ever, Roberts and Roberts (*7*) suggested the possible adjuvant role of an infectious agent in the etiology of osteosarcomas in reporting the simultaneous development of this neoplasm in three members (one brother and two sisters) of the same family. Additional clinical evidence consistent with a viral etiology of osteosarcoma are reports of the simultaneous occurrence of osteosarcomas at multiple sites in the same patient (*8*) and the predilection of this disease for young people.

All animal neoplasms induced by the same virus have been found to contain a common tumor antigen (*9*). It is possible that the presence of a common antigen in human osteosarcomas demonstrated by this study similarly indicates a common virus etiology. A viral etiology is also suggested by the recent isolation of a murine osteosarcoma virus that induces osteosarcomas in mice which have many similarities to the human disease (*10*).

This study suggests the close association of an infectious agent with human osteosarcomas. Whether this agent is an incidental passenger in these neoplasms or related to their etiology remains to be determined. However, the clinical evidence in favor of a viral etiology, when combined with the implications of this study, strongly suggests that attempts should be made to isolate a viral agent from this neoplasm.

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Human Monocytes: Distinct Receptor Sites for the Third Component of Complement and for Immunoglobulin G

Abstract. *Human monocytes contain two distinct receptor sites, one specific for the third component of complement (C'3), the other for immunoglobulin G (γG). The two receptors may function either independently or cooperatively in the induction of phagocytosis. Ingestion of erythrocytes coated with immunoglobulin M antibody requires a relatively large number of bound C'3 molecules per cell. Ingestion of erythrocytes sensitized with γG antibody is independent of complement; however, the reaction is inhibited by concentrations of γG far below those in normal serum. Inhibition by γG-globulin is overcome by a relatively small number of bound C'3 molecules per cell. The two monocyte receptors exert a cooperative effect on ingestion by monocytes of erythrocytes coated with γG antibody in the presence of inhibitory amounts of free γG.*

Human monocytes contain a receptor site for immunoglobulin G (γG) (*1*, *2*) which facilitates attachment and then ingestion of red cells coated with γG antibodies. This receptor is present not only on monocytes from the peripheral blood, but also on macrophages (*2*, *3*). The reaction between monocytes or macrophages and red cells sensitized with γG antibody occurs in vitro in the absence of complement (*1–3*). These γG receptors, however, bind specifically

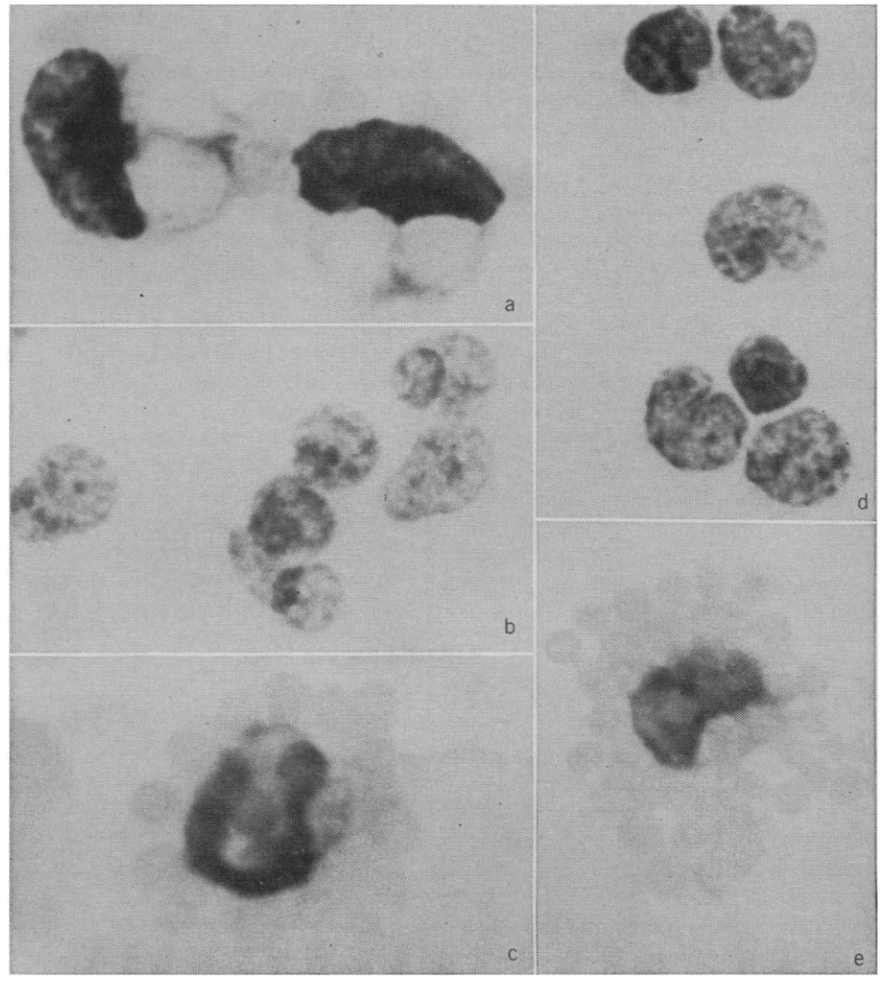

Fig. 1. Effect of antibody type and complement on interaction of isolated monocytes with sensitized sheep erythrocytes. (a) Positive reaction with erythrocytes sensitized with γG antibody in the absence of complement; (b) inhibition of reaction (a) by γG-globulin in medium (1 mg/ml); (c) neutralization of inhibition (b) by erythrocyte-bound complement (C'1,4,2,3); (d) negative reaction with erythrocytes sensitized with γM antibody in the absence of complement; (e) positive reaction with erythrocytes (d) after their reaction with complement components C'1, C'2, and C'3.

only those subclasses of guinea pig γG (*3*) and human γG (*1*) which react efficiently with complement (*3*, *4*). We now show that there is a second receptor on human monocytes which is specific for complement. This receptor facilitates the attachment and ingestion of cells coated with either γG or γM antibodies after their reaction with the first four complement components (C′1, C′4, C′2, C′3).

Human monocytes in high purity were obtained from healthy donors (*5*) and planted in Leighton tubes (*6*) as described (*1*). Sheep red cells were sensitized with purified γG or γM from rabbit antiserums to boiled red cells from sheep (*1*). The sensitized red cells were then reacted with purified human complement components in a sequential manner (*7*). Monocytes and red cells were allowed to react at 37°C for 60 minutes, and attachment and ingestion were measured quantitatively on cover slips (*8*).

In the absence of complement, purified human monocytes bound and readily ingested red cells sensitized with γG antibodies (Table 1 and Fig. 1a). The reaction was inhibited by pooled normal γG in the fluid phase (Fig. 1b). The amount of γG required to abolish this reaction was proportional to the amount of antibody on the red cells (*1*); in a concentration comparable to that of normal serum, γG was always strongly inhibitory. If the red cells, however, were coated with γG antibody and with C′1, C′4, C′2, and C′3, free γG was no longer inhibitory. The complex of sheep red cell γG antibody and complement is ingested despite presence of γG in the fluid phase (Fig. 1c and Table 1). Thus, addition of γG to the fluid phase made it possible to distinguish the complement-dependent immunophagocytosis from the reaction mediated only by the monocyte γG receptor. Prior treatment of the monocyte layer with trypsin (0.2 mg/ml at 37°C for 1 hour) reduced the complement-dependent reaction, but did not inhibit the uptake of red cells coated with γG antibody alone. Complement-dependent attachment of red cells to monocytes was due to C′3 bound to red cells; cells that had reacted with antibody and C′1, C′1,4, or C′1,4,2 did not attach to monocytes. Addition of C′5,6,7 did not enhance the reaction. Fluid phase γG did not interfere with ingestion, only with attachment. If added after attachment had already occurred, it was without effect (*9*).

Table 1. Effect of complement on ingestion by human monocytes of erythrocytes sensitized with γG or γM antibody.

Erythrocyte complex	Addition to the medium	Monocytes with ingested red cells (%)
EA(γG)	None	94
EA(γG)	γG	6
EA(γG)C′1,4,2,3	γG	92
EA(γM)	None	0
EA(γM)	γG	0
EA(γM)C′1,4,2,3	None	18
EA(γM)C′1,4,2,3	γG	77

* The γG added to the reaction medium was a preparation of pooled, normal γG-globulin at a concentration of 1 mg/ml.

Complement-dependent attachment to and ingestion by isolated monocytes was also produced by γM antibody. However, complexes of sheep red cell and γM antibody became attached to monocytes only after reaction with C′1, 4,2, and 3 (Fig. 1, d and e; Table 1). Bound γM alone, whether of rabbit or human origin, or in conjunction with either C′1, C′1,4, or C′1,4,2 failed to induce uptake of red cells to monocytes.

Quantitative studies of ingestion of red cells, with ^{125}I-labeled C′3 (*7*), revealed a marked difference in C′3 requirement, depending on the type of antibody coating the red cells. In the complement-dependent reaction, γG antibody required much less C′3 than γM antibody for effective ingestion. Approximately 100 molecules of bound C′3 per cell were sufficient, in the case of γG antibody, to induce ingestion by 50 percent of the monocytes. In contrast, under the same experimental conditions, approximately 1000 bound C′3 molecules per red cell were needed to produce the same effect with γM antibody. If the experiment with γM antibody was performed in a protein-free medium (not containing either γG or albumin), approximately 20,000 molecules of bound C′3 per red cell were required to induce ingestion of these cells by 50 percent of the monocytes (Fig. 2).

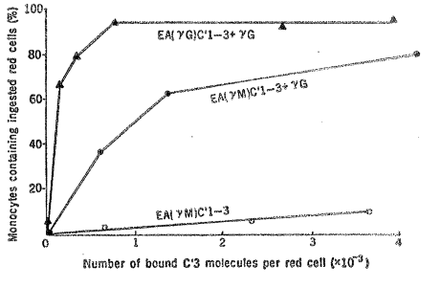

Fig. 2. Quantitative relation between erythrocyte-bound C′3 and phagocytosis of erythrocytes by isolated human monocytes, with differing C′3 requirement of γG and γM antibody. Sheep erythrocytes were optimally sensitized with the γG or γM fraction of an antiserum to sheep erythrocytes [EA(γG), EA(γM)]. Red cells coated with antibody and components of complement up to C′3 are designated EAC′1–3. The number of C′3 molecules per cell was determined with isolated ^{125}I-labeled human C′3. In the phagocytic assay, the incubation medium was free of serum proteins, except if indicated (+ γG). Under these conditions, the final concentration of γG was 1 mg/ml.

In the γM antibody system, attachment of red cells to monocytes was observed with approximately the same number of molecules of bound C′3 per cell as with γG (about 100 molecules per cell). However, with γM antibody this amount of C′3 did not cause ingestion (Fig. 2), far larger numbers of C′3 molecules being required for this effect. In the γG antibody system, attachment and ingestion of red cells had low C′3 requirements.

Complexes of antigen, antibody, and complement adhere to a variety of primate and nonprimate cells that have the so-called immune adherence receptor (*10*). The following observations suggest a possible intimate relationship between the immune adherence receptor and the monocyte C′3 receptor. Both require the participation of C′1,4,2,3; both are destroyed by trypsin, and approximately the same number of bound C′3 molecules is required in both instances (*11*). However, since the chemical nature of these receptors is unknown, such conclusions must be interpreted with caution.

In addition to demonstrating two distinct receptors on monocytes for γG and C′3, respectively, our study revealed the following fact which may be highly significant for the ingestion of antibody-coated particles by monocytes in vivo. C′3-dependent cellular adherence is not sufficient to induce ingestion of red cells by monocytes. In the case of γM antibodies, many more C′3 molecules are required for ingestion of red cells than for their mere adherence to monocytes. In the case of γG antibodies, C′3-dependent adherence brings into play the reaction between the antibody and the respective monocyte receptor which in turn appears to initiate ingestion. Thus, the two receptors on monocytes and presumably on other macrophage-like cells appear to fulfill a cooperative function

in phagocytosis. The C′3 receptor compensates for the inability of γM antibodies to react with monocytes and for the inhibition of the reaction of γG antibodies by free γG (*12*). The γG receptor, on the other hand, compensates for the lower complement-binding capacity of γG antibodies relative to γM antibodies (*13*).

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6. The isolated monocytes in a concentration of 10^6 cell/ml were allowed to settle in Leighton tubes containing a cover slip. After 2 hours at 37°C, the cell layer was washed three times with warm Hanks balanced salt solution, which removed contaminating lymphocytes and proteins present in the culture medium used in the earlier steps of the separation (*1*). The final cell layer consisted of at least 95 percent of phagocytic cells, as judged by their ingestion of polystyrene particles or red cells optimally sensitized by γG antibodies. Unsensitized, sensitized, and complement-coated red cells were added to the monocytes in a final concentration of 0.5 percent in warm, balanced salt solution. Unless otherwise stated, this incubation medium was free of serum proteins.
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8. After the monocytes had reacted with the red cells for 60 minutes, the coverslip with the monocyte layer was removed from the Leighton tubes and washed twice in warm balanced salt solution. To distinguish attachment of the red cells to the monocytes from intracellular localization, hypotonic lysis (0.06*M* NaCl for 45 seconds) was applied to some of the monocyte preparations, which lysed the bound, but not the ingested red cells. The preparations were finally fixed and stained. At least 200 monocytes were evaluated on each slide (*1*). With unsensitized red cells, the range of ingestion was 0 to 5 percent (*1*). Under optimum conditions more than 90 percent of the monocytes contained at least one sensitized red cell. At a lesser degree of sensitization this percentage decreased, reaching an end point comparable to that obtained by hemagglutination caused by specific antiserums (*1*).
9. Red cells sensitized by γG antibody were added to monocytes and incubated at room temperature for 30 minutes. Attachment to most of the monocytes, but almost no ingestion, was demonstrable. Then, γG in a final concentration of 1 mg/ml was added, and the cells were incubated at 37°C for 60 minutes; after this time most of the monocytes contained intracellular red cells. In control tubes, in which γG was added to the monocytes together with red cells, no attachment or ingestion was observed.
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Zymogen Granules in Enzyme Liberation and Activation in Pea Seeds

Abstract. *Pea seeds have zymogen-like granules that contain an inactive form of the enzyme amylopectin-1,6-glucosidase. The enzyme can be liberated from the particles in the inactive form and can then be activated by limited proteolysis with trypsin.*

A particulate fraction of cells of pea seeds contains amylopectin-1,6-glucosidase (E.C. 3.2.1.9) which is released into the soluble fraction of the cell during germination (*1*). We now describe the particles and demonstrate that activation of the enzyme does not involve synthesis of new protein.

A particulate fraction was prepared by centrifugation at 30,000*g* for 30 minutes from homogenates (*1*) of seeds soaked for 4 hours. The precipitate was resuspended, layered on a gradient with a concentration of 20 percent Ficoll (Pharmacia) at the top of the tube and 50 percent Ficoll at the bottom and centrifuged for 1 hour at 10,000*g* at 0°C; the fractions at various densities were then recovered. Since the amylopectin-1,6-glucosidase in the particulate fraction can be activated and released by addition of supernatant fractions of the same homogenate (*1*), the activities of the fractions were assayed either immediately on recovery, or after suitable incubation with the supernatant fraction obtained from the initial homogenate of the seeds. The fraction in equilibrium with 20 percent Ficoll contained all the amylopectin-1,6-glucosidase activity (Table 1).

Table 1. Density-graient fractionation (in Ficoll medium) of particles from which the amylopectin-1,6-glucosidase is released. Control is supernatant plus buffer. Incubation is at 26°C.

Ficoll (%)	Activity (IDC unit/ml) after addition of supernatant at	
	0 time	2½ hr
0	0.175	0.182
20	.192	1.740
30	.225	0.215
40+50	.287	.325
Control	.165	.170

To study the appearance of the particulate fraction, we rinsed it with buffer in the presence of lauric acid (*2*) to remove the Ficoll. The particles were then fixed with glutaraldehyde, treated with osmic acid, embedded in Luft's Epon (*3*), and sectioned. The sections were stained with uranyl acetate and examined under the electron microscope, before and after activation (Fig. 1). The intact, purified particulate fraction is composed of well-defined particles with granular content. The particles lose their granular content, and subsequently also their structure, after treatment with trypsin which releases the amylopectin-1,6-glucosidase. It is therefore legitimate to regard the structural changes and the activation as related processes and to refer to these particles as zymogen granules. It was now necessary to establish whether release and activation of the enzyme involved synthesis of new protein.

Seeds were germinated for 72 hours in the presence of ^{35}S-labeled K_2SO_4 (*4*) and homogenized; the supernatant was recovered after centrifugation of the homogenate at 30,000*g*. The supernatant was subjected to electrophoresis on agar gel. Amylopectin-1,6-glucosidase, α-amylase, and β-amylase were located on the gel. Radioactivity in the various sections of the gel was determined in parallel. No radioactivity was