Ia Antigens and Fc Receptors of Mouse Peritoneal Macrophages as Determinants of Susceptibility to Lactic Dehydrogenase Virus

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(Accepted 14 March 1985)

SUMMARY

The relationship between susceptibility of mouse peritoneal macrophages to lactic dehydrogenase-elevating virus (LDV) infection and expression of I region-coded antigens (Ia) on these cells was investigated. The proportion of Ia-positive cells in resident peritoneal macrophages from adult and suckling mice were 4 to 10% and 50 to 70% respectively. Approximately the same percentage of the cells were susceptible to LDV, as detected by fluorescent antibody staining. In adult mice, double-labelling experiments showed that most of the Ia-positive cells were LDV-infected. When the cells were cultured for more than 24 h in vitro, Ia-positive cells rapidly disappeared and the culture became resistant to LDV. Removal of Ia-positive cells by treatment with anti-Ia plus complement or enrichment using an anti-Ia-coated Petri dish simultaneously removed or enriched for LDV-susceptible cells. Treatment of cells with trypsin (1 mg/ml) removed their I-A and I-E antigens and simultaneously abolished susceptibility for LDV. When LDV was preincubated with subneutralizing amounts of antibody, infectivity for macrophages was enhanced and the proportion of LDV-infected cells was higher than that of Ia-positive cells. This suggests that Fc receptors on macrophages can act as receptors for LDV coated with antiviral IgG.

INTRODUCTION

Lactic dehydrogenase-elevating virus (LDV) causes a non-lethal infection in mice and no significant pathological alterations are reported. Infection is persistent and life-long, certain plasma enzymes are elevated, and there are immunological perturbations. Elevated plasma enzymes appear to be due to disturbance of reticuloendothelial cell clearance of enzymes in infected mice (Rowson & Mahy, 1975). The replication of LDV is limited to a specific subpopulation of macrophages (Stueckemann et al., 1982a) and occurs in only 5 to 20% of starch-induced peritoneal macrophages, even when multiplicities of infection (m.o.i.) of 10^4 ID_{50}/cell or more were used. These authors suggested that infected cells were eliminated and that continuous generation of additional susceptible cells was the factor accounting for persistent infection in vivo. We have confirmed the results of Stueckemann et al. (1982a) in showing that only 4 to 10% of resident peritoneal macrophages are susceptible to LDV, and that this correlates very well with the percentage of I region-coded antigen (Ia)-positive cells. Inhibition of infection by pretreatment of susceptible macrophages with monoclonal anti-Ia antibody or pretreatment of the virus with purified Ia antigens indicated that the Ia antigens are receptors for LDV (Inada & Mims, 1984). We report here further studies on the close correlation between Ia antigen expression in macrophages and susceptibility to LDV.

METHODS

Mice. Outbred CD-1 and inbred C57BL/6 mice were used as adults (4 to 8 weeks old) or as sucklings of various ages. All mice were maintained as specific pathogen-free animals and obtained from the Animal Breeding Centre of Guy's Hospital Medical School.

Virus. LDV (kindly donated by Dr K. E. K. Rowson) in the form of serum was obtained from mice injected intraperitoneally (i.p.) 24 h previously. The sera were collected by heart puncture and stored at −70 °C. The
pooled serum contained approximately $10^{11}$ median infectious doses (ID$_{50}$) per ml. Virus titres were estimated from measurements of plasma lactic dehydrogenase activity 5 days after inoculation of tenfold dilutions of material into groups of mice (Wroblewski & Landu, 1955).

*Preparation of cells and LDV injection* in vitro. Peritoneal macrophages from normal mice were collected by peritoneal lavage using MEM supplemented with 10% newborn calf serum (NCS) and 5 U/ml heparin, and incubated in culture dishes as described previously (Inada & Mims, 1984). After 3 h, the cultures were washed vigorously with phosphate-buffered saline (PBS) to remove unattached cells. At various times, the cells were infected with LDV at an m.o.i. of $10^2$ to $10^4$ ID$_{50}$/cell at 37°C for 1 h, washed three times with warmed MEM and cultured in medium (MEM supplemented with 10% NCS, 100 µg/ml penicillin and 100 units/ml streptomycin) in 5% CO$_2$ in air at 37°C for 7 to 10 h.

**Anti-Ia antibodies.** To detect Ia antigens monoclonal mouse anti-rat Ia antibodies which cross-react with mouse I-A (OX-6, OX-3) kindly donated by Dr A. F. Williams (Fukumoto et al., 1982) and anti-I-E antibody (H-10.81.10), kindly donated by Dr M. Pierres (Pierres et al., 1980) were used. These antibodies were reported to be specific for Ia.17,18 (OX-6), and Ia.9 (OX-3) of the I-A-coded molecule, and Ia.7 of the I-E-coded molecule.

For complement (C)-dependent cytolyis of Ia antigen-positive macrophages, or double-labelling experiments, rabbit anti-rat Ia serum was used [kindly donated by Dr A. F. Williams (Fukumoto et al., 1982)]. This serum cross-reacts with mouse I-E-coded molecules. In double-labelling experiments with fluorescein and rhodamine, using rabbit antiserum to I-E and mouse monoclonal antibody to I-A, it had been shown that I-A and I-E antigens were present on the same macrophages (data not shown). Therefore, the antiserum reacting with mouse I-E plus C removes simultaneously the cells expressing I-E and I-A antigens.

**Anti-LDV serum.** Anti-LDV hyperimmune serum was prepared in mice by i.p. injection of $10^9$ to $10^{10}$ ID$_{50}$ of LDV at 2-week intervals after the last injection the sera were collected, inactivated at 56°C for 30 min, pooled and stored at −20°C. A large proportion of the virus and virus–antibody complexes present in the sera were removed by ultracentrifugation at 105000 g for 1 h.

**Immunofluorescence (IF) staining.** For detection of Ia antigen on the cell surface, the cells were fixed with 1% paraformaldehyde for 10 min at room temperature, washed with PBS and stained with monoclonal anti-Ia antibody (OX-6) and anti-I-E antibody (H-10.81.10) at a concentration of 10 µg/ml followed by fluorescein isothiocyanate (FITC)-labelled anti-mouse IgG (1/300 to 1/600, Nordic Immunological Laboratories, Maidenhead, U.K.).

Negative controls included normal mouse serum instead of OX-6 or OX-3, and also macrophages from C57BL/6 (H-2b) mice stained with OX-6, and with anti-I-E antibody which has been shown not to react with H-2b strain macrophages (Fukumoto et al., 1982). Fluorescence-positive cells were not seen with any of these negative controls, in contrast to positive staining of C57BL/6 macrophages with OX-3, and CD-1 mouse macrophages with OX-3, OX-6 and anti-I-E monoclonal antibodies. For LDV antigens, infected cells were fixed with acetone for 10 min and stained with antiserum (1/100) followed by FITC-labelled anti-mouse IgG (1/100). There was no staining in uninfected cells stained with anti-LDV serum or in infected cells stained with normal mouse serum or hyperimmune anti-mouse cytomegalovirus serum.

Double-labelling experiments were performed using macrophages infected with LDV 7 h previously. The infected, fixed, cells were first labelled with rabbit anti-Ia and rhodamine-labelled goat anti-rabbit IgG, then washed, dried and fixed with acetone followed by staining with anti-LDV serum and FITC-labelled anti-mouse IgG.

The mean number of Ia or LDV antigen-positive cells was calculated after counting 3000 to 5000 cells on each of two to four ring cultures.

**Complement-dependent cytotoxicity.** To remove Ia-positive macrophages, peritoneal exudate cells were incubated with serially diluted rabbit anti-Ia serum at 4°C for 20 min. The cells were washed once with ice-cold MEM and incubated with 1/7 diluted guinea-pig complement (Wellcome) at 37°C for 45 min. Complement was dialysed against PBS overnight at 4°C before use. The cells were washed with cold MEM three times and $2 \times 10^5$ viable cells (i.e. which excluded trypan blue) seeded to each ring culture.

**Enrichment of Ia-positive cells.** Ia-positive cells in peritoneal macrophage preparations were enriched by attachment to an anti-Ia antibody-coated Petri dish (Wysocki & Sato, 1978). Monoclonal anti-I-A (OX-6) or I-E antibody, with normal mouse serum as control, 1/10 diluted with PBS, was placed in a Polystylen plastic dish (Sterilin) and incubated at 4°C overnight. The plate was then washed five times with PBS and peritoneal exudate cells (10⁶ per ml) from CD-1 or C57BL/6 mice added. After 1 h incubation at 4°C, the plate was gently swirled to remove unattached cells. The attached cells were washed gently twice with PBS, detached by pipetting, washed once and seeded to ring culture as described above. After 3 h, the cultures were washed vigorously with PBS to remove non-adherent cells.

**Antibody-mediated enhancement of LDV for macrophages.** LDV (10⁸ to 10⁹ ID$_{50}$ in 0·1 ml MEM supplemented with 4% NCS) was preincubated with 0·1 ml of serially diluted antiserum, with normal mouse serum or PBS as control at 4°C for 1 h. Macrophage cultures were infected (0·1 ml; m.o.i. 10⁻¹ ID$_{50}$/cell) at 37°C for 1 h, washed
three times with warmed MEM and incubated in medium. At 7 h post-infection, the cells were dried, fixed with acetone and stained with anti-LDV serum.

For inhibition of antibody-mediated enhancement, macrophages were pretreated with monoclonal anti-Fc receptor antibody (anti-Fc rec) for 20 min at 4 °C, washed with MEM and then infected with antiserum-treated virus. Control cultures were pretreated with culture medium of myeloma P3U.1. Both reagents were kindly provided by Dr J. S. Porterfield (Peiris et al., 1981); anti-Fc rec has been shown to recognize Fc receptor II which binds mouse IgG2b and IgG1 (Unkeless, 1979).

Preparation of F(ab')2 fragment of anti-LDV IgG. Anti-LDV IgG was precipitated three times by one-third saturated (NH₄)₂SO₄, digested with pepsin (Sigma, 1:60000), and purified F(ab')₂ obtained with a Sephadex G200 column (Madsen & Rodkey, 1976). Specificity of purified F(ab')₂ was confirmed by Ouchterlony double-immunodiffusion test using rabbit anti-mouse IgGFc fragment antibody (Nordic Immunological Laboratories).

Trypsin treatment of macrophages. The cultured macrophages were incubated with various concentrations of trypsin (Cambrian Chemicals Ltd., Croydon, U.K.) in MEM at 37 °C for 30 min. The cells were washed three times with warmed MEM and infected with LDV at an m.o.i. of 10³ ID₅₀/cell. Separate cultures were fixed and stained for Ia antigens.

RESULTS

Expression of Ia antigens on macrophages in relation to their susceptibility to LDV

Between 4 and 10% of resident peritoneal macrophages express Ia antigens (Beller & Unanue, 1981; Inada & Mims, 1984) and the same proportion of cells are susceptible to LDV infection. The expression of Ia antigens decreases rapidly on culture of macrophages in vitro, becoming undetectable by 24 to 48 h (Beller & Unanue, 1981; Snyder et al., 1982). LDV (10³ ID₅₀/cell) was added to cultures at various times after their initiation and at the same time the proportion of Ia-positive cells was determined. Susceptibility to LDV was scored by fluorescent antibody staining 8 h after infection. Fig. 1 shows that the decreased expression of Ia antigens was paralleled by a decreased susceptibility to LDV infection. There were virtually no Ia-positive and no LDV-infectible cells in cultures maintained for 48 h.

Age-dependent susceptibility of peritoneal macrophages to LDV infection

The peritoneal macrophages from mice of different ages were infected with LDV and the percentage of LDV-infectible and Ia-positive cells determined. Fig. 2 shows that peritoneal macrophages from neonatal mice were highly susceptible to LDV infection and there was a rapid decrease in susceptibility until at 4 to 8 weeks of age the proportion of LDV-infected cells reached the adult level (10%). Changes in LDV susceptibility were paralleled by changes in expression of Ia antigen and at any age the proportion of Ia antigen-positive cells was similar to LDV-infectible cells. Fluorescence staining of Ia antigens on macrophages from suckling mice was clearly seen, but was very faint when compared to that on macrophages from adult mice. In this experiment, the m.o.i. was 10⁴ ID₅₀/cell. When LDV was used at 10³ or 10² ID₅₀/cell considerably fewer macrophages from suckling mice were infected.

The high proportion of suckling mouse peritoneal cells infected in vitro was confirmed in an experiment in vivo. Mice (7 days old) were given LDV i.p., and 40 to 50% of peritoneal cells were found to be infected 24 h later.

Removal or enrichment of Ia-positive macrophages is accompanied by parallel changes in LDV susceptibility

Fresh peritoneal cells from CD-1 mice were treated with various dilutions of rabbit anti-I-E serum plus C and then infected with LDV (10² ID₅₀/cell) while duplicate cultures were fixed for Ia staining. Treatment with anti-Ia serum diluted up to 1/100 killed 10 to 15% of cells as determined by trypan blue exclusion. The proportion of I-A-positive macrophages stained with OX-6 fell from about 12% to 1 to 2% and there was a similar fall in the number of LDV-susceptible cells (Table 1). When macrophage cultures were enriched for Ia-positive cells by the use of an anti-Ia-coated Petri dish, as described in Methods, the proportion of LDV antigen-positive cells was increased to a similar extent (Table 2). In C57BL/6 (H-2b) peritoneal exudate cells used as control, there was no increase of Ia-positive and LDV-infected macrophages (Table 2).
Fig. 1. Expression of Ia antigens on peritoneal macrophages and susceptibility to LDV. Peritoneal macrophages from CD-1 mice were cultured in vitro and at various times after initiation of the culture, cells were infected with LDV at an m.o.i. of $10^3$ ID$_{50}$/cell. At the time of initiation of infection, separate cultures were stained for Ia antigen (●). Susceptibility to LDV (○) was scored 8 h after infection by fluorescent antibody staining. Each value represents the mean from counts on two ring cultures.

Fig. 2. Age-dependent susceptibility of peritoneal macrophages to LDV. Peritoneal macrophages from CD-1 mice of different ages were collected, cultured and infected with LDV at an m.o.i. of $10^3$ ID$_{50}$/cell. The LDV-susceptible (○) and Ia-positive (●) cells were scored as described in Fig. 1. Each point represents the mean value obtained from counts on two or three ring cultures. Each ring was derived from pooled macrophages collected from three to six mice.

Fig. 3. Effect of trypsin treatment of macrophages on LDV susceptibility and expression of Ia antigens. Peritoneal macrophages from CD-1 mice were incubated with trypsin (0.1 to 1 mg/ml) in MEM at 37 °C for 30 min and infected with LDV at an m.o.i. of $10^3$ ID$_{50}$/cell. Eight h later the cells were scored for LDV infection (○) by fluorescent staining. At the time of initiation of infection, separate cultures were stained with anti-I-A (●) and anti-I-E (▲) antibodies. Each value represents the mean from counts on two ring cultures. C, Control macrophages incubated without trypsin.
Ia antigens as LDV receptors

Table 1. Removal of LDV-susceptible macrophages by anti-Ia plus complement

<table>
<thead>
<tr>
<th>Anti-Ia dilution</th>
<th>Ia-positive macrophages (%)</th>
<th>LDV-positive macrophages (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/5</td>
<td>1.2†</td>
<td>1.3</td>
</tr>
<tr>
<td>1/10</td>
<td>1.5</td>
<td>1.6</td>
</tr>
<tr>
<td>1/20</td>
<td>1.3</td>
<td>1.2</td>
</tr>
<tr>
<td>1/80</td>
<td>1.1</td>
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</tr>
<tr>
<td>1/100</td>
<td>1.9</td>
<td>2.3</td>
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<tr>
<td>1/120</td>
<td>9.1</td>
<td>8.3</td>
</tr>
<tr>
<td>1/160</td>
<td>12.5</td>
<td>11.6</td>
</tr>
<tr>
<td>NRS (1/5)*</td>
<td>11.0</td>
<td>10.5</td>
</tr>
</tbody>
</table>

* NRS, Normal rabbit serum.
† Each figure represents the mean value of two ring cultures.

Table 2. Enrichment of LDV-susceptible macrophages by anti-Ia-coated Petri dish

<table>
<thead>
<tr>
<th>Petri dish coated with</th>
<th>Ia-positive macrophages (%)</th>
<th>LDV-positive macrophages (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-I-A (OX-6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expt. 1</td>
<td>40.2†</td>
<td>32.9</td>
</tr>
<tr>
<td>Control (NMS)*</td>
<td>12.1</td>
<td>14.4</td>
</tr>
<tr>
<td>Expt. 2</td>
<td>35.4</td>
<td>37.3</td>
</tr>
<tr>
<td>Control (NMS)</td>
<td>11.4</td>
<td>9.6</td>
</tr>
<tr>
<td>Expt. 3†</td>
<td>16.4</td>
<td>13.9</td>
</tr>
<tr>
<td>Control (NMS)†</td>
<td>17.2</td>
<td>15.8</td>
</tr>
<tr>
<td>Anti-I-E (H-10,81,10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expt. 1</td>
<td>29.2</td>
<td>22.1</td>
</tr>
<tr>
<td>Control (NMS)</td>
<td>7.8</td>
<td>8.5</td>
</tr>
<tr>
<td>Expt. 2</td>
<td>35.0</td>
<td>30.8</td>
</tr>
<tr>
<td>Control (NMS)</td>
<td>8.4</td>
<td>9.8</td>
</tr>
<tr>
<td>Expt. 3†</td>
<td>15.3</td>
<td>16.1</td>
</tr>
<tr>
<td>Control (NMS)†</td>
<td>17.2</td>
<td>15.8</td>
</tr>
</tbody>
</table>

* NMS, Normal mouse serum.
† Peritoneal exudate cells from C57BL/6 mice.
‡ Each figure represents the mean value of two ring cultures.

Table 3. Double-staining of infected macrophages with anti-Ia and anti-LDV serum

<table>
<thead>
<tr>
<th>Time after initiation of cell culture (h)</th>
<th>Ia-positive macrophages (%)</th>
<th>LDV-positive macrophages (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3* (0)†</td>
<td>11.0</td>
<td>12.5 (52.1)§</td>
</tr>
<tr>
<td>10 (7)†</td>
<td>5.2 (84.6)‡</td>
<td></td>
</tr>
</tbody>
</table>

* Each figure represents the mean value derived from two ring cultures.
† Figures in parentheses indicate time after infection.
‡ Figure in parentheses indicates the percentage of Ia-positive cells that were LDV-positive.
§ Figure in parentheses indicates the percentage of LDV-positive cells that were Ia-positive.

Double-labelling experiment

To see whether Ia antigen-positive cells and LDV-susceptible cells belong to the same population, double-staining experiments were carried out. Peritoneal macrophages from adult mice were infected with LDV and 7 h later double-labelled with anti-LDV and anti-Ia antibodies. As shown in Table 3, most of the Ia-positive cells (84.6%) were LDV-infected. There were, however, a substantial proportion of LDV-infected cells which did not express Ia antigen (Table 3). This is probably due to decreased expression of Ia antigen that occurs after in vitro culture of cells for 10 h. Thus, at the time of infection, 11.0% of cells were Ia-positive, which is very similar to the proportion that were LDV-infected 7 h later (12.5%).
Fig. 4. Antibody-mediated enhancement of LDV infection for macrophages. LDV (10^9 IDs₀/ml) was preincubated with serially diluted anti-LDV serum (●) or NMS (○) at 4 °C for 1 h, then added to macrophages from CD-1 mice which had been incubated in vitro for 24 h to reduce the number of Ia-positive cells. The treated cells were then tested for infection as described in Methods. For inhibition of antibody-mediated enhancement of virus infectivity, macrophages were pretreated with anti-Fc receptor antibody (culture medium of hybridoma) (▲) or culture medium of myeloma P3U.1 (▲) at 4 °C for 20 min, washed with PBS and infected with antibody-treated virus. The percentage of Ia antigen-positive cells at the time of initiation of infection was 1.3% ± 0.3 (mean ± 1 s.d.) and about the same proportion of LDV-infected cells was observed 7 h after infection with control (PBS-treated) virus (1.4% ± 0.3). Each bar represents 1 standard error of the mean calculated from separate counts on four ring cultures.

Fig. 5. No enhancement by antiviral F(ab')₂ fragment. Preparations of anti-LDV antibody IgG (○) and its F(ab')₂ (■) fragments were diluted and preincubated with LDV (10^8 ID₅₀/ml) and infection scored by FA staining 7 h post-infection. Number of infected cells as percentage of control (PBS-treated) value was calculated as in Fig. 4. Each point represents the mean derived from counts from two ring cultures.

Effect of trypsin treatment of macrophages on their susceptibility to LDV and on their Ia antigens

Peritoneal macrophages from adult mice were treated with various concentrations of trypsin (0.1 to 1 mg/ml). The cells were infected with LDV and at the time of infection the percentage of Ia-positive cells were scored. As shown in Fig. 3, cells treated with trypsin at concentrations of 0.5 to 1 mg/ml showed decreased Ia expression, which is paralleled by decreased LDV susceptibility. Ia antigens are therefore trypsin-sensitive and removal of these antigens abolishes susceptibility to LDV. We have observed that Ia antigens were not re-expressed until 7 h after trypsin treatment. LDV susceptibility reappeared at the same time (data not shown).

Antibody-mediated enhancement of LDV replication in macrophages

There is substantial evidence for antibody-mediated enhancement of virus infectivity (Halstead et al., 1973; Peiris & Porterfield, 1979). For instance, the infectivity of certain togaviruses for human monocytes or mouse macrophage-like cell lines is enhanced by subneutralizing amounts of antibody or by non-neutralizing antibody. Antibody-mediated enhancement has been shown to be mediated via interaction between the Fc portion of antiviral
IgG antibody and Fc receptors on macrophages (Peiris et al., 1981). In a brief report, antibody-mediated enhancement was described for LDV (Cafruny & Plagemann, 1982). We therefore tested for the phenomenon with a view to determining whether Ia-negative macrophages are infectible with LDV via Fc receptors. The result (Fig. 4) shows a significant increase in the percentage of infected cells (about 3-5 times) in macrophages cultured for 24 h at antibody concentrations of $5 \times 10^{-1}$ and $10^{-2}$. This increase was inhibited by pretreatment with monoclonal anti-Fc receptor antibody.

No enhancement by antiviral F(ab')$_2$ fragment

F(ab')$_2$ fragment of anti-LDV IgG was prepared and adjusted to the original protein concentration of the separated IgG (10 mg/ml). The IgG and F(ab')$_2$ preparations were diluted and preincubated with LDV ($10^{10}$ ID$_{50}$/ml) and infection scored. Whereas the enhancement was observed when virus was treated with antiviral IgG diluted to $10^{-2}$ and $2 \times 10^{-2}$, there were no such effects when the F(ab')$_2$ fragment was used (Fig. 5).

DISCUSSION

LDV has been reported to replicate exclusively in macrophages (Herman et al., 1966; Porter et al., 1968) but only a few percent of these cells are susceptible (Tong et al., 1977; Stueckemann et al., 1982). In a previous report, we showed that the percentage of Ia-positive macrophages from various organs corresponded closely with that of LDV-susceptible cells and produced evidence that Ia antigens acted as receptors for LDV (Inada & Mims, 1984). The above experiments support the close relation between expression of Ia antigens on macrophages and their susceptibility to LDV.

In peritoneal macrophage cultures the percentage of Ia-positive cells rapidly decreased from 4 to 10~ to undetectable levels over 24 to 48 h (Beller & Unanue, 1981). This was accompanied by a progressive decrease in susceptibility to LDV infection. Similar observations were reported by Stueckemann et al. (1982a), who scored viral replication by viral RNA synthesis as determined by autoradiography.

Susceptibility of macrophages to LDV infection is age-dependent, peritoneal macrophages from neonatal mice being highly susceptible and susceptibility gradually decreasing with age. This was closely correlated with the decline in the proportion of Ia-positive peritoneal macrophages with age. The intensity of staining for Ia antigen on macrophages from neonatal mice was very faint, suggesting a low density of antigen on these cells. It has been reported by Lu et al. (1979) that the proportion of Ia-positive cells in peritoneal macrophages from suckling mice is low when compared to that of adult mice. If, however, the density of Ia antigens on neonatal mouse macrophages is low, the complement-dependent cytotoxicity test employed by these workers might not have been sensitive enough to detect them. A high susceptibility of macrophages from neonatal or suckling mice has been observed for several viruses (Mogensen, 1979). In the case of LDV it is suggested that this is due to Ia antigen expression in a high proportion of these macrophages. There is evidence that the limited susceptibility of 4-week-old mouse peritoneal macrophages to vesicular stomatitis virus or encephalomyocarditis virus is due to endogenously produced interferon (Belardelli et al., 1984). The proportion of macrophages susceptible to infection with these viruses increased from 4 to 6~ to over 80~ when anti-interferon serum was injected intraperitoneally. In the case of LDV, however, treatment of mice with anti-interferon serum (from immune sheep, kindly donated by Dr I. Gresser) did not affect the susceptibility to infection of peritoneal macrophages from 4-week-old mice (unpublished observation).

Pretreatment of peritoneal exudate cells with anti-Ia antibody plus complement led to more than a 90~ reduction in susceptibility to LDV. Conversely, enrichment of Ia-positive cells led to a similar enrichment in LDV-susceptible cells. Double-labelling experiments suggested that most of the Ia-positive cells are infected with LDV. Ia antigens are trypsin-sensitive and removal of these antigens simultaneously abolished LDV susceptibility. All these results extend and confirm the original finding that LDV selectively infects Ia-positive cells. If infected Ia-positive macrophages suffer functional impairment or death, profound effects on immunological function are to be expected (Unanue, 1981; Isakov et al., 1982).
When LDV was preincubated with appropriately diluted antiviral antibody there was a significant increase in the percentage of infected macrophages, confirming the results of Cafruny & Plagemann (1982). The enhancement was not seen when the F(ab')2 fragment of antiviral antibody was used or when macrophages were pretreated with monoclonal anti-Fc receptor antibody. It is concluded that enhancement is mediated via interaction between the Fc portion of IgG and Fc receptors on macrophages, and is not dependent on Ia antigens acting as receptors.

Although there was a report which suggests that Ia antigens and Fc receptors are closely located on the membrane (Dickler & Sachs, 1974), later studies indicate that these molecules are separate entities and move independently on the cell surface (Schirrmacher et al., 1975; Rask et al., 1975). Helenius et al. (1978) reported that H-2 antigens are receptors for Semliki Forest virus (SFV). H-2 antigens, however, are not the only receptors for SFV because H-2-negative mouse cells are susceptible to this virus (Oldstone et al., 1980). It is possible that Ia antigens are not the sole receptors for LDV. The use of Fc receptors by LDV when coated with enhancing antibody is a separate but interesting phenomenon, which could be relevant in vivo.

What implications do these findings have for the mechanism of persistent infection with LDV? Persistently infected mice have viraemias of about $10^4$ ID$_{50}$/ml, the reduction from peak titres of $10^{10}$ or $10^{11}$ ID$_{50}$/ml occurring largely during the first few days after infection (Rowson & Mahy, 1975). There is evidence that Ia-positive macrophages are selectively eliminated following infection in vitro (Tong et al., 1977; Inada & Mims, 1985) and for a day or two after initial infection of mice, Ia-positive macrophages in spleen and peritoneal cavity are almost undetectable. This is shortly followed by recovery to one-half to one-third of normal levels (Inada & Mims, 1985) presumably due to constant regeneration of Ia-positive cells. Depletion of susceptible cells, therefore, could only account for a small reduction in viraemia levels. The striking fall from $10^{10}$ or $10^{11}$ to $10^5$ ID$_{50}$/ml is likely to be due to the operation of host defences. Du Buy et al. (1971) showed that viraemia levels in persistently infected mice increased 100-fold after treatment with cyclophosphamide, suggesting that virus replication is controlled by immune mechanisms. There is so far no evidence for cell-mediated immunity in LDV infection. Neutralizing antibodies are detectable by in vivo methods about 2 months after infection, although the virus present in plasma at that time resists neutralization because it is complexed with non-neutralizing antibody (Notkins et al., 1966; Rowson et al., 1966). Furthermore, the data in Fig. 5 suggest that antisera can neutralize LDV infection of macrophages in vitro. Replication of LDV in macrophages in persistently infected mice, therefore, is probably partly controlled by antibody, but protection of circulating infectious virus by non-neutralizing antibody is a major factor in persistence.

REFERENCES


Ia antigens as LDV receptors


(Received 11 January 1985)