The Physiological Role of the Lymphoid System. VI. The Stimulatory Effect of Leucophilic γ-Globulin (Leucokinin) on the Phagocytic Activity of Human Polymorphonuclear Leucocyte*

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ABSTRACT: Human leucocytes, like human erythrocytes, bind specific γ-globulin (γG) in low ionic strength isotonic medium. Leucocyte-bound γ-globulin is readily demonstrable *in situ*. It is of the γG class and has the chromatographic properties on cellulose phosphate columns that correspond to serum γ-globulin fraction IV. A significant portion but not all of the latter binds to leucocytes. This leucophilic portion has leucokinin activity as manifested by its stimulatory effect on the phagocytic function of the autologous polymorphonuclear leucocyte. In the absence of this fraction only minimal phagocytosis is obtained.

It was shown recently that a specific leucophilic γ-globulin is bound *in situ* to dog white blood cells and as such stimulates their phagocytic ability to a considerable extent (Fidalgo and Najjar, 1967). This fraction is identical in its physicochemical properties with fraction IV of autologous serum γ-globulin as obtained by chromatography on cellulose phosphate columns (Thomaidis *et al.*, 1967). *In vitro*[1] binding of this fraction occurs in low ionic strength isotonic sucrose medium and can be eluted with 0.15 M NaCl. None of the other fractions (I–III) showed comparably significant binding nor any augmentation of the phagocytic rate. The stimulatory effect of leucophilic γ-globulin on phagocytosis of *Staphylococcus aureus* accounts for almost all that exhibited by autologous serum rendered devoid of any complement or specific antibody (opsonin) activity.

Like the erythrophilic components of autologous γ-globulin leucophilic γ-globulin fraction IV is also formed in part by the spleen. Its level in serum was significantly reduced in 9 out of 15 dogs 6–8 weeks after removal of the spleen (Fidalgo *et al.*, 1967a,b). It has since been found in this laboratory that the serum of such animals or fraction IV thereof lose their former stimulatory effect on the phagocytic activity of the autologous leucocyte. However, samples of the same cell preparation were stimulated to the fullest extent by the serum or its fraction IV obtained from the same dog prior to the removal of the spleen. This will be the subject of a separate communication (B. V. Fidalgo and V. A. Najjar, manuscript in preparation).

Thus far, studies on the binding of leucophilic γ-globulin to white blood cells and its effect on their functional activity have been reported in the dog. The purpose of this communication is to report similar studies in man. The findings run parallel to those obtained in the dog and show that the human leucocyte binds leucophilic γ-globulin fraction IV *in situ* and *in vitro*. The binding is ionic in character and is readily demonstrable in media of low ionic strength. Conversely, the bound γ-globulin can be dissociated at higher ionic strength. As will be shown later, only a portion of fraction IV binds to the leucocyte and of this only a part is actively concerned with phagocytosis. In the interest of simplicity and in order to avoid undue cumbersome expressions, we shall refer to this phagocytosis active factor as leucokinin inasmuch as the major element in phagocytosis is the kinetic activity of the engulfing process and pseudopod formation.

Materials and Methods

Fresh human blood was obtained from adult volunteers of both sexes with heparin as anticoagulant (25 mg/100 ml). The γ-globulin used in all binding and phagocytosis experiments was autologous with respect to the leucocytes. The various fractions were isolated with column chromatography (Thomaidis *et al.*, 1967) on cellulose phosphate (CP)[2] (Selectacel, cellulose phosphate, Carl Scheicher & Schuell Co., Keene, N. H.). Columns of 1.5 × 12 cm were used for samples containing 20–25 mg and 0.5 × 12 for samples 4–6

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[1] *In vitro* refers to the binding of γ-globulin to isolated washed white blood cells. *In situ* refers to the finding of bound γ-globulin on white blood cells in freshly drawn blood with no prior manipulation of the white blood cells.

[2] Abbreviations used: CP, cellulose phosphate; γA, γ-globulin A; γM, γ-globulin M; γG, γ-globulin G.

mg of γ-globulin. Immunoelectrophoresis and immunodiffusion (Crowle, 1961) studies were performed with goat antihuman serum and horse antihuman γ-globulin (Orthodiagnostic, Raritan, N. J.). Protein was measured by optical density at 280 mμ using an extinction of 1.12 or by the Folin–Lowry method (Lowry *et al.*, 1951). The preparation of γ-globulin from serum or solutions of its components was done by ammonium sulfate precipitation at 0.33 saturation, then reprecipitation at 0.6 saturation. In all experiments relating to binding of γ-globulin to the cell, the sucrose phosphate solution used for washing was composed of sucrose (0.27 M) in sodium phosphate buffer (5×10^{-3} M, pH 7.4). The NaCl solution was 0.15 M. The media used for phagocytosis were either Hank's medium (McKinney *et al.*, 1953), usually employed for this type of experiment and containing in addition $MgCl_2$ (5×10^{-3} M) at pH 7.4, or our own sucrose medium composed of sucrose (0.27 M), glucose (5.5×10^{-2} M), KCl (1.3×10^{-2} M), $MgCl_2$ (5×10^{-3} M), $CaCl_2$ (5×10^{-4} M), and sodium phosphate buffer (5×10^{-3} M, pH 7.4). In each case, 100 mg of heparin/l. of medium was included. All media, solutions, and glassware were sterile.

The Phagocytic Reaction. In all phagocytosis experiments, leucocytes were isolated from the buffy coat. In some, the cells were washed three times with three volumes each of the sucrose medium, separating the leucocyte layer in the process. In this case they remained coated with γ-globulin fraction IV and will be referred to as *coated cells.* Alternatively, the cells were washed with Hank's medium which is considerably higher in ionic strength. Consequently, the leucophilic γ-globulin was eluted and washed off. These cells will be referred to as *naked cells.* Washing was performed three times each with three volumes of the medium and the cells in all cases were sedimented at 250*g*. After each washing, the leucocyte layer was separated from the red cells. However, after the third washing the leucocyte layer was about 0.4 of the combined cell volume.

The phagocytic reaction was carried out at 37° in siliconed roller tubes with continuous shaking at 8 cycles/min. Coagulase-positive *S. aureus* from an 18-hr culture was added at zero time in a ratio of 1.5 bacteria/polymorphonuclear leucocyte. All protein components added to the reaction mixture were previously dialyzed in the particular medium used in the experiment. Furthermore, all sera and their components used in this study, other than the cellular elements, were heated at 56° for 30 min to destroy complement activity. Because of the possible presence of so-called *opsonizing* antibody, all samples were absorbed at 37° for 30 min at least with 1.0 mg dry weight of staphylococci/mg of γ-globulin. Further precautions against possible unequal sensitization of the *Staphylococcus* organism by the various samples or fractions, the bacteria were incubated at 30° for 30 min in 1 ml of the particular serum from which the components under study were isolated. The reaction mixture, unless otherwise indicated, was composed of 0.3 ml of leucocytes suspended in the desired medium containing 2–2.5 $\times 10^4$ cells/cmm mixed with 0.05 ml of the serum component or fraction and allowed to interact for 10 min. *Staphylococcus* culture (0.05 ml) was then added to start the reaction.

The rate of the phagocytic reaction was measured by removing a loopful of the mixture at zero time and at various times thereafter for staining and microscopic examination under high power. For proper spreading of the cells on the slide, a loopful of bovine serum albumin, 60 mg/ml was mixed with the sample immediately before smearing. In all cases reported in this study, 200 polymorphonuclear leucocytes were observed. The extent of phagocytosis was recorded as the number of cells containing 1 or more organisms/100 cells. Zero-time samples were removed 15–20 sec after the addition and thorough mixing of the bacteria. In this sample leucocytes, seemingly containing bacteria, were considered to result from random adherence of bacteria to the cell surface. This represented 3–9% of the cells. This varied with the individual serum under study.

Results

Leucocyte-Bound γ-Globulin. Human leucocytes isolated in low ionic strength isotonic sucrose medium retain bound γ-globulin which readily dissociates in higher ionic strength isotonic NaCl or Hank's medium. Consequently, the associative forces are primarily ionic in character. In general therefore, the characteristics of binding are similar to those observed with human and dog erythrocytes as well as dog leucocytes. The leucophilic γ-globulin displays chromatographic characteristics of serum γ-globulin fraction IV. Purified leucocytes were prepared *in vitro* by separating the buffy coat from 500 ml of blood. These were taken up in 0.27 M sucrose buffered with sodium phosphate (5×10^{-3} M, pH 7.4) and resedimented four to five times for further separation of the contaminating erythrocytes. In some experiments the sucrose-suspended cells were purified with dextran (Skoog and Beck, 1956). In either case, some erythrocyte contamination remained, about 10–15% by count. These *coated* leucocytes were then eluted two times each with three volumes of 0.15 N NaCl. The protein in the combined eluates was concentrated either by surface evaporation in narrow dialysis bags in the cold room or precipitated in 0.6 saturation of ammonium sulfate by adding the solid salt. The saline-treated *naked* cells were then used for binding studies. In this case, they were washed in the isotonic sucrose phosphate solution and whole autologous γ-globulin was added and mixed at 0–4°. After 10 min, the cells were centrifuged and the supernatant was also concentrated either by evaporation or by ammonium sulfate precipitation. The sedimented cells with bound γ-globulin were washed with the sucrose phosphate solution, eluted with NaCl, and the protein of the eluate was concentrated as before. All concentrated protein samples were dialyzed against sodium acetate buffer (0.05 M, pH 4.8) at 4° for 4 hr

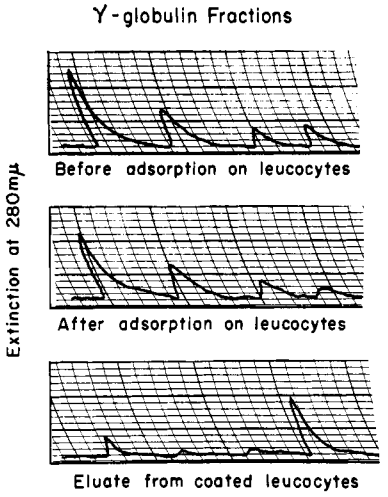


FIGURE 1: Shows that naked leucocytes bind mainly γ-globulin IV from serum γ-globulin causing a reduction in the fraction. Serum γ-globulin was first dialyzed against sucrose solution. Five milliliters containing 15 mg was then mixed with 2 ml of packed *naked* leucocytes and incubated for 10 min. The cells were then sedimented and the supernatant was dialyzed against sodium acetate buffer (0.05 M, pH 4.8). The leucocytes were then washed three times each with 6 ml of the sucrose solution and finally eluted with 6 ml of 0.15 M NaCl two times. The combined eluates were concentrated by evaporation and precipitated in ammonium sulfate at 0.6 saturation. This was followed by dialysis against the acetate buffer. Aliquots containing 4 mg of γ-globulin derived from the sample before adsorption (top graph), after adsorption (middle graph), and that derived from the eluate of the leucocytes (bottom graph) were chromatographed in 12 × 0.5 cm CP column at room temperature. All other manipulations were performed at 0–4°. For details, see text.

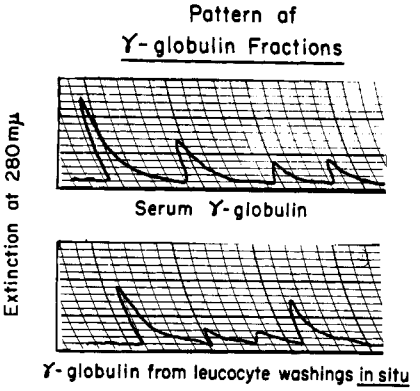


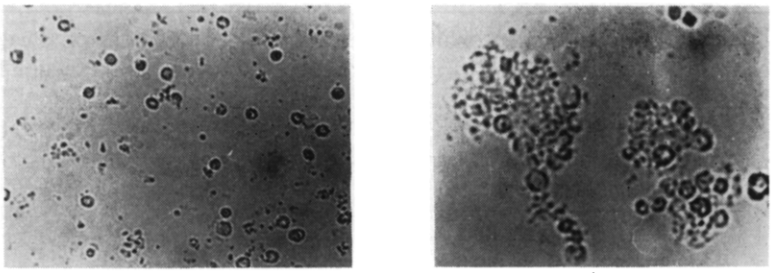
FIGURE 2: Shows the presence of leucophilic γ-globulin bound to leucocytes *in situ*. The pattern of the eluate (lower graph) from saline-washed leucocytes shows augmentation of fraction IV as compared with the pattern of whole γ-globulin (upper graph). Heparinized blood (500 ml) was centrifuged at 1000*g* for 5 min. The plasma and buffy coat were removed separately. The leucocyte layer (3.6 ml) was resuspended in 15 ml of plasma and centrifuged. Again the leucocyte layer was separated from the contaminating red cells. This process was repeated five more times. The packed cells (1.8 ml) along with the contaminating plasma were then washed twice each with 4 ml of saline. The pooled washings were then precipitated with solid ammonium sulfate at 0.33 saturation and the γ-globulin recovered was dialyzed against 0.05 M acetate buffer (pH 4.8) and chromatographed. For details, see text.

and chromatographed on CP columns.

Figure 1 shows the fractionation pattern of γ-globulin before adsorption on naked cells and illustrates the usual proportions of the four fractions. After adsorption on the naked cells, only fraction IV showed significant reduction. It should be pointed out here that only a portion of fraction IV binds to leucocytes. Exhaustive adsorption with naked leucocytes fails to reduce the level of that fraction beyond 40–50% of its original level. The protein eluted from *coated* leucocytes, either from the original sucrose-washed cell isolate of whole blood or from the binding experiment with whole γ-globulin, showed identical patterns with predominance of fraction IV and minor components representing the other three fractions. The pattern of the eluted γ-globulin is shown in the bottom of the figure. The amount of γ-globulin bound is about 2.0 mg/ml of packed leucocytes of which about 1.8 mg is fraction IV.

It can be further shown that leucophilic γ-globulin is bound *in situ* to the white blood cell in much the same relative amounts as that obtained *in vitro*. Inasmuch as leucocytes normally survive and function in plasma, this finding lends considerable credence to the concept that leucophilic γ-globulin is intimately concerned with the phagocytic activity of the polymorphonuclear leucocyte in the circulating blood (Fidalgo and Najjar, 1967).

The experiment was carried out as follows. The buffy coat from 500 ml of freshly drawn blood was separated from the sedimented cells and resuspended in its own clear plasma. This was again centrifuged and the leucocyte layer was separated from the contaminating red cells. This was repeated several times (four to six) until minimal contamination with erythrocytes was obtained, 10–15% by count. At this point, the supernatant plasma was removed. The remaining sedimented cells were washed twice with three volumes of 0.15 N NaCl. The washings were concentrated in a dialysis bag and the protein was precipitated at 0.33 saturation of ammonium sulfate. This was followed by dialysis against acetate buffer (0.05 M, pH 4.8) for 6 hr. The precipitated fibrin and denatured protein were eliminated by centrifugation and the sample was finally chromatographed. Under these circumstances, it would be expected that the washings with the isotonic salt would contain all the usual four γ-globulin fractions of the contaminating plasma in their relative



a b

FIGURE 3: Shows the binding of leucophilic γ-globulin to blood leucocytes. Rabbit antihuman γG-globulin serum was exhaustively absorbed with saline-washed blood cells until all nonspecific hemaglutinins were removed. *Naked* leucocytes as well as *coated* leucocytes were separately incubated at room temperature with the antiserum. After 2 hr, the tubes were gently centrifuged and a loopful of the sedimented cells was examined under the microscope. *Naked* cells (a) were not agglutinated whereas *coated* cells (b) show marked agglutination.

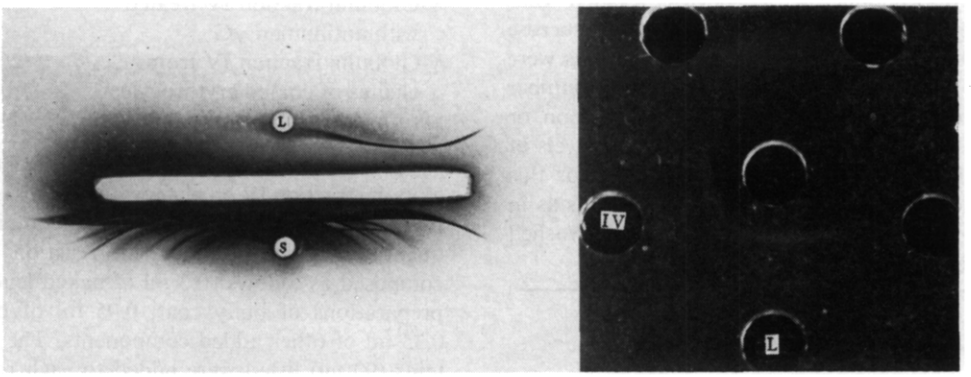


FIGURE 4: Shows that the leucophilic γ-globulin eluted from coated leucocytes is of the γG class and has the characteristics of identity with fraction IV obtained from serum γ-globulin. Leucophilic γ-globulin was concentrated and dialyzed against saline. Fraction IV was prepared as usual. Each (100 μg) was used for immunoelectrophoresis and gel diffusion studies. Immunoelectrophoresis, shown on the left, was performed in 1% agar. Horse antihuman serum was placed in the trough. Leucophilic γ-globulin (L) and normal serum (S) were placed in the wells. Microdiffusion analysis was carried out at 37° for 30 min followed by 48 hr at 4°. Fraction IV (IV) and leucophilic γ-globulin (L) are shown on the right.

proportions. In addition, it would also contain that portion of γ-globulin that is bound onto the leucocytes. The latter would be reflected in the relative increase in the corresponding fractions. It is clear from Figure 2 that fraction IV is the fraction that is primarily augmented. This indicates that the leucocytes, in their native plasma, possess a γ-globulin coat which is predominantly of fraction IV. This confirms and validates the parallel finding of bound fraction IV observed *in vitro*. It is reasonable to conclude, therefore, that these cells exist and function in the circulating blood with bound γ-globulin forming an intimate part of the outer structure and directly relating to the phagocytic activity of the cell (Fidalgo and Najjar, 1967). The existence of bound γ-globulin onto the leucocytes in the face of the normal physiological ionic strength in the serum is due both to the strong affinity to the leucocyte membrane and the considerable concentration of fraction IV in the serum, about 250 mg/100 ml of serum. This contrasts with a value of about 2.0 mg of leucocyte-bound fraction IV/ml of cells. Considering the small volume of leucocytes in the blood, the amount bound to leucocytes/100 ml of serum is correspondingly small.

Immunochemical identification of cell-bound leucophilic γ-globulin was demonstrated by agglutination. These experiments were performed by reacting leucocytes with horse or rabbit antihuman γ-globulin in the buffered sucrose solution. *Coated* leucocytes were readily agglutinated by the antiserum while salt-washed *naked* cells showed no agglutination (Figure 3).

Immunoelectrophoresis performed on salt eluates of coated leucocytes yielded one precipitin line characteristic of γG. No γM or γA was detectable (Figure 4). Agar diffusion studies on fraction IV isolated from these eluates and fraction IV of serum γ-globulin showed characteristics of identity (Figure 4). It can be assumed therefore that leucophilic fraction IV derives from the same fraction of serum γ-globulin and that the two are in a dynamic state of equilibrium.

TABLE I: Phagocytic Activity of Human Leucophilic γ-Globulin-Coated Polymorphonuclear Cells.[a]

Blood Type and Sex	Extent of Phagocytosis in Sucrose Medium after 30-min Incubn (%)	
	Naked Leucocytes[b]	Coated Leucocytes[c]
O^+ ♂	16	49
B^+ ♂	20	46
A^+ ♂	17	47
O^+ ♀	17	48
B^+ ♂	19	50
A^+ ♂	18	49

[a] Shows the comparative efficiency of naked and coated polymorphonuclear leucocytes. Coated cells were isolated by washing the buffy coat with the sucrose medium of low ionic strength. Naked leucocytes were isolated by washing with Hank's medium. The number of leucocytes at zero time showing organisms on or within the cells ranges from 3 to 9%. The levels of phagocytosis in the table were not corrected for this value. Values are to the nearest per cent (details as in the text). [b] Washed with Hank's medium. [c] Washed with sucrose medium.

TABLE II: Stimulation of Phagocytosis by Human Leucophilic γ-Globulin Fraction IV.[a]

Components Added	Extent of Phagocytosis in Sucrose Medium after 30-min Incubn (%)	
	Expt 1	Expt 2
Bovine serum albumin	24	29
Serum proteins (0.5–0.9) $(NH_4)_2SO_4$ saturation	23	26
γ-Globulin fraction I	19	26
γ-Globulin fraction II	21	24
γ-Globulin fraction III	25	27
γ-Globulin fraction IV	44	50
γ-Globulin fraction IV treated with antihuman γG	5	16
γ-Globulin fraction IV from eluates of coated erythrocytes	26	28
Serum (0.03 ml)	44	55

[a] Shows the stimulatory effects of leucophilic γ-globulin fraction IV on phagocytosis. This is comparable to that obtained by whole serum containing similar quantities of the fraction. The reaction mixture was composed as follows: 0.3 ml of naked leucocytes from preparations of buffy coat, 0.05 ml of bacteria, and 0.35 ml of other added components. The various proteins (90 μg) listed were added to each reaction tube. The volume of serum chosen (0.03 ml) contains 90 μg of γ-globulin fraction IV. The sample treated with antihuman γG was prepared as follows. Serum γ-globulin fraction IV (180 μg) in 0.3 ml of sucrose medium was mixed with 0.3 ml of rabbit antihuman γG representing an excess of antibody. The precipitate was centrifuged down and 0.3 ml of the supernatant was used for the experiment. As control, the rabbit antiserum was absorbed with 180 μg of γ-globulin fraction I. The extent of phagocytosis was similar to that obtained with bovine serum albumin. The final volume in each tube was brought up to 0.7 ml by the addition of sucrose medium. The ammonium sulfate cut at 0.5–0.9 saturation was dialyzed against the sucrose medium and 0.3 ml representing 90 μg of protein was used for the test. γ-Globulin fraction IV from coated erythrocytes was obtained by ammonium sulfate precipitation of the eluate at 0.6 saturation and dialysis in the acetate buffer. It was then separated by CP chromatography. This fraction (90 μg) was used in 0.3 ml. The rabbit antiserum used was first rendered hemagglutinin free by absorption with erythrocytes of the same leucocyte donor (see Figure 3).

The Leucokinin Effect of Fraction IV on Phagocytosis. Based on the principle that the presence of cell-bound γ-globulin represents an expression of the *physiological intent* of the organism, it was assumed that leucophilic γ-globulin must relate to the survival and function of the leucocyte. Experiments designed to study the survival of the cell *in vitro* in the presence and absence of leucophilic γ-globulin as well as *in vivo* in splenectomized dogs are in progress. However, it has been possible to show that fraction IV is necessary for the full expression of the phagocytic activity of dog polymorphonuclear leucocytes. In our sucrose medium, coated leucocytes approached maximal phagocytic rates of 42–55%. By contrast, naked cells showed only 1–7%. These cells, however, effected maximal phagocytosis upon the addition of fraction IV. No other fraction showed any specific stimulatory effect (Fidalgo and Najjar, 1967). Essentially similar results have been obtained with human leucocytes which are detailed herein.

Coated human leucocytes were prepared by washing the buffy coat three times each with three volumes of the sucrose medium. *Naked* leucocytes, on the other hand, were treated in the same manner except that Hank's medium was used instead. The high ionic strength of this medium strips off the γ-globulin coat. Aliquots of both kinds of cells obtained from various blood samples were tested for their ability to engulf *S. aureus* which had been exposed to the individual autologous serum (opsonized). The serum and all its components that were investigated were in turn heated for 30 min at 56° to destroy complement activity. They were also rendered free of any possible antibody that might react with the *Staphylococcus*

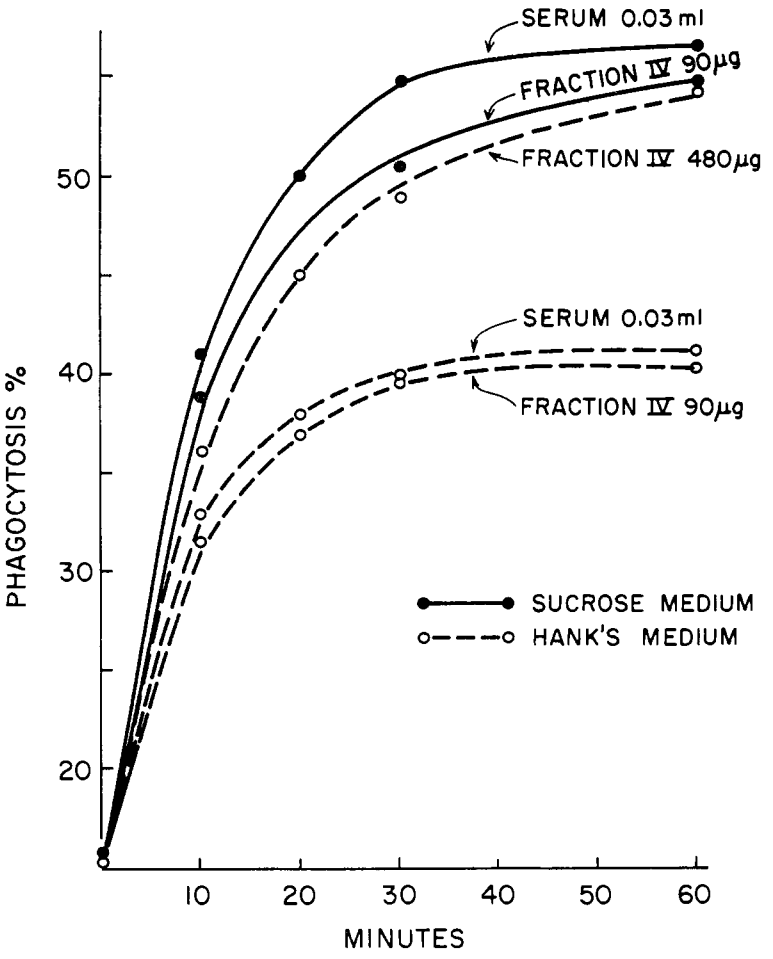


FIGURE 5: Shows the relative stimulatory effect on phagocytosis of isolated γ-globulin fraction IV as compared to serum containing the same amount of the fraction. It also emphasizes the advantages of the sucrose medium over Hank's medium. In order to attain a level of phagocytosis in Hank's medium comparable to that obtained in the sucrose medium, five times the amount of fraction IV was needed. The reaction was carried out as described in the text. The ordinate represents the number of polymorphonuclear leucocytes containing 1 or more staphylococci/100 cells.

by exhaustive absorption with the organism. Table I shows the results of this experiment. It is obvious that *naked* cells are much less efficient than *coated* cells. The extent of phagocytosis with the former 16–20% contrasts markedly with 46–50% with coated cells. These latter values are near maximal levels under the circumstances of the experiment, since at this level relatively few bacteria remain free in the medium.

The specificity of the *leucokinin* activity of fraction IV in the stimulation of phagocytosis is apparent when comparisons are made between the various fractions of serum including serum albumin and the various fractions of γ-globulin. A further quantitative comparison of the *leukokinin* effect of whole serum with fraction IV reveals that the latter is responsible for most of the activity present in serum. Table II shows that only fraction IV of all serum and γ-globulin components is alone responsible for this stimulatory activity. The effect of leucokinin is almost completely abolished by pretreatment with rabbit antihuman γ-globulin. Such values approached the nonspecific basal value obtained with serum albumin.

The Kinetics of Leucokinin-Induced Phagocytosis. The rate of phagocytosis with various concentrations of fraction IV and whole serum containing comparable quantities of the fraction was studied in low ionic strength sucrose medium as well as with Hank's higher ionic strength medium. The latter or some variation thereof is the classical medium ordinarily used in this type of study. The purpose of this study is to compare the relative efficiency of leucokinin in its native state in serum and as isolated by the procedure outlined before (Thomaidis *et al.*, 1967). In addition, the use of two media with markedly different ionic strengths, one favoring maximal binding of the γ-globulin and the other favoring dissociation, should allow an evaluation of the efficiency of phagocytosis relative to the amount of bound leucokinin. Figure 5 is illustrative of the results obtained. The rate of phagocytosis as influenced by whole serum is similar to that obtained

by an amount of fraction IV equivalent to that present in serum. This was the case in both Hank's medium and our sucrose medium. In all instances studied, the latter medium was more conducive to a higher rate than Hank's. This was not unexpected since it is reasonable to assume that leucokinin can only exert its effect by intimate contact with the cell rather than by its free presence in the solution. This is further emphasized by a series of experiments in which a variable amount of fraction IV was added to Hank's medium. In order to attain a rate of phagocytosis in Hank's medium comparable to that attained in the sucrose medium, five times the amount of fraction IV was needed. In other words, it requires a fivefold concentration of leucokinin in the high ionic strength medium to approach the same level of binding obtained in the sucrose medium.

Discussion

The results of this study on human leucocytes confirm in every detail those already reported on dog leucocytes (Fidalgo and Najjar, 1967). Of the four fractions of γ-globulin that can be separated on CP chromatography only fraction IV is bound to any significant extent to the leucocyte. The binding is not an experimental artifact resulting from the unphysiological manipulation procedures. Rather, it is evident *in situ* under conditions of minimum manipulation. It is therefore expected that the same applies *in vivo* in the circulating blood. Nor is the γ-globulin bound to the white cell a sterile anatomical or structural appendage to the cell membrane. On the contrary, it is an expression of what we have termed the *physiological intent*. It has a functional assignment—the augmentation of a complex process that finally results in the remarkable stimulation of the phagocytic activity of the polymorphonuclear blood cell. It does not appear to exert this effect by increasing the viability of the cell and therefore its phagocytic potential. Naked leucocytes incubated in Hank's medium or the sucrose medium for 3 hr do not show any diminution of the phagocytic response to leucokinin when compared to the stimulation observed with freshly isolated cells, coated or naked. This is not to say that the presence of a γ-globulin coat does not prolong viability irrespective of its leucokinin activity.

It is certain that only a portion of serum γ-globulin fraction IV is responsible for leucokinin activity. Fraction IV isolated from coated erythrocytes contains no leucokinin. Leucokinin, by all the criteria we have used, is of the γG class of γ-globulin, as shown by immunoelectrophoresis, immunodiffusion, immunofluorescence, and column chromatography. It is unlikely that the leucokinin effect is due to a minor contaminant of small molecular weight. Unless this is covalently bound to the γ-globulin molecule, it could not survive the series of procedures used in the fractionation: salt precipitation, chromatography, dialysis, etc. Furthermore, treatment of fraction IV with antihuman γ-globulin destroys its leucokinin activity.

Acknowledgment

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