Acute Infection of Mice with Lactate Dehydrogenase-elevating Virus Enhances Fc and Complement Receptor Activity of Peritoneal Macrophages

By NANCY LUSSENHOP, BEULAH HOLMES, WILLIAM A. CAFRUNY AND PETER G. W. PLAGEMANN*

Department of Microbiology, Medical School, University of Minnesota, Minneapolis, Minnesota 55455, U.S.A.

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SUMMARY

Peritoneal macrophages isolated from Balb/c mice 1 day after infection with lactate dehydrogenase-elevating virus (LDV) exhibited a 5- to 10-fold enhancement of attachment and ingestion of sheep red blood cells coated with immunoglobulin (EA_{IgG}) or immunoglobulin plus complement (EA_{IgM}C). Macrophages isolated from mice 7 days after LDV infection or macrophages infected with LDV in culture were also slightly more active than macrophages from uninfected mice, but the differences were not significant. The results indicate that a specific increase in the number of Fc and C3 receptors on macrophages occurs during the acute phase of infection. This increase correlates with the transient appearance of interferon in acutely infected mice. We postulate that during the acute phase the productive infection of a subpopulation of macrophages that is permissive for LDV results in the synthesis of sufficient interferon to cause activation of the remaining non-permissive macrophages in the animal.

INTRODUCTION

The replication of lactate dehydrogenase-elevating virus (LDV), a non-flavi, non-alpha togavirus of mice (Brinton-Darnell & Plagemann, 1975), is limited to a subpopulation of macrophages (Stueckemann et al., 1982). Infection of mice with LDV results in a rapid and massive virus production resulting in peak plasma virus titres of up to 10^{10} median infectious doses (ID_{50}) per ml at about 1 day post-infection which thereafter begin to decrease (Notkins & Shochat, 1963; Plagemann et al., 1963). This acute infection is associated with the development of an impaired blood clearance of lactate dehydrogenase and some other enzymes which results in the plasma elevation of these enzymes (Rowson & Mahy, 1975). It is also associated with a transient enhancement of the humoral antibody response of infected mice to both T cell-dependent and -independent antigens (Michaelides & Simms, 1980; Notkins et al., 1966a) and the depression of the cellular immune response (Howard et al., 1969; Michaelides & Schlesinger, 1974; Notkins, 1971; Turner et al., 1971). The acute infection invariably progresses into a life-long persistent infection which is characterized by a continuing elevation of plasma enzyme levels and a low-level viraemia (Notkins & Shochat, 1963; Rowson & Mahy, 1975). During the chronic stage, however, the immune functions of the host have largely returned to normal and the virus is present mainly in a complex with IgG (Michaelides & Schlesinger, 1974; Notkins et al., 1966b; Turner et al., 1971).

Recent evidence suggests that during the acute infection most of the macrophages in the body that are permissive for LDV are destroyed as a consequence of the productive infection with LDV and that life-long persistence of LDV is maintained by passage of the virus to new
permissive cells that are continuously generated from non-permissive precursor cells (Stueckemann et al., 1982). Infection of mice with LDV generally does not result in any gross pathological changes, except in certain strains of mice (C58 and AKR) when they are immunosuppressed either as a result of old age or chemical treatment (Martinez et al., 1980; Nawrocki et al., 1980). In such mice, acute primary infection with LDV results in the rapid development of an inflammatory destruction of motor neurones (poliomyelitis) and death of the animals (Martinez et al., 1980; Nawrocki et al., 1980), but the interaction between LDV infection and immunological and genetic factors of the host in causing this disease is not understood.

In order to learn more about the relationship between LDV infection and the host immune system we have examined the expression of Fc and C3 receptors on macrophages from mice acutely and chronically infected with LDV. We find that peritoneal macrophages harvested from mice 24 h post-infection show a greatly enhanced capacity to bind and ingest immunoglobulin- and complement-coated erythrocytes which correlates with the transient production of interferon in acutely infected mice.

METHODS

Macrophage cultures. Peritoneal cells were harvested from 2- to 4-month-old unstimulated Balb/c mice (Lyons Laboratory mouse colony, University of Minnesota) and cultured on 12 mm diam. glass coverslips as described previously (Brinton-Darnell et al., 1975; Tong et al., 1977), except that the coverslips were placed into wells of a leukocyte migration plate (Cooke Laboratories Products, Alexandria, Va., U.S.A.) rather than Petri dishes. Each coverslip was seeded with \(5 \times 10^6\) peritoneal cells in medium 199 plus 20% (v/v) foetal calf serum.

Virus and virus infection. LDV titres were estimated in mice as described previously (Plagemann et al., 1963). Mice were infected with LDV by intraperitoneal injection of \(10^5\) to \(10^6\) ID\(_{50}\)/mouse and macrophage cultures with about 20 ID\(_{50}\)/cell as described previously (Brinton-Darnell et al., 1975; Brinton-Darnell & Plagemann, 1975).

Preparation of IgG to sheep erythrocytes. Ascites fluid was induced in Swiss Webster mice (Bio Lab, St. Paul, Mn., U.S.A.) as described by Tung et al. (1976). The mice were injected intraperitoneally three times each week with 0.2 ml of an equal mixture of a 10% (v/v) suspension of saline-washed sheep erythrocytes (obtained in Alsevers’ solution from Texas Biological Laboratories, Fort Worth, Tex., U.S.A.) and complete Freund’s adjuvant. After 8 weeks the ascites fluid was harvested and its antibody titre determined by a conventional haemagglutination assay. The ascites fluid when absorbed with goat anti-mouse heavy chain-specific (G1, G2a, G2b and G3) 7S immunoglobulins (Bionetics Laboratory Products, Kensington, Md., U.S.A.) lost more than 97% of its haemagglutinating activity.

Preparation of EA\(_{IgG}\) and EA\(_{IgM}\). Washed sheep erythrocytes suspended to 5% (v/v) in medium 199 were incubated at 37 °C for 15 min with subagglutinating concentrations of antibody: with a 1/320 dilution of ascites fluid containing the mouse anti-sheep red cell IgG to obtain EA\(_{IgG}\) or with a 1/40 dilution of rabbit anti-sheep red cell IgM (lot S0365, Cordis Laboratories, Miami, Fla., U.S.A.) to obtain EA\(_{IgM}\). The sensitized erythrocytes were washed once in medium 199 and suspended at 0.5% (v/v) in the same medium. Control sheep red blood cells (E) were incubated and washed in the same manner, except that the antibody was omitted.

Preparation of EA\(_{IgM}\) C. EA\(_{IgM}\) were suspended at a concentration of 5% (v/v) in complement fixation diluent (Consolidated Laboratories, Chicago Heights, Ill., U.S.A.) containing 1% (w/v) gelatin in barbitol buffer. The suspension was mixed with an equal volume of a 1/10 dilution of B10-Old mouse serum deficient in C5 component (Lyons Laboratory mouse colony, University of Minnesota) and incubated at 37 °C for 10 min. The cells were then washed twice in the same diluent and suspended to 0.5% (v/v) in medium 199.
Assay of attachment and ingestion of red blood cells. Monolayers of macrophages were incubated with sensitized or control sheep red blood cells in a ratio of about 1:100 for 30 min in a humidified 5% CO₂ incubator at 37 °C. The coverslips were then washed vigorously with normal saline, air-dried quickly, stained with Wright's and Giemsa stains and examined with a Zeiss microscope at a magnification of × 1000. The macrophages with bound red cells (rosettes) were counted and scored for the number of red cells attached or ingested. Attached and ingested red blood cells were readily distinguishable on the basis of their morphological appearance; because of their envelopment by endocytotic vesicles ingested red cells appeared significantly larger than cells attached to the surface of the macrophage and also were less intensely coloured. Ten repeated counts of a macrophage from an LDV-infected mouse that was reacted with EAIgG yielded a mean ± S.E. of 20 ± 0.38 ingested and 48 ± 0.44 attached red cells. Corresponding values for an EAIgMC-reacted cell were 20 ± 0.33 ingested and 67 ± 0.99 attached red cells. At least 100 macrophages were counted on each of duplicate coverslips. The statistical significance of observed differences was assessed by Student's t-test.

Interferon assay. Interferon levels in plasma were measured by an endpoint dilution assay similar to that described by Rubinstein et al. (1981). The pH of plasma samples was adjusted to 2 by addition of 1 m-HCl and after storage at 4 °C for 5 days to about pH 7 by addition of 1 m-NaOH. Twofold dilutions of the plasma were prepared in Falcon 96-well microtitre plates in minimal essential medium which was supplemented with 10% (v/v) calf serum (growth medium). To each well with 100 μl plasma dilution were added 1 × 10⁴ L929 cells in 100 μl growth medium. The plates were incubated in a CO₂ incubator at 37 °C for 24 h. The medium was then aspirated and 5000 p.f.u. vesicular stomatitis virus (VSV) in 100 μl growth medium was added per well. L929 cells and VSV were propagated and VSV was titrated by plaque assay as described by Stueckemann et al. (1982). The plates were incubated for another 32 h and the cells then stained with 0.1% (w/v) crystal violet in 50% (v/v) ethanol. One unit of interferon was defined as the amount that reduced cell killing by VSV roughly 50% and was, within experimental error, equivalent to 1 international unit, in terms of the WHO international reference preparation of mouse interferon G-002-904-511 (provided by the Institute of Allergy and Infectious Diseases, NIH, Bethesda, Md., U.S.A.).

RESULTS

A comparison of the data in Table 1 shows the following. (i) A small percentage of peritoneal macrophages from LDV-infected or uninfected mice attached or ingested a small number of E or EAIgM, whereas the majority of the macrophages attached and ingested EAIgG and EAIgMC. (ii) In all instances, however, a greater proportion of macrophages from 1-day LDV-infected than from uninfected mice attached and ingested EAIgG and EAIgMC and in greater numbers per macrophage. For comparative purposes we have combined both parameters in the calculation of an index number. The differences between the index values for macrophages from uninfected and 1-day LDV-infected mice were highly significant for both attachment and ingestion (P < 0.02). (iii) Macrophages from 7-day LDV-infected mice, on the other hand, bound and ingested only a slightly greater number of sensitized red blood cells than those from uninfected mice and the differences were not significant.

Similar differences between macrophages from uninfected and LDV-infected mice were obtained whether the cells were cultured for 2 h (Table 1) or 24 h (data not shown). The cultivation of macrophages for 24 to 48 h per se caused an increase in the number of EAIgG and EAIgMC attached and ingested (compare macrophages from uninfected mice, Table 1, with mock-infected macrophages, Table 2), but macrophages from uninfected and LDV-infected mice were stimulated to about the same extent during 24 h in culture, so that the difference between them remained unaltered (data not shown). On the other hand, infection of
Table 1. Attachment of antibody- and complement-coated and untreated sheep red blood cells (SRBC) to and their ingestion by macrophages (MP) from uninfected and LDV-infected mice*

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Mice</th>
<th>Attachment</th>
<th>Ingestion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MP with bound</td>
<td>Bound</td>
<td>Ingested</td>
</tr>
<tr>
<td></td>
<td>SRBC (% of total)</td>
<td>SRBC (mean/MP)</td>
<td>Index†</td>
</tr>
<tr>
<td>EA</td>
<td>Uninfected</td>
<td>4 ± 2</td>
<td>1 ± 2</td>
</tr>
<tr>
<td></td>
<td>1-day infected</td>
<td>13 ± 4</td>
<td>2 ± 0</td>
</tr>
<tr>
<td></td>
<td>7-day infected</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>EA_{1G}</td>
<td>Uninfected</td>
<td>73 ± 13</td>
<td>7 ± 3</td>
</tr>
<tr>
<td></td>
<td>1-day infected</td>
<td>97 ± 1</td>
<td>27 ± 7</td>
</tr>
<tr>
<td></td>
<td>7-day infected</td>
<td>91</td>
<td>9</td>
</tr>
<tr>
<td>EA_{1M}</td>
<td>Uninfected</td>
<td>3 ± 1</td>
<td>1 ± 0</td>
</tr>
<tr>
<td></td>
<td>1-day infected</td>
<td>11 ± 0</td>
<td>1 ± 0</td>
</tr>
<tr>
<td></td>
<td>7-day infected</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>EA_{1G}</td>
<td>Uninfected</td>
<td>98 ± 2</td>
<td>26 ± 2</td>
</tr>
<tr>
<td></td>
<td>1-day infected</td>
<td>100 ± 0</td>
<td>38 ± 3</td>
</tr>
<tr>
<td></td>
<td>7-day infected</td>
<td>99</td>
<td>28</td>
</tr>
</tbody>
</table>

* Macrophages from uninfected and 1-day or 7-day LDV-infected mice were cultured for 2 h and then assayed for the binding and ingestion of EA, EA_{1G}, EA_{1M}, and EA_{1G}C as described in Methods. Values represent means from three or more repeat experiments ± S.E. of the mean or averages from two repeat experiments.

† Index: percentage of total macrophages with bound or ingested red cells × mean number of red cells bound or ingested/macrophage. The index was calculated for each experiment and the values represent the means ± S.E.

‡ Calculated by Student’s t-test for index values for macrophages from uninfected and 1-day infected mice.
Table 2. Attachment of antibody- and complement-coated and untreated sheep red blood cells (SRBC) to and their ingestion by macrophages (MP) infected with LDV or mock-infected in culture*

<table>
<thead>
<tr>
<th>Reagent</th>
<th>MP with bound SRBC</th>
<th>Bound SRBC (mean/total) MP)</th>
<th>Index†</th>
<th>MP with ingested SRBC</th>
<th>Ingested SRBC (mean/total) MP)</th>
<th>Index†</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>Mock-infected</td>
<td>1 ± 0</td>
<td>2 ± 0</td>
<td>2 ± 0</td>
<td>2 ± 1</td>
<td>2 ± 2</td>
</tr>
<tr>
<td></td>
<td>LDV-infected</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>EA_{IgG}</td>
<td>Mock-infected</td>
<td>86 ± 8</td>
<td>12 ± 5</td>
<td>1156 ± 446</td>
<td>91 ± 2</td>
<td>7 ± 1</td>
</tr>
<tr>
<td></td>
<td>LDV-infected</td>
<td>93</td>
<td>12</td>
<td>1116</td>
<td>88</td>
<td>8</td>
</tr>
<tr>
<td>EA_{IgM}</td>
<td>Mock-infected</td>
<td>1 ± 1</td>
<td>1 ± 0</td>
<td>2 ± 1</td>
<td>3 ± 1</td>
<td>1 ± 0</td>
</tr>
<tr>
<td></td>
<td>LDV-infected</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>EA_{IgM}C</td>
<td>Mock-infected</td>
<td>100 ± 0</td>
<td>39 ± 2</td>
<td>3878 ± 163</td>
<td>51 ± 9</td>
<td>4 ± 1</td>
</tr>
<tr>
<td></td>
<td>LDV-infected</td>
<td>100</td>
<td>33</td>
<td>3300</td>
<td>70</td>
<td>4</td>
</tr>
</tbody>
</table>

* Macrophages from uninfected mice were cultured for 2 h and then infected with LDV at an m.o.i. of about 20 ID_{50}/cell or mock-infected. After another 22 h incubation, the cells were assayed for the binding and ingestion of E, EA_{IgG}, EA_{IgM}, and EA_{IgM}C as described in Methods. Values represent means ± s.e. from three experiments with mock-infected cultures and a single experiment with LDV-infected cultures.

† Index: percentage of total macrophages with bound or ingested red cells x mean number of red cells bound or ingested/macrophone.

macrophages with LDV after they had been put in culture had no significant effect on their binding of EA_{IgG} or EA_{IgM}C (Table 2).

LDV infection of mice had no significant effect on the composition of the population of cells harvested from the peritoneum. The cell yields from uninfected, 1-day or 7-day LDV-infected mice were 2 × 10^6 to 4 × 10^6 cells/mouse and consisted on average of about 1% polymorphonuclear cells, 1% eosinophils, 7% lymphocytes, 3% basophils and 88% macrophages. Intraperitoneal and intravenous injection of about 3 × 10^{13} washed polystyrene latex particles per mouse (diam. 85 nm; Dow Chemical Co., Midland, Mich., U.S.A.) increased the cell yield after 1 day to about 1 × 10^7 cells/mouse which consisted of 34% polymorphonuclear cells, 12% eosinophils, 4% lymphocytes, 1% basophils and 49%
macrophages. When cultured, the latter cells attached and ingested sensitized sheep red blood cells to a similar or even slightly lesser extent than macrophages from uninfected mice (data not shown).

The increased Fc and C3 receptor activity of peritoneal macrophages from LDV-infected mice correlated with plasma LDV and interferon levels in these mice (Fig. 1). The latter attained a maximum 1 day post-infection and then decreased precipitously during the next 2 days. Significant levels of interferon were not detected beyond 3 days post-infection.

**DISCUSSION**

The enhanced ability of macrophages from 1-day infected mice to attach and ingest EA$_{\text{IgG}}$ and EA$_{\text{IgM}}$C indicates an increased density of Fc and C3 receptors on these cells (Bianco et al., 1975; Kerbel, 1976; Michl et al., 1976), but an increased binding affinity of the receptors and increased general phagocytic activity of the macrophages may be contributing factors. These changes probably reflect an activation of these cells. Activation does not result from the intraperitoneal injection *per se* since injection of latex particles which greatly increased the yield of peritoneal cells had no enhancing effect on the receptor activity of the macrophages. In fact, receptor concentration was slightly reduced as also observed with latex-treated human monocytes (Schmidt & Douglas, 1972). A 24 h cultivation of macrophages *in vitro* also caused a slight increase in receptor activity, probably as a result of ingestion of serum components (Bianco et al., 1975; Cohn & Benson, 1965), but macrophages from uninfected and LDV-infected mice were stimulated to a similar degree.

Increased receptor concentration was also not a response of macrophages to infection with LDV *per se*, since practically all cells in these populations showed enhanced Fc and C3 receptor activity, whereas generally only 6 to 10% of the total peritoneal macrophages are permissive for LDV and can be productively infected (Tong et al., 1977). Furthermore, we have observed that few, if any, peritoneal macrophages isolated from mice 24 h after infection with LDV (<0.1% of total) produce virus RNA or infectious virus or can be productively infected in culture (Stueckemann et al., 1982). These and other results have suggested that most of the permissive cells in a mouse become infected with LDV upon primary infection and are lost 24 h after infection (Stueckemann et al., 1982).

Thus, macrophage activation results from an indirect effect of LDV infection and is thus clearly distinct from the induction of Fc receptors on non-phagocytic cells by herpes simplex virus type 1 (Para et al., 1980; Westmoreland & Watkins, 1974) or cytomegalovirus (Keller et al., 1976; Westmoreland et al., 1976). In the latter cases a virus glycoprotein is an Fc receptor (Para et al., 1980) and an increase in receptor activity is, therefore, confined to cells that are actively infected with virus. In mice acutely infected with LDV, on the other hand, macrophage activation is most likely caused by interferon which is rapidly, but transiently, produced in mice after a primary infection with LDV (Du Buy et al., 1973; Evans & Riley, 1968; see also Fig. 1) and has been clearly shown to cause an activation of macrophages (Schultz & Chirigos, 1978; Schultz et al., 1977) and also to enhance the Fc and C3 receptor activity of peritoneal macrophages (Hamburg et al., 1978). In fact, the production of interferon in infected mice correlates well with the enhanced Fc and C3 receptor activity of their macrophages. The interferon level in LDV-infected mice reaches a maximum about 1 day post-infection and then decreases rapidly until none can be detected by about 3 days post-infection. Our data are in complete agreement with those reported previously (Du Buy et al., 1973; Evans & Riley, 1968).

The interferon is probably produced in infected mice by the macrophages that are productively infected with LDV since in the case of positive-strand viruses, such as LDV (Brinton-Darnell & Plagemann, 1975), gene expression is required for interferon induction (Fuller & Marcus, 1980a, b). We have estimated that $10^6$ to $10^7$ macrophages become
Productively infected in a mouse after a primary infection with LDV (Stueckemann et al., 1982). During the chronic stage of LDV infection, which is sustained by passage of the virus to newly formed permissive cells, too few of such cells become generated and productively infected (100 to 1000/day) to raise the blood interferon titre to detectable levels (Stueckemann et al., 1982). In agreement, we found that the Fc and C3 receptor activity of macrophages of infected mice had returned to close to normal 7 days post-infection. In cell cultures also, an insufficient number of macrophages becomes productively infected to raise interferon levels in the medium significantly above background, even during the acute phase of infection (Stueckemann et al., 1982) and, therefore, LDV infection does not cause macrophage activation.

An increased phagocytosis of $\text{EA}_{\text{IgG}}$ by macrophages from LDV-infected mice has also been observed by Stevenson et al. (1980), but their data differ significantly from ours. The investigators found that the phagocytic activity of macrophages from C3H mice for $^{51}\text{Cr}$-labelled sheep red blood cells sensitized with rabbit anti-Forssman antibody was greatest 4 to 6 days post-infection and remained elevated for more than 25 days post-infection, but the reasons for this prolonged effect of LDV infection are not known.

Interferon-activated mouse macrophages have been shown to possess non-antibody-dependent tumouricidal activity in vitro (Schultz & Chirigos, 1978; Schultz et al., 1977). The activated macrophages in mice acutely infected with LDV, however, do not seem to play a significant role in the suppression of tumour cell growth in these animals since the survival of tumour cells is enhanced rather than depressed during the acute phase of infection, most likely as a result of the depression of the cellular immune response (Michaelides & Schlesinger, 1974). On the other hand, the depression of the cellular immune response, as well as the enhanced humoral antibody response in acutely infected mice, coincide with or closely follow the postulated destruction of the productively infected macrophages and the activation of the remaining macrophages by interferon, and could be causally related. For example, soluble factors, possibly interferon or other products released from macrophages, have been implicated in causing the slight destruction of lymphocytes in thymus-dependent areas of lymphatic tissues observed in 1 and 2 day infected mice (Rowson & Mahy, 1975). Lymphocyte depletion is associated with an accumulation of macrophages, many containing cell debris, in the affected areas (Rowson & Mahy, 1975).

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