaplan *et al.*, 1971) was dialysed for 6 h at 4° against 0·0035 M sodium phosphate buffer (pH 8·0) with 0·0001 M EDTA, applied to a 5×40 cm DEAE (A50) Sephadex column equilibrated in the same buffer and eluted with a linear NaCl gradient to a final concentration of 0·3 M NaCl in EDTA–phosphate buffer. Pools from the column were concentrated to 10 ml, dialysed against 0·01 M Tris-buffered 0·14 N NaCl and assayed for phagocytosis-enhancing activity (Fig. 1). Prekallikrein and pre-PTA (not shown) which were over 90 per cent in their respective proenzyme forms appeared together in two discrete areas, a minor peak in the effluent and the major peak in the early portion of the eluate. Only one peak of plasminogen proactivator was found

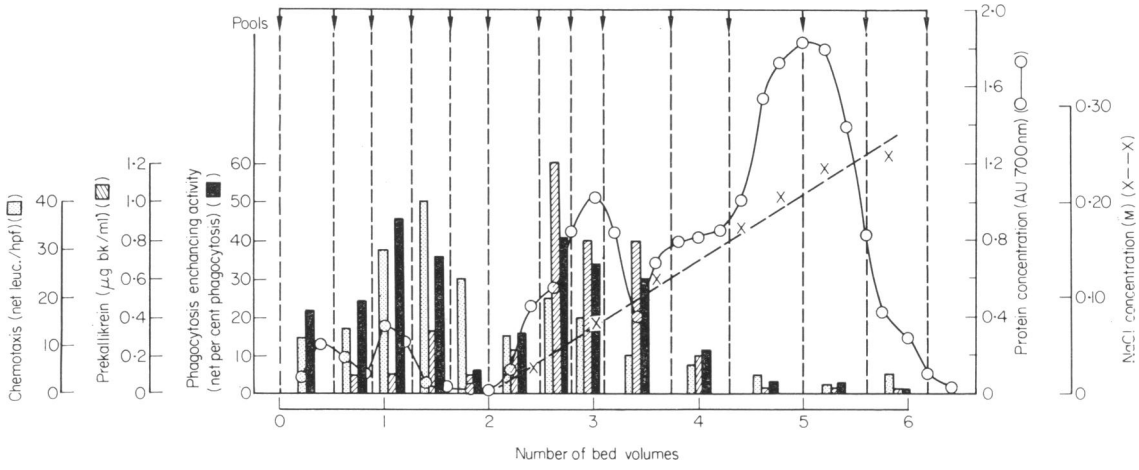

Figure 1. DEAE–Sephadex fractionation of human plasma PEF activity. The vertical dashed lines dividing the chromatogram designate the pools prepared for functional assays. The column bed volume was 790 ml and the volume of each fraction was 16 ml. Prekallikrein and chemotactic activities were assessed after Hageman factor activation of portions of each pool. bk = Bradykinin; leuc = leucocytes; hpf = high power field)

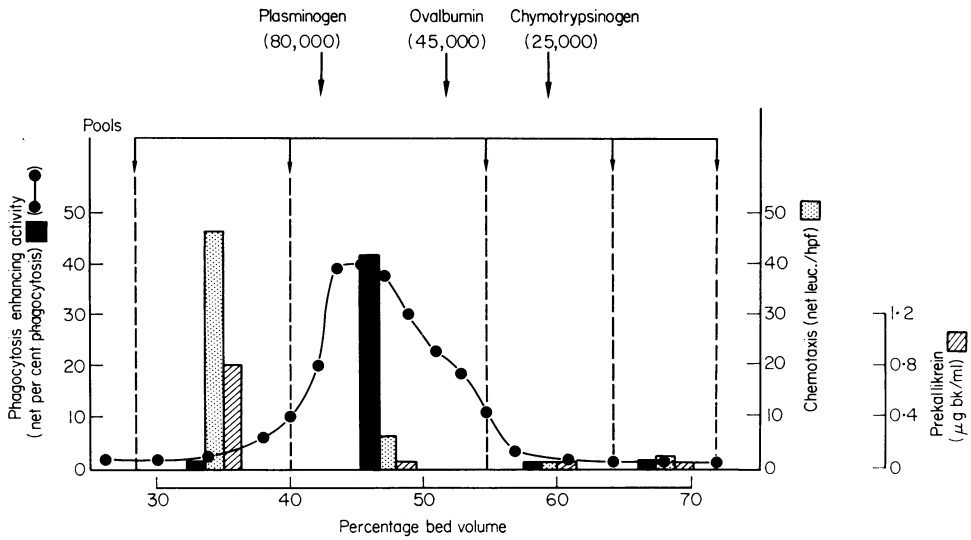

Figure 2. Sephadex G-100 filtration of PEF activity from the later DEAE–Sephadex peak. The 2·8 × 83 cm column had a bed volume of 525 ml and a fraction volume of 6·4 ml. Prekallikrein and chemotaxis were assessed after Hageman factor activation. bk = Bradykinin; leuc = leucocytes; hpf = high power field.

(not shown) superimposed on the second peak of prekallikrein and pre-PTA. Before the introduction of Hageman factor, chemotactic activity was restricted to the minor peak of prekallikrein and pre-PTA in the effluent, and incubation with Hageman factor both increased the effluent peak over three-fold and resulted in the appearance of chemotactic activity in the eluate peak of prekallikrein containing plasminogen proactivator (Fig. 1). The phagocytosis-enhancing activity of plasma appeared in two regions of the chromatogram which overlapped both peaks of prekallikrein and pre-PTA, but the enhancing

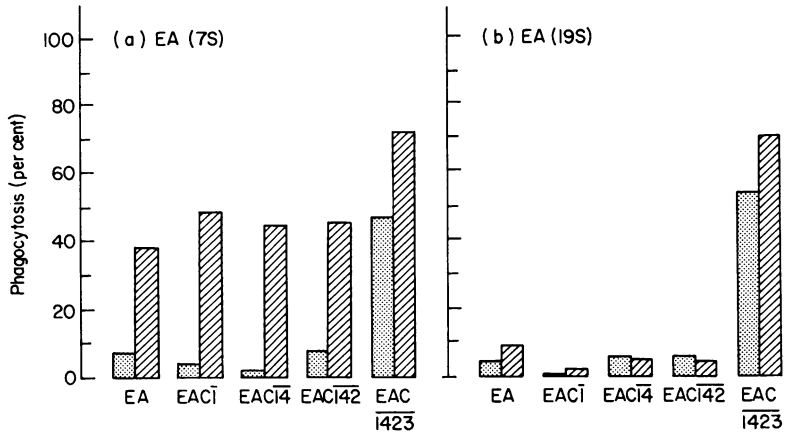

Figure 3. Target cell requirements for PEF action. Stippled columns, buffer; hatched columns, PEF.

activity was not influenced by preincubation with Hageman factor fragments. This pattern was reproduced in ten other chromatographic runs.

Filtration of either DEAE–Sephadex pool of PEF on Sephadex G-150 yielded the same single peak of phagocytosis-enhancing activity which was distinct from any chemotactic activity. PEF from Sephadex G-150 was filtered on Sephadex G-100 and yielded one peak of phagocytosis-enhancing activity which had an apparent molecular weight of approximately 70,000 daltons and was free of pre-PTA, prekallikrein, and plasminogen proactivator (Fig. 2). PEF obtained by sequential purification on DEAE-Sephadex, Sephadex G-150, and Sephadex G-100 was utilized for subsequent studies.

Antibody and complement requirements for PEF action

Sensitized sheep erythrocyte intermediates were prepared with the 7S or 19S fraction of purified rabbit anti-sheep E antisera at optimal antibody doses for haemolysis and utilizing the following number of effective haemolytic complement molecules per cell: C1, 200; C4, 100; C2, 200; and C3, 30 (Nelson *et al.*, 1966; Gigli and Nelson, 1968). Phagocytosis by human mixed peripheral leucocytes of any of the four EA (7S) intermediates lacking C3 was in a range of $5{\cdot}0 \pm 2{\cdot}5$ per cent which increased to a maximum of $43{\cdot}5 \pm 5{\cdot}0$ per cent after the introduction of PEF (Fig. 3a). In contrast, phagocytosis of the EA (19S) intermediates lacking C3 exhibited a range of $5{\cdot}5 \pm 2{\cdot}5$ per cent with no apparent effect of PEF at concentrations ranging up to five-fold higher than that yielding optimal phagocytosis of the EA (7S) intermediates (Fig. 3b). The addition of C3 to the cellular intermediates raised baseline phagocytosis of EA (19S) $\overline{C1423}$ as well as EA (7S) $\overline{C1423}$ and permitted additional enhancement by PEF (Fig. 3a and b). It therefore appears that the enhancing effect of PEF is dependent upon the presence of either 7S antibody or C3b bound to the target cell.

The influence of the sensitizing dose of 7S antibody on the levels of phagocytosis and complement-induced haemolysis was analysed over a range of 4·1–66·7 ng of protein per 5×10^8 erythrocytes (Fig. 4). In the absence of PEF, maximal phagocytosis of 25–30 per cent was seen at an antibody dose of 66·7 ng (1/50) which fell at a plateau antibody dose in the haemolytic assay. Phagocytosis at approximately the same 25–30 per cent level in the presence of PEF occurred with one-fourth to one-third the amount of antibody required without PEF, an antibody dose that also resulted in nearly complete complement-induced haemolysis. A 1/400 dilution of 7S antibody, which alone failed to promote phagocytosis, allowed 10–15 per cent phagocytosis in the presence of PEF and led to 70–75 per cent complement-induced haemolysis.

Batches of 5×10^8 E were sensitized in 1 ml with varying dilutions of either $F(ab')_2$ or the molar equivalent of undigested (7S) IgG antibody from which the $F(ab')_2$ had been prepared. Portions of 1×10^8 EA 7S or EA 7S-$F(ab')_2$ were incubated with human leucocyctes in the presence or absence of PEF (Fig. 5). EA (7S-$F(ab')_2$) were not phagocytosed nor was their phagocytosis promoted by PEF, while erythrocytes sensitized with intact 7S antibody

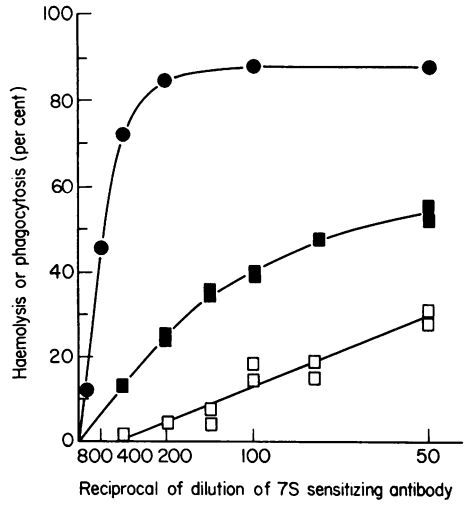

Figure 4. Influence of sensitizing dose of 7S fraction of anti-sheep erythrocyte antibody on erythrophagocytosis (□), PEF enhancement of erythrophagocytosis (■), and haemolysis (●). The undiluted preparation of antibody contained 3·28 μg protein/ml.

showed promotion of phagocytosis by PEF which was related to the concentration of sensitizing 7S antibody.

Characteristics of PEF enhancement of phagocytosis

Dose–response of PEF action

Portions of 1×10^8 EA (7S) were incubated with mixed leucocytes and PEF at dilutions ranging from 1/300 to 1/1000. Phagocytosis rose from 32 to 88 per cent with a steep linear dose–response relationship; phagocytosis for duplicate specimens at each dose of PEF was within ±5 per cent of the mean value (Fig. 6).

Time course of PEF-promoted phagocytosis

PEF at a final dilution of 1/350 or buffer alone was preincubated with 7×10^8 EA (7S) for 5 min at 37°; a suspension of 25×10^6 leucocytes was then added which maintained the standard ratio of reagents. The mixtures were incubated in a shaker at 37° and duplicate portions consisting of one-seventh of the initial suspension were removed for assay at intervals from 3–20 min (Fig. 7). The greater than four-fold increase in phagocytosis at 20 min was apparent at earlier time points beginning within 3 min. In the same experiment, a 1 min preincubation of EA (7S) with PEF gave comparable four-fold enhancement of phagocytosis by 20 min while less enhancement was seen at 3 and 6 min (Fig. 7). Employing a lower concentration of PEF also revealed a time-dependence of its action on EA (7S) within the initial 5 min. The period of preincubation of EA (7S) and PEF, at a final dilution of 1/1000, was varied from 0 to 5 min before a 9 min incubation with leucocytes. Phagocytosis increased from 7·5 per cent in the absence of PEF to 12·5 per cent without preincubation to a plateau value of 27·5 per cent with preincubation times of between 1 and 5 min.

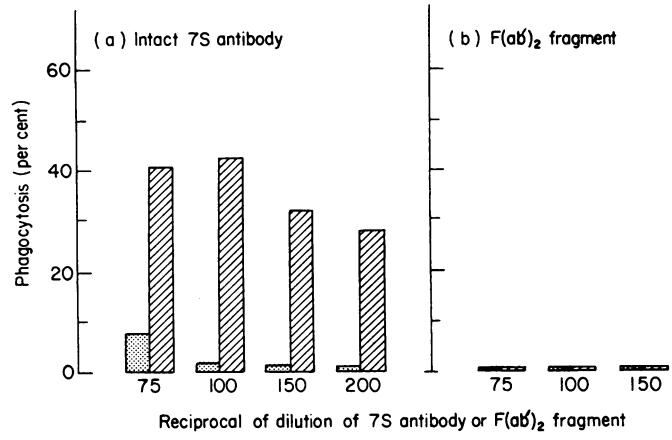

Figure 5. Dependence of PEF enhancement of erythrophagocytosis on the Fc fragment of the sensitizing (7S) antibody. The stock antibody preparation was identical to that used in Fig 4. Stippled columns, buffer; hatched columns, PEF.

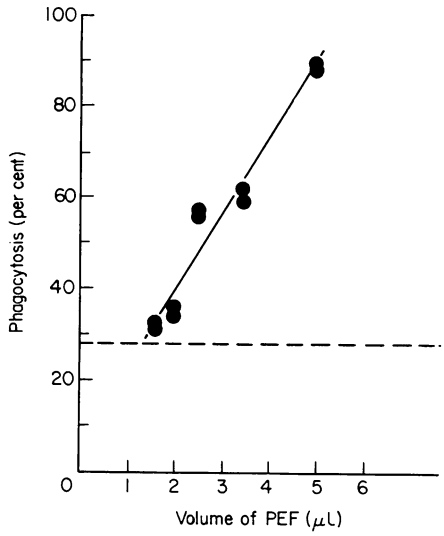

Figure 6. Dose–response relationship for PEF enhancement of phagocytosis of EA (7S). (●) EA (7S)+PEF. (– – –) EA (7S)+buffer.

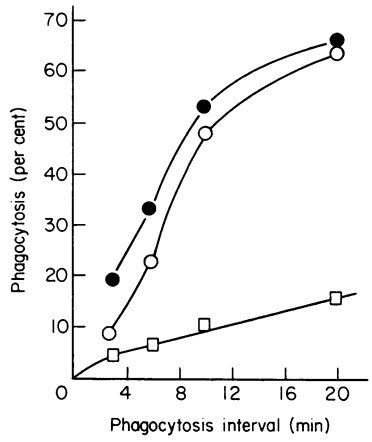

Figure 7. Time course of PEF enhancement of phagocytosis of EA (7S). Times indicated in the key represent the preincubation period of PEF with EA (7S). (●) EA (7S)+PEF, 5 min; (○) EA (7S)+PEF, 1 min; (□) EA (7S).

Mechanism of PEF effect

Persistence of PEF action on target cells

EA (7S) and PMN leucocytes were separately preincubated with PEF, washed, and divided into two portions, of which one received another dose of PEF before the phagocytosis interval (Table 1). Washing the PEF-treated EA (7S) only partially reversed the promotion of phagocytosis (sample 3) seen with unwashed PEF-treated EA (7S) (sample 2). Replicates of EA (7S) identically treated with PEF, washed and then resuspended in fresh PEF manifested a striking increment in phagocytosis (sample 4) to a level exceeding that of EA (7S) only exposed to a single dose of PEF (sample 2). Although separated preincubation of both suspensions of EA (7S) and leucocytes with PEF followed by washing (sample 7) gave slightly higher levels of phagocytosis than similar treatment of only EA (7S) (sample 3), preincubation of leucocytes alone with PEF followed by washing gave no appreciable enhancement of phagocytosis (sample 5) over baseline levels (sample 1). Addition of a second dose of PEF to phagocytosis mixtures with PEF-treated and washed leucocytes

Table 1. Reversibility of PEF action

Sample	Cells preincubated with PEF*	PEF in final phagocytosis mixture†	Phagocytosis (per cent)‡
1	0	0	5·6, 1·9
2	0	+	42·2, 45·9
3	EA (7S)	0	26·9, 20·3
4	EA (7S)	+	59·2, 62·7
5	PMN	0	7·7, 9·4
6	PMN	+	45·1, 40·3
7	EA (7S), PMN	0	32·8, 31·5
8	EA (7S), PMN	+	61·4, 60·8

* Leucocytes and/or target cells preincubated separately for 20 min at 37° with PEF, diluted 1/60, and washed three times in Hanks's solution; 0 indicates that both cell types were preincubated in buffer alone.

† + = PEF at 1/60 dilution added to final combined suspensions EA (7S) and PMN.

‡ Values for duplicate tubes are presented.

Table 2. Effect of PEF on phagocytosis of EA (7S) by neutrophils and monocytes

	Phagocytosis (per cent)	
	Neutrophils	Monocytes
Buffer	3·9	2·9
PEF 1:1	45·5	32·5
PEF 1:3	29·8	17·4

(sample 6) allowed augmentation of the ingestion of target cells which was even greater when both leucocytes and EA (7S) had been preincubated with PEF (sample 8).

PEF promotion of phagocytosis by polymorphonuclear and mononuclear leucocytes

Human leucocytes from a single donor were fractionated into mononuclear leucocytes and polymorphonuclear leucocytes, washed and suspended in phagocytosis buffer at a concentration of 4×10^6 cells/ml. Quadruplicate 1 ml portions were incubated with 10^8 EA (7S) or E and either a dose of PEF or buffer. PEF enhanced the ingestion of EA by both cell types with a proportionally greater promotion of polymorphonuclear leucocyte than mononuclear leucocyte phagocytosis (Table 2).

Stability of PEF activity

PEF purified by the standard three-column procedure was analysed for its susceptibility to specific treatments (Table 3). PEF activity resisted acid pH and exposure to DFP. Phosphoryl choline which blocks the activity of C-reactive protein had no influence on the function of PEF. PEF was partially inactivated by heating, and largely destroyed by digestion with insoluble trypsin (Miles Laboratories Inc., Kankakee, Illinois).

Table 3. Inactivation of PEF enhancement of human leucocyte phagocytosis of EA (7S)

Conditions of treatment	Phagocytosis (per cent)*
Buffer	13
PEF untreated	63
PEF heated 60°, 30 min	38
PEF + insoluble trypsin, 1 h, 37°	25†
PEF dialysed at pH 3·5, 4°	59‡
PEF + DFP 10^{-4} M, 1 h, 37°	65
PEF + 10 μg phosphoryl choline	56

* Mean value for duplicate samples; PEF dilution was 1/350.

† 0·2 mg of trypsin in 0·05 M Tris (pH 8·0) made 0·01 M in $CaCl_2$.

‡ 0·1 M glycine–HCl buffer, pH 3·5.

DISCUSSION

Human plasma contains an activity termed the phagocytosis-enhancing factor (PEF) which is capable of enhancing the ingestion by human peripheral blood polymorphonuclear and mononuclear leucocytes of sheep erythrocytes sensitized with the 7S fraction of rabbit anti-sheep erythrocyte antiserum, EA (7S), or prepared with the first four components of complement, $EAC\overline{1423}$ (Fig. 3, Table 2). Partial purification of PEF by chromatography on DEAE A50-Sephadex (Fig. 1) revealed two discrete regions of PEF activity, and filtration of pools from each region on Sephadex G-150 and G-100 (Fig. 2) permitted the identification of a single peak of PEF which had an apparent molecular weight of approximately 70,000, was completely separated from the Hageman factor-dependent plasma enzymes, prekallikrein, plasminogen proactivator and pre-PTA, and was devoid of Hageman factor-activatable chemotactic activity (Fig. 2).

The availability of 7S and 19S fractions of rabbit anti-sheep erythrocyte antiserum and the ability to generate erythrocyte-bound C3b by the introduction of the first four components of complement permitted an analysis of target cell requirements for the action of partially purified PEF. Enhancement of human leucocyte phagocytosis by PEF was evident for EA (7S) but not EA (19S) in the absence of complement, whereas there was comparable enhancement of phagocytosis by PEF for both 7S and 19S $EAC\overline{1423}$ (Fig. 3). The specific recognition by PEF of target cell-bound C3b or intact 7S antibody is corroborated by the failure of PEF to enhance leucocyte phagocytosis of either EA (19S) $C\overline{142}$ (Fig. 3) or erythrocytes sensitized with a molar equivalent of the $F(ab')_2$ fragment of 7S anti-sheep erythrocyte antibodies (Fig. 5). Leucocyte phagocytosis of EA (7S-$F(ab')_2$) was uninfluenced by PEF in the same dose-range where the phagocytosis of EA (7S) by the same leucocyte preparation was enhanced more than six-fold by PEF. The degree of

sensitization of erythrocytes which first allows an appreciation of PEF enhancement of leucocyte phagocytosis of Ea (7S) occurs at an antibody dose which provides for 70–75 per cent of maximal complement-induced erythrocyte lysis (Fig. 4). At 7S antibody concentrations leading to maximal lysis by complement, PEF enhancement of EA (7S) phagocytosis was also approaching a plateau which resulted in an approximately three- to eight-fold increase in phagocytosis of EA (7S). Thus PEF enhancement of leucocyte phagocytosis of EA (7S) and complement-induced lysis of similarly sensitized erythrocytes share both a comparable antibody dose–response (Fig. 4) and dependence on the integrity of the Fc fragment (Fig. 5), whereas erythrocytes sensitized by 19S antibodies will only undergo complement-induced lysis (Humphrey and Dourmashkin, 1965).

The steep linear dose–response relationship between the concentration of PEF and the extent of enhancement of phagocytosis (Fig. 6) permits quantitative conclusions regarding not only the effects of a variety of treatments on PEF activity but also the interactions of PEF with both the phagocytic and target cells. A partial characterization of the stability of PEF revealed it to be a protein which was largely destroyed by digestion with trypsin and partially inactivated by heating for 30 min at 60° (Table 3). No interaction between PEF and human leucocytes could be demonstrated by assessing the influence of preincubation time or cell washing on enhancement of phagocytic activity by PEF (Fig. 7, Table 1). In contrast, PEF did appear to bind to sensitized erythrocytes, and washing portions of EA (7S) preincubated with PEF only partially reversed their enhanced phagocytosis by leucocytes (Table 1). The interaction of EA (7S) and PEF occurred rapidly since a 1 min preincubation interval of EA (7S) and a low concentration of PEF resulted in maximal enhancement of phagocytosis and the omission of preincubation still permitted the manifestation of 25–35 per cent of maximal PEF-enhancing effect.

Two other plasma proteins unrelated to the complement system, C-reactive protein and α2-HS glycoprotein, have previously been shown to enhance phagocytosis of bacteria by human neutrophils through an apparent action on the target particles (Ganrot and Kindmark, 1969; van Oss *et al.*, 1974). C-reactive protein is present in functionally significant quantities only in acute phase sera and has a molecular weight approximately twice that of PEF (Gotschlich and Edelman, 1965). In addition, the ability of C-reactive protein to activate complement or to enhance phagocytosis is blocked by phosphorylcholine which has no effect on PEF function (Table 3). The molecular weight of PEF differs from that of α2-HS glycoprotein and, further, PEF is heterogeneous with a probable gamma-globulin component revealed by DEAE–Sephadex chromatography (Winzler and Bocci, 1972) (Fig. 1). Definitive identification of the nature of PEF and its relationship to other phagocytosis-enhancing factors of plasma must await further purification and development of specific antisera to PEF.

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