

ISOLATION OF VARIANTS IN PHAGOCYTOSIS OF A MACROPHAGE-LIKE CONTINUOUS CELL LINE*

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Phagocytosis has long been recognized as one of the differentiated functions of the macrophage and yet many of the cellular and biochemical mechanisms underlying this process remain unclear. Rabinovitch has separated the process of phagocytosis into two phases: attachment and ingestion (1). The former is dependent both on the surface properties of the cell and on the particle and is independent of energy and divalent cations, while the ingestion phase requires energy and divalent cations. Attachment can be mediated through binding to receptors on the macrophage surface for the C3 component of complement and the Fc portion of immunoglobulins. In studies on Fc-mediated and C3-mediated phagocytosis, Griffin et al. (2) observed that a particle will be ingested only if the macrophage receptors for that particle are able to bind the entire surface of the particle. Thus, the initial necessary attachment at a single site is not sufficient for phagocytosis, but binding must occur on other sites of the particle during ingestion. In addition, these investigators found that erythrocytes attached to the macrophage surface with cross-linking antibody would not be ingested during the phagocytosis of other particles (3). Thus, phagocytosis was shown to occur not through a general activation of the membrane but rather through a localized or segmental response. In further studies, Griffin et al. demonstrated that particles bound to the macrophages by the C3 receptors were not phagocytized coincidentally during phagocytosis mediated through the Fc receptor (4). These studies have greatly extended our understanding of Fc-mediated phagocytosis by describing the selective interactions between particles and the phagocytic cell and additionally by distinguishing various types of phagocytosis: nonspecific phagocytosis, Fc-mediated phagocytosis, and C3-mediated phagocytosis, the latter being carried out exclusively by activated macrophages (5).

We have studied the mechanism of phagocytosis in a cloned continuous macrophage-like cell line by isolating variant clones which differ from the parental cell in phagocytosis of opsonized sheep red blood cells (SRBC).¹ The genetic approach has two advantages. First, the use of a cloned cell line eliminates the variation in properties among the different cell types in a heterogeneous population such as primary macrophages. Second, because the isolation of mutants is possible, one can dissect complex mechanisms into individual steps.

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¹ *Abbreviations used in this paper:* cAMP, adenosine 3':5' cyclic monophosphoric acid; 8 Br-cAMP, 8-bromoadenosine 3':5' cyclic monophosphoric acid; E[IgG], sheep red blood cells coated with anti-sheep red blood cell IgG; MIBX, 3-isobutyl-1-methylxanthine; NG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; SRBC, sheep red blood cells.

A reticulum cell sarcoma, J774 described by Ralph et al. (6, 7), displays a number of characteristics of primary macrophages, such as lysozyme synthesis and secretion, phagocytosis of carbon particles, expression of Fc receptors, and ingestion of erythrocytes coated with IgG. Using the continuous cloned cell line derived from this tumor, we have developed a "Trojan horse" selection scheme utilizing a toxic drug contained within the ingested particles. The rationale was that phagocytosis of opsonized erythrocytes containing a lethal substance should result in the death of all phagocytic cells leaving as survivors a population of variant cells either defective in expression of the receptor, in the process of phagocytosis, in the ability to degrade erythrocytes when phagocytized, or resistant to the toxin. The toxic agent chosen for these studies was tubercidin, an adenosine analog, which is known to be sequestered by red blood cells and is found phosphorylated in the red cell (8). By analogy with the four common ribonucleotides which are not transported in their phosphorylated forms (9), we reasoned that if during the selection the phosphorylated tubercidin should leak out of the red blood cells, it would probably not re-enter other cells and could simply be washed away. Using this selection scheme we have isolated a number of variants in phagocytosis and, in some instances, we have been able pharmacologically to correct the defect.

Materials and Methods

Cells. J774.2, the cell line used in these experiments, is a cloned cell line which was adapted to in vitro culture from the tumor J774 (6) and selected for high levels of lysozyme secretion by L. Frank and M. Scharff, and generously provided by them. It was recloned 1 mo before the selection experiments to be described. J774.2 and its derivatives were grown both in suspension and in monolayer in Dulbecco's Modified Eagle's medium (DME) (Grand Island Biological Co. [Gibco], Grand Island, N. Y.) supplemented with penicillin, streptomycin, glutamine, nonessential amino acids, and 20% heat-inactivated horse serum (Gibco). The cells were kept in a humid incubator with an air mixture containing 5% CO₂ at 37°C. Cells in suspension were counted directly in a hemocytometer. Cells in monolayer were trypsinized in a known volume and then counted.

Cloning. All cloning was done in soft agar in 25-cm² tissue culture dishes (Falcon Plastics, Oxnard, Calif. no. 3007) by the method of Coffino et al. (10). A monolayer of the rat fibroblast cell line 1706 (a gift from M. Scharff) was used as a feeder layer. A solution of 0.32% wt/vol agarose (Seakem, Marine Colloids, Inc., Rockland, Me.) in medium was maintained at 42°C. 5 ml of this solution were layered onto the feeder layer. Then the dishes were placed on a level surface at 4°C for 5 min. After the agarose had solidified, the cells to be cloned were applied to the top of the dishes in a 0.29% wt/vol solution of agarose in medium. The dishes were again cooled at 4°C to solidify the agarose and then placed in a moist incubator at 37°C. 200 cells per dish were plated and the cloning efficiencies observed were between 40% and 80%.

Preparation of SRBC Coated with IgG (E[IgG]). The method used was that described by Bianco et al. (5). SRBC in Alsever's solution (obtained weekly from the Animal Blood Centre, Syracuse, N. Y.) were washed three times in Hanks solution, resuspended to 5% (vol/vol), and incubated with an equal volume of Hanks containing anti-SRBC IgG (Lot S0245, Cordis Laboratories, Miami, Fla.) so that the final dilution of antiserum was 1:1,000. This antiserum agglutinated a 1% suspension of SRBC at a titer of 1:400. The red cell antibody mixture was incubated for 30 min at 37°C, washed three times, and resuspended to 0.5% (vol/vol) in DME for use.

Fc Rosettes and Fc-Mediated Phagocytosis. Cover slips were flamed in alcohol and placed in 25-cm² petri dishes to which approximately 5×10^5 cells were added in 5 ml of medium and incubated overnight. For the assay, the cover slips were removed and covered with the suspension of E[IgG] and incubated for 1 h at 37°C. At that time, the cover slips were rinsed in Hanks and examined for the number of cells forming rosettes. At least 100 cells were counted. A cell was considered positive if three or more erythrocytes were bound to it. Then adsorbed red blood cells

were lysed by a 10-s dip in Hanks diluted 1:5 with distilled water, and rinsed in full strength Hanks. The cells were then evaluated according to the number of erythrocytes ingested by each cell. Again, at least 100 cells were counted. Controls of red blood cells incubated without IgG were always included and never more than 4% of the cells ingested or bound any SRBC.

Latex Particles Phagocytosis. Cells were prepared as for the Fc-mediated phagocytosis assay. Latex particles, 1.1 μm (Dow Diagnostics, Indianapolis, Ind.), were dialyzed extensively against distilled water before use. For the assay, latex particles suspended to 45×10^6 particles/ml in Hanks were applied to the cover slips. After 1 h at 37°C the cover slips were rinsed and phagocytosis was evaluated. Cells containing three or more particles were considered positive. At least 100 cells were counted for each determination.

8 Br-cAMP Incubation. Cover slips and cells were incubated overnight in medium containing 0.5 mM 8-bromoadenosine 3':5' cyclic monophosphoric acid (8 Br-cAMP) (Plenum Laboratories, Hackensack, N. J.) and 0.05 mM MIBX, a phosphodiesterase inhibitor (Aldrich Chemical Co., Inc., Milwaukee, Wis.). The suspension of SRBC coated with IgG used to test these cells also included these concentrations of the drugs.

Selection Procedure. The cells to be treated were plated in 5 ml at 5×10^5 cells per 25-cm² tissue culture dish and cultured overnight. For the selection procedure some aspects of the preparation of SRBC coated with IgG (E[IgG]) were altered. In particular, during the incubation of SRBC with antiserum, tubercidin (a generous gift of Dr. G. Whitfield, The Upjohn Co., Kalamazoo, Mich.) was included at a final concentration of 500 $\mu\text{g/ml}$ and the incubation time was lengthened to 1 h. Then the E[IgG] containing tubercidin were washed five times in Hanks. E[IgG] containing tubercidin (2 ml of a 0.5% suspension) were added to the dishes after the medium had been aspirated. After 2 h of incubation at 37°C, the free E[IgG] were aspirated, the dishes were washed once with Hanks, the adsorbed erythrocytes were hypotonically lysed for 20 s as above, and the dishes were washed four times with Hanks solution, after which medium was added. 4 h after the end of the first selection, 2 ml of the complexes containing tubercidin were again added to each dish and the procedure repeated. The dishes were washed 2 days after the selection.

Mutagenesis. *N*-methyl-*N'*-nitro *N*-Nitrosoguanidine (NG) (Aldrich) was dissolved in ethanol and diluted in 0.01 M sodium acetate, pH 5, at a concentration of 30 $\mu\text{g/ml}$. Cells were suspended in Hanks at a density of 1×10^6 cells/ml with 0.3 μg NG/ml for 30 min. The cells were then washed and suspended in medium. After 2 days of incubation approximately 50% of the cells treated with NG survived as compared to control cultures without NG.

Results

Toxicity of SRBC Coated with IgG Containing Tubercidin. The selection scheme depended on the ability of J774.2 to phagocytize E[IgG] containing tubercidin, and of the ingested tubercidin to result in the death of these cells. As shown in Table I, a 2-h exposure of J774.2 to E[IgG] containing tubercidin, followed by lysis of the red blood cells and extensive washing, resulted in the death of 95% of the cells at 3 days. Since this amount of killing would not be sufficient to select for a variant present at low frequencies, we tried to maximize killing by a second cycle of the selective procedure 4 h after the first selection. This resulted in less than 0.1% survival observed on day 3. To show that the killing was specific for Fc receptor-mediated phagocytosis the anti-SRBC IgG was omitted from the selection mixture. By such a procedure no Fc-mediated phagocytosis should occur and the extent of nonspecific killing could be assessed. In the experiment illustrated in Table I, only 25% of the cells were nonspecifically killed, and in subsequent experiments this figure was reduced to 5–10% by washing the cells more thoroughly. Thus, the tubercidin was not killing the cells after being released from nonphagocytized red blood cells or by adhering to the surface of the red blood cells.

TABLE I
Effect of SRBC Containing Tubercidin

Number of applications	IgG*	Number of cells [‡] × 10 ⁵		Control (%)	
		day 1	day 3	day 1	day 3
0	—	2.7	6.0	100	100
1	+	0.66	0.03	25	5
2	+	0.06	<0.01	2	0
2	—	2.3	4.5	82	75

* Some SRBC containing tubercidin were prepared in the absence of anti-SRBC IgG. + or — indicates the presence or absence of the antiserum.

‡ 1 day and 3 days after treatment duplicate plates were counted.

Application of the Selection Scheme. J774.2 cells were mutagenized with nitrosoguanidine (NG), then plated at 5×10^5 cells per dish and subjected to two cycles of the selection procedure. After 9 days, 37 discrete adherent clones with from 8 to 16 cells each were noted. We tested these clones directly on the dish for Fc receptors and Fc-mediated phagocytosis by removing the medium and incubating with 2 ml of E[IgG] for 1 h. 36 clones clearly made rosettes and so had Fc receptors. One clone appeared negative. The SRBC were lysed and the clones examined for phagocytosis. Of these clones, 25 had phagocytized almost no red blood cells, 6 contained cells which had ingested many red blood cells and therefore resembled the parental type, and 6 clones appeared to be made up of both phagocytic and nonphagocytic cells. The loose adherence of these clones to tissue culture dishes prevented the recovery of these clones by cloning cylinder methods.

Isolation of Cloned Variant Populations. To recover the potential mutants as clonal populations, cells were again exposed to NG and plated, and the selection procedure was performed in two cycles on these dishes. Four independent selections were carried out. After 2 wk of growth, the selection procedure was repeated on each dish. 2 wk later, when each dish contained about 3×10^6 cells, the populations were examined for Fc-mediated phagocytosis and each selection was found to have resulted in cells which were minimally phagocytic as compared to the parental cells. While 19% of J774.2 phagocytized no E[IgG], 80–93% of the selected cells failed to phagocytize. Accordingly, cells from each of the four selections were cloned in soft agar and several clones from each were picked, grown, and independently maintained.

We tested 40 clones drawn from each of the four selections. All clones appeared to have Fc receptors. (Greater than 90% of the cells in each formed rosettes.) 31 clones, however, were found to have a reduced ability to phagocytize E[IgG]. 9 of the 40 were wild-type in regard to phagocytosis. The phagocytic patterns of sample clones are shown in Fig. 1. These examples demonstrate the variation in the extent of phagocytosis between the individual clones, although all are clearly deficient in phagocytosis when compared to the parental cell line. These differences are a stable and reproducible property of the clones. For example, cells of clone 12 from selection no. 4 (designated clone 4.12) contained 95% nonphagocytic cells, clone 4.5, about 80% nonphagocytic cells, and clone 1.6

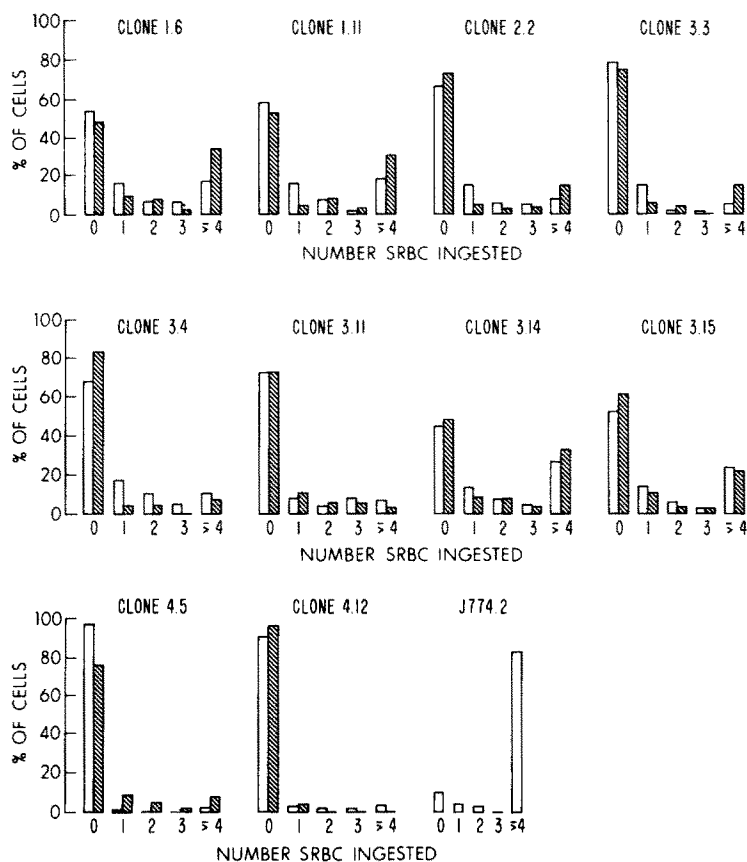


FIG. 1. Fc-mediated phagocytosis by J774.2 and variants in phagocytosis. The parent clones are shown with open bars and the subclones of the parent clones with hatched bars.

averaged about 50% nonphagocytic cells. No clone has yet been found in which there is a total absence of phagocytic cells.

Recloning of Variant Clones. We considered the possibility that the remaining low level of phagocytosis in the mutants might be due to mixed clones rather than representative of a true clonal phenotype, although this was unlikely since only 200 cells were cloned per dish. To eliminate this possibility, we randomly chose 10 of the original parent clones to be recloned and tested. We found that subclones did not differ from their parent clone in the pattern of phagocytosis to any noticeable degree (Fig. 1). This experiment demonstrates the heritable nature of the nonphagocytic trait.

When these recloned populations were additionally tested for phagocytosis of latex particles, 92–100% of the cells in each clone showed latex phagocytosis (Table II). These levels are comparable to the level of 95% shown by J774.2.

Effect of Tubercidin on the Variants. The selection procedure described in this paper resulted in the isolation of clones showing reduced levels of phagocytosis. The possibility was considered that the selection might have allowed survival of clones either resistant to tubercidin or unable to degrade red blood

TABLE II
Phagocytosis of Latex and E[IgG] by 10 Variant Clones

Clone tested	Cells phagocytizing latex	Cells phagocytizing >3 E[IgG]
	%	%
J774.2 (parent)	95	75
1.6	94	23
1.11	100	20
2.2	92	11
3.3	100	6
3.4	96	15
3.11	100	15
3.14	98	31
3.15	95	23
4.5	95	2
4.12	97	0

cells. Since mutants in the degradative processes would presumably not be altered in phagocytosis, this possibility could be eliminated at least for the nonphagocytic variants. However, to confirm that the selection of cells resistant to tubercidin did not account for our results, we tested the susceptibility of J774.2 to tubercidin as well as that of the 10 lines which had been recloned. All were exposed to tubercidin in the medium at 1 $\mu\text{g}/\text{ml}$. We found that 4 days after placing approximately 2×10^5 cells/ml in suspension culture in this concentration of tubercidin there were no detectable survivors, while there were approximately 6×10^5 cells/ml in all control dishes without drug. Note that 0.1 $\mu\text{g}/\text{ml}$ is not toxic to these cells. Thus, the selection procedure employed appears to yield cells defective in phagocytosis and not resistant to tubercidin.

Analysis of Wild-Type Clones. The clones with wild-type phenotypes with respect to phagocytosis might be candidates for cells resistant to tubercidin or deficient in red blood cell degradation. These two possibilities could be tested simultaneously by repeating the selection procedure on these cells. This was done on the nine clones which were wild-type in regard to phagocytosis, on clone 4.5, a nonphagocytic variant, and on J774.2 itself (Table III). Eight of the phagocytic clones were completely killed by the selection procedure as was J774.2. Clone 4.5, the nonphagocytic variant, survived, while the phagocytic line 1.15 was not completely killed. We have not further analyzed this partial resistance of clone 1.15.

Effect of 8 Br-cAMP on Variants. In other experiments we have found that 8 Br-cAMP at 0.5 mM concentrations is capable of enhancing Fc-mediated phagocytosis by J774.2 under appropriate conditions.² Consequently, we tested the effect of 8 Br-cAMP on the variant clones. Some clones showed a marked increase in phagocytosis on addition of 8 Br-cAMP while others were unaffected in regard to phagocytosis. The phagocytic pattern of some clones in response to 8 Br-cAMP is shown in Fig. 2. It can readily be seen that clone 1.6, which showed intermediate levels of phagocytosis, maintained the same pattern after the

² R. J. Muschel, O. M. Rosen, N. Rosen, and B. R. Bloom. Manuscript in preparation.

TABLE III
Effect of the Selection Procedure on Phagocytic Clones

Cell tested	Survival at 3 days*
	%
J774.2	0
Phagocytic clones	
1.10	0
1.15	10
2.1	0
3.1	0
3.2	0
3.7	0
3.12	0
4.3	0
4.8	0
4.16	0
Nonphagocytic clone	
4.5	80

* Percent survival was calculated by comparison with the number of cells on a dish treated with SRBC containing tubercidin but not coated with IgG.

incubation with 8 Br-cAMP. Conversely, clone 1.11, which had a similar pattern under the original conditions, was highly phagocytic after treatment with the drug. Clone 3.13, which was only negligibly phagocytic under the original conditions, was unaltered in phagocytosis by 8 Br-cAMP, whereas others such as 4.12 or 2.2 which were both nonphagocytic were markedly enhanced in phagocytosis of E[IgG] although to different extents; clone 4.12 had only 30% of its cells ingesting four or more red blood cells in 8 Br-cAMP, while clone 2.2 acquired phagocytic activity comparable to the parental type. Of 19 of the original 31 clones defective in phagocytosis, 11 showed some substantial degree of restoration of Fc-mediated phagocytosis upon treatment with 8 Br-cAMP, while 8 showed no enhancement. 0.5 mM AMP was also tested and did not enhance Fc-mediated phagocytosis on two of the variants which were enhanced by 0.5 mM 8 Br-cAMP. All 19 variants and J774.2 showed at least 90% of their cells forming Fc rosettes in 8 Br-cAMP. In addition, we tested the 10 recloned lines in this way and compared their behavior to the 10 parental lines and again found no differences between the parent clone and the subclone.

Discussion

In the present experiments we have isolated variants in the mechanism of phagocytosis by means of somatic cell genetical techniques using a cloned continuous macrophage-like cell line, J774.2. These variants were stable and showed different phagocytic patterns, with some being correctable by 8 Br-cAMP. They were isolated by a strategy using IgG-coated SRBC (E[IgG]) containing the toxic drug tubercidin. A similar approach has recently been reported by Refsnes and Munthe-Kaas (11) who used immune complexes of ricin to kill primary macrophages. We have demonstrated that J774.2 is susceptible

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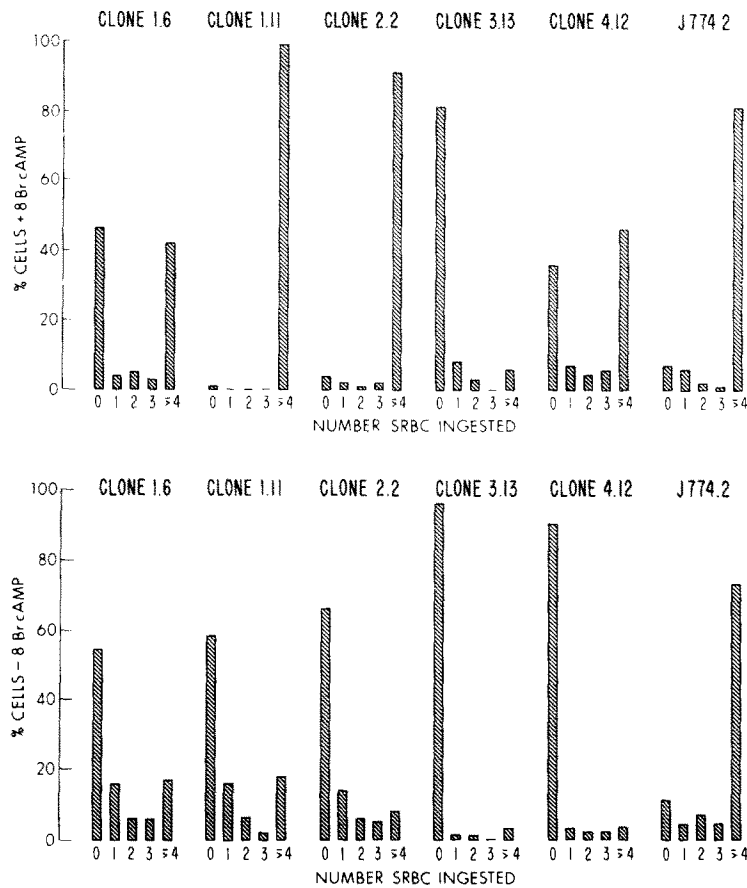


FIG. 2. Patterns of Fc-mediated phagocytosis by J774.2 and some variant clones plus and minus 8 Br-cAMP. Cells grown in the absence of 8 Br-cAMP are shown in the lower panel; cells grown in the presence of 0.5 mM 8 Br-cAMP and 0.05 mM MIBX are shown in the upper panel.

to lethal effects of tubercidin contained in E[IgG] and that the killing was due specifically to Fc-mediated phagocytosis since the presence of sheep erythrocytes containing tubercidin in the absence of IgG had only a slightly toxic effect (Table I). This method has permitted us to isolate variants which are in some way defective in the process of phagocytosis. It is to be noted that in order to isolate variants which occur at low frequencies, it has been empirically found that two cycles of treatment are more effective. The requirement for two selection cycles may be related to the cell cycle or may be due to random variation in phagocytosis among cells in the population.

Variants which survived the selection procedure were cloned and compared to the parental cell in regard to various processes in which predicted defects might have allowed survival. In particular, the following were considered: (a) resistance to the drug tubercidin; (b) defects in the intracellular degradation of erythrocytes; (c) complete loss of Fc receptors; (d) defects in phagocytosis per se.

We found only one clone which might fit into categories (a) or (b), clone 1.15,

which was phagocytic but weakly survived the reapplication of the selection scheme. All other phagocytic clones appeared to be wild-type in these respects.

Cells defective in Fc-mediated phagocytosis were also anticipated and found in relative abundance. Variants with defects in Fc-mediated phagocytosis were detected at a frequency of 31 per 5×10^5 or 6×10^{-5} after NG treatment. This is a frequency consistent with mutation, especially if the assumption is made that several genes are involved in the process. The property of defective phagocytosis has been shown to be stable and heritable by recloning. By a simple definition of mutation as a heritable change in phenotype, these clones could be judged to be mutants. We prefer, however, to use the term variant rather than mutant when referring to these cells, until a mutant gene product can be demonstrated.

Fc-mediated phagocytosis has been experimentally separated into two phases: attachment via the Fc receptor, and ingestion (1). One could predict that the variants might prove to be defective in one or other phase. All of the isolated clones exhibited Fc receptors, although one clone was found which appeared to lack the Fc receptor but it could not be recovered. However, the results of Unkeless and Eisen (12) and of Walker (13) suggest that there are different receptors for the Fc portions of IgG 2a and IgG 2b. Therefore, a positive result in the rosetting assay used in these experiments does not definitely exclude the loss of one type of Fc receptor if J774.2 had both types. If any of the variants had lost one receptor but not both, it would represent a cell which might still form Fc rosettes but in which phagocytosis might be impaired due to the lack of a Fc receptor for a different class of immunoglobulin. We cannot at present conclude that the variants have a full complement of Fc receptors. Experiments are in progress to examine the Fc receptors for the IgG subclasses in the variants.

This work also extends the observations of Griffin and Silverstein (3) in regard to the relationship of attachment to ingestion. They have elegantly shown the necessity of binding via the Fc receptor for phagocytosis of red blood cells by normal macrophages. Other sorts of artificial attachments did not result in phagocytosis. Our results supplemented these observations by showing attachment to an Fc receptor is not itself a sufficient condition for phagocytosis. This result may be interesting in regard to possible distinctions between Fc receptors and their functions in binding, in phagocytosis and in cytolysis.

While most studies of phagocytosis have assumed that the process was independent of the nature of the particle studied, the mechanisms of latex phagocytosis and Fc receptor-mediated phagocytosis have recently been experimentally differentiated by Michl et al. (14) using the drug 2-deoxy-glucose, which markedly inhibited Fc-mediated phagocytosis without altering Fc rosetting or ingestion of latex particles. This has been confirmed in the present experiments, in which we took variant clones defective in phagocytosis of E[IgG] and tested them for the ability to phagocytize latex particles. All were positive. From these data, it seems clear that latex- and Fc-mediated phagocytosis occur by different mechanisms, since pharmacologic agents can block the latter process, leaving the former intact, and since variants in Fc-mediated phagocytosis are not altered in their ability to phagocytize latex particles.

Finally, we found that Fc-mediated phagocytosis by some but not all of the variants would be enhanced by 8 Br-cAMP (Fig. 2). We have noted essentially

five classes of variants in regard to Fc-mediated phagocytosis in the presence and absence of 8 Br-cAMP. Fig. 2 shows one example of each type: a variant which has intermediate levels of Fc-mediated phagocytosis and another which has almost no Fc-mediated phagocytic ability are both not altered in phagocytosis by 8 Br-cAMP. On the other hand, one intermediate type (1.11), and (2.2) almost completely lacking in phagocytosis were both fully converted to wild-type levels of Fc-mediated phagocytosis by 8 Br-cAMP, while the fifth class was boosted to an intermediate level only. In addition to suggesting the involvement of the cAMP system in phagocytosis, these results make it necessary to consider that some of the variants may have defects in cAMP metabolism.

A large amount of literature exists on changes in intracellular levels of cyclic nucleotides in neutrophils and macrophages after phagocytosis (15-18), but there has been up to now no formal evidence that cAMP is required in the phagocytic process. At least two reports in the literature have concluded that cAMP inhibits Fc rosetting and phagocytosis. Zuckerman and Douglas (19) reported that cholera toxin, a drug known to activate adenylate cyclase, reduced the number of Fc rosetting cells in a lymphoblastoid cell line. However, the concentration of cholera toxin required for this effect was 1,000 times greater than that needed optimally to elevate cAMP in their hands, and it may be questioned whether the effect was primarily related to the action of cholera toxin in raising cAMP levels or to some secondary effect. Secondly, Cruchard et al. (20) have reported an inhibitory action of dibutyryl cAMP on Fc-mediated phagocytosis at a minimum concentration of 10^{-2} M. Since the enhancement we have observed occurs at a concentration of 5×10^{-4} M, it is difficult directly to compare these observations.

Since we have isolated some variants that were enhanced in phagocytosis by 8 Br-cAMP and some that were not, it might not be unreasonable to consider two components of Fc-mediated phagocytosis, one dependent on cAMP, the other independent. If we accept the hypothesis of Kuo and Greengard (21), that in eukaryotes cAMP acts exclusively through its interaction with cAMP binding protein phosphokinases, it becomes apparent that mutations within the cAMP system itself could produce both effects. If cAMP truly enhances Fc-mediated phagocytosis physiologically, mutations either in adenylate cyclase or in the appropriate cAMP binding protein phosphokinase might lead to the phenotypes which we have observed; adenylate cyclase mutants being enhanced by 8 Br-cAMP and cAMP binding protein phosphokinase mutants perhaps being uninfluenced by the drug. Viable mutants in cAMP metabolism have been isolated in other cell lines, e.g., mutants in adenylate cyclase (22) and cAMP binding phosphokinase (23, 24). On the other hand, both classes of variants described here could result from mutation outside the cAMP system itself. These possibilities are susceptible to experimental analysis.

Summary

We have isolated cloned variants in phagocytosis from a cloned continuous murine macrophage-like cell line, J774.2. A selection procedure against Fc receptor-mediated phagocytosis was devised using IgG-coated SRBC containing a toxic drug, tubercidin, as the lethal agent. A series of variant clones deficient in Fc receptor-mediated phagocytosis were isolated. Such variants occurred at

low frequency ($\sim 6 \times 10^{-5}$), were stable, and appeared to possess Fc receptors. The degree to which they were defective in phagocytosis of IgG-coated SRBC varied from clone to clone, yet all clones were able to phagocytize latex particles. The phagocytic defect in some variants could be corrected by the addition of 8 Br-cAMP, in others, the drug was without effect. It is likely, therefore, that different variants are defective in several distinct steps critical to Fc receptor-mediated phagocytosis.

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