MACROPHAGE ACTIVATION: INCREASED INGESTION OF IgG-COADED ERYTHROCYTES AFTER ADMINISTRATION OF INTERFERON INDUCERS TO MICE

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Systemically administered inducers of interferon (IF) appear to activate the macrophage system. This is suggested by increased in vivo clearance of colloids from the plasma (1) and by enhanced spreading on glass of peritoneal macrophages from mice treated with the inducers (2). We show here that inducers of IF also increase the in vitro uptake by peritoneal macrophages of erythrocytes coated with graded numbers of IgG molecules (EA) (3, 4). The increase is similar to that elicited by the administration of lipopolysaccharide (LPS) or Corynebacterium parvum, well known activators of the mononuclear phagocyte system.

Materials and Methods

Animals. Swiss Webster female mice, 20-25 g were obtained from Taconic Farms, Inc., Germantown, N.Y.

Interferon Inducers and Other Agents. Newcastle disease virus (NDV), Hickman stain, was grown in 10- to 11-day-old chick embryos for 48 h. 1 x 10⁹ plaque-forming units (PFU) in 0.2 ml allantoic fluid were given i.v. to mice 24 h before sacrifice. Controls received phosphate-buffered saline (PBS) since in previous studies no macrophage activation resulted from the injection of allantoic fluid from uninfected embryos. Vesicular stomatitis virus (VSV) was produced by L cells. 1 x 10⁷ PFU in 0.2 ml were injected i.v. 24 h before sacrifice. Control mice received conditioned Dulbecco's modified Eagle medium (DMEM) containing 5% fetal bovine serum from uninfected cultures. Tilorone was given by gavage in water at 25 mg/100 g, 24 or 28 h before sacrifice. Polyinosinic-polycytidylic acid (poly I. poly C), was supplied by Dr. Jan Vilcek (N.Y.U. School of Medicine). Mice received 100 μg i.v. 24 h before collection of the macrophages. Polyadenylic-polyuridylic acid (poly A. poly U) was used similar to poly I. poly C. Salmonella abortus equi lipopolysaccharide (Difco Laboratories, Detroit, Mich.) was injected subcutaneously at 50 μg 24 h before sacrifice. C. parvum vaccine was obtained from Wellcome Research Laboratories, Beckenham, Kent, England, and given i.v. in a 1 mg dose 2 wk before sacrifice. All of the agents were tested in at least two separate experiments.

Collection of the Macrophages. Peritoneal washings were collected and total and differential counts were performed as in reference 2. Washings from control mice contained an average of 7.4 x 10⁶ cells per mouse, with 76% macrophages, 22% lymphocytes, and 1.5% or less neutrophils.

Phagocytosis Assay. IgG-coated erythrocytes were prepared by a modification of the procedure given in reference 4. 1 ml of a 5% suspension of washed erythrocytes was added to a 1 ml dilution of anti-sheep erythrocyte IgG (Cappel Laboratories, Inc., Downingtown, Pa.). The final IgG concentrations were 13.7, 3.4, and 1.7 μg/ml and correspond to dilutions of 1:1,000, 1:4,000, and 1:8,000, respectively. The suspensions were incubated 30 min at 0°C, and then 15 min at 37°C, washed once in DMEM and resuspended at 1 x 10⁸ erythrocytes per ml. The average numbers of IgG molecules per erythrocyte, determined by capture of ¹²⁵I-labeled anti-sheep erythrocyte IgG
Ingestion of sheep erythrocytes by peritoneal macrophages as a function of the concentration of anti-sheep erythrocyte IgG. Abscissae: $\log_{10}$ antibody titer. Ordinates: percentage of macrophages ingesting four or more opsonized erythrocytes. Vertical bars represent standard errors. Closed circles indicate averages of treated animals and open circles those of controls. A, NDV; B, VSV; C, tilorone; D, poly I, poly C and poly A, poly U (A—△); E, LPS; F, C. parvum.

Macrophage monolayers were rinsed and overlaid with 0.2 ml of the EA suspensions and phagocytosis proceeded at 37°C for 30 min. Erythrocytes attached to the macrophages were lysed with hypotonic saline and the cells fixed in 2% glutaraldehyde and examined by phase contrast microscopy. 200 cells were scored in each of two coverslips for the ingestion of four or more erythrocytes and the counts were averaged.

Serum IF activity was determined as in reference 2. Animals were bled from the orbital plexus at sacrifice rather than at the peaks of the IF responses.

Data were analyzed by regression techniques and response curves compared by covariant analysis.

Results

Ingestion of opsonized erythrocytes by peritoneal macrophages was significantly increased after systemic administration of NDV, VSV, tilorone, or poly I, poly C. This stimulation of phagocytosis was found with each of the IgG concentrations used for opsonization (Fig. 1 A–D). In contrast, poly A, poly U, a weak interferon inducer, did not significantly stimulate the ingestion of EA
TABLE I
Slopes and Equations of the Lines Shown in Figure 1

| Agent       | Slope* | P value* | Equation       |
|-------------|--------|----------|----------------|
| NDV         | 42.1 ± 10.5(8) | >0.2 | y = 42.1 x + 179.7 |
| Control     | 20.9 ± 2.3(6)  | >0.2 | y = 20.9 x + 80.4 |
| VSV         | 24.8 ± 3.7(8)  | <0.01| y = 24.8 x + 102.2 |
| Control     | 10.8 ± 1.2(5)  | <0.01| y = 10.8 x + 42.2 |
| Tilorone    | 40.5 ± 12.0(6) | >0.2 | y = 40.5 x + 195.5 |
| Control     | 27.2 ± 8.7(4)  | >0.2 | y = 27.2 x + 109.0 |
| Poly I.poly C | 35.0 ± 14.1(5) | >0.2 | y = 35.0 x + 169.8 |
| Control     | 20.0 ± 5.3(8)  | >0.2 | y = 20.0 x + 76.5 |
| Poly A.poly U | 23.6 ± 6.4(6)  | >0.2 | y = 23.6 x + 94.1 |
| C. parvum   | 57.8 ± 12.0(8) | <0.01| y = 57.8 x + 249.7 |
| Control     | 20.3 ± 3.9(6)  | <0.01| y = 20.3 x + 73.9 |
| LPS         | 29.4 ± 7.7(6)  | >0.01| y = 29.4 x + 136.9 |
| Control     | 17.8 ± 3.2(4)  | >0.01| y = 17.8 x + 68.2 |

* Slope ± standard error of mean (no. mice).
† Level of significance is taken as P > 0.05.
§ Ingestion of erythrocytes (Y) as a function of antibody dilution (X).
¶ Equations for treated groups were significantly different from the appropriate controls (P < 0.001) except for the poly A.poly U-treated mice (P > 0.3)

(Fig. 1D). LPS and C. parvum also enhanced the ingestion of EA after systemic administration (Fig. 1 E–F). For all the agents used a linear dose response was obtained relating the logarithms of the concentrations of IgG to percent phagocytosis of four or more EA. The slopes of the curves may be taken to reflect the sensitivity of the endocytic response of the macrophages to the number of IgG molecules present on the erythrocytes. The slopes fell within a relatively narrow range for both activated and control macrophages. Lines for the activated macrophages were found to be statistically parallel to controls for all the agents used except VSV and C. parvum (Table I).

Fig. 2 shows that the number of erythrocytes ingested by macrophages from an NDV-treated animal (2 A) is clearly greater than that ingested by macrophages from a mouse injected with allantoic fluid (2 B).

Total and differential cell counts did not significantly change after the administration of NDV, VSV, or poly A. poly U in these experiments. Total counts were decreased after poly I. poly C or tilorone administration by 50 and 30%, respectively, and were increased by 170% after treatment with C. parvum. The changes involved both lymphocytes and macrophages. Neutrophils were not elevated in the fluids obtained at sacrifice except with LPS where an increase from 8 x 10⁴ to 50 x 10⁴ was found. No changes in spleen weights were recorded except in C. parvum and LPS-treated mice, where increases of 350 and 60%, respectively, were found.

Serum interferon levels at 24 h were found to be markedly elevated in mice given NDV, VSV, or tilorone but not poly I. poly C or poly A. poly U. (data not shown). Significant correlation between interferon and phagocytosis was obtained for VSV-treated animals (r = 0.681, P < 0.02, degrees of freedom = 10). The correlation coefficients for NDV and tilorone were not statistically significant.
Fro. 2. Macrophages from mice injected with $2 \times 10^6$ PFU of NDV (A) or with control allantoic fluid (B) and sacrificed after 24 h. Macrophages on coverslips were incubated for 30 min with sheep erythrocytes coated with an estimated average of 2,100 molecules of anti-sheep erythrocyte IgG per erythrocyte. Erythrocytes attached to the macrophages were lysed in hypotonic saline before fixation. The number of ingested erythrocytes is markedly higher in macrophages from NDV injected mice (A) than those of controls (B). Phase contrast ($\times$ 500).

Discussion

Several viral and nonviral IF inducers systemically administered to mice increased the uptake by macrophages of erythrocytes opsonized with IgG antibody. The curves relating phagocytosis and IgG concentrations were all displaced in treated animals above those for controls. Similar displacement was observed with the macrophage activators, LPS, and C. parvum. The results confirm and extend with more reliable methodology, previous conclusions based upon the macrophage spreading assay.¹

The macrophage features responsible for the increased uptake of EA have not been defined. Increased ingestion could result from increased numbers, availability or avidity of Fc receptors (5, 6) from enhanced spreading of the macrophage surface over the particles, or from heightened function of the cytoskeletal apparatus (7).

For all the inducers used a logistic distribution of the peritoneal macrophage population capable of ingesting four or more erythrocytes was observed (Fig. 1). This type of distribution reflects the heterogeneity of the population under

¹ In the present experiments, neither spreading nor increased ingestion of EA followed the administration of cycloheximide to mice; the cause of our previously reported (2) increase in spreading by cycloheximide could not be ascertained.
study and the slope of such a curve is indicative of that heterogeneity (6, 8). As shown in Table I the slopes of the curve obtained with macrophages from mice treated with NDV, tilorone, poly I. poly C, and LPS are all similar to that of controls. The upward displacement of the curves indicates increased phagocytic ability at all antibody titers as compared to controls. This represents a shift of the entire population of macrophages to a new status, "activated", with respect to their phagocytic ability. The parallelism of the curves for the activated and control macrophages indicates that the heterogeneity of the cellular populations has not changed. Rhodes (6) arrived at a similar conclusion in experiments on the attachment of EA to normal or inflammatory macrophages from guinea pigs. The activation resulted in an increase in overall avidity of macrophages for EA without a substantial change in the distribution of avidities for the population.

In contrast to the macrophage stimulation obtained by intraperitoneal administration of several agents (9), little influx of the blood-borne macrophages appeared to follow systemic administration of the IF inducers. Thus, total numbers of macrophages were either not increased or actually decreased, and accumulation of PMNs was rarely found at the time of sacrifice. It is thus probable that we have observed a change in phagocytic ability of resident macrophages without a contribution from the influx of additional phagocytes. Such an influx, however, may have occurred after administration of C. parvum, and this could explain the increased slope of the log dose-response curve (Fig. 1E) with this inducer.

As NDV, VSV, and tilorone are known interferon inducers, interferon could indirectly or directly modulate macrophage activation. Activation could also result from the release of migration inhibitory factor (MIF) or other lymphokines by lymphocytes or other cells triggered by the IF inducers. Activation by IF has received support from experiments in which incubation of macrophages with IF-rich preparations increased the uptake of carbon (10) or of opsonized erythrocytes (3). The possibility that these preparations also contain MIF (11) has not been excluded.

The present results emphasize the need for further investigation of the role of interferon in macrophage activation.

Summary

In vitro phagocytosis of IgG-opsonized sheep erythrocytes (EA) was used to measure the in vivo activation of mouse peritoneal macrophages. Uptake of EA was enhanced by the extraperitoneal administration of Newcastle disease virus, vesicular stomatitis virus, tilorone or polyinosinic-polycytidylic acid. Ingestion of EA was similarly stimulated by lipopolysaccharide or killed Corynebacterium parvum. Dose-response curves relating concentrations of IgG to phagocytosis were parallel for both treated and control animals. This indicates that the heterogeneity of the macrophage populations did not change and that the overall populations were activated with respect to phagocytic ability. Numbers of macrophages were not increased (except in C. parvum-treated mice), suggesting that resident, rather than newly recruited macrophages, were activated by the different agents.
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