Early Interaction between Mouse Hepatitis Virus 3 and Cells

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SUMMARY

The interaction between mouse hepatitis virus 3 (MHV3) and cells was studied in order to investigate whether or not early events occurring after infection could be involved in the difference in virus replication seen between mouse strains with different genetic sensitivities to MHV3 infection. Kinetic data showed that MHV3 uptake by both macrophages and L cells was time- and temperature-dependent. In addition, treatment of cells with cytochalasin B or prostaglandin E1, prior to virus infection, resulted in a strong inhibition of sheep red blood cell phagocytosis without any effect on MHV3 uptake. Similar uptake of radiolabelled MHV3 was shown by whole spleen cells, purified T lymphocytes and thymocytes. Furthermore, no difference in 3H-labelled MHV3 uptake was seen between macrophages originating from resistant A/J mice, semi-susceptible (C57Bl/6 × A/J)F1 and susceptible animals. These results indicate, therefore, that genetically related in vivo sensitivity toward MHV3 infection is not related to differential uptake of virus by cells.

INTRODUCTION

Mouse sensitivity to MHV3 infection is genetically determined (Le Prevost et al., 1975a, b) and the degree of virus replication varies from one strain of mice to another (Le Prevost et al., 1975a). Macrophages support MHV3 replication and represent target cells for the virus (Mallucci, 1965).

In previous studies, we observed that virus replication in non-immune macrophages was similar in susceptible and in resistant mouse strains upon in vitro infection, but was different when animals were infected in vivo prior to collection of peritoneal exudate cells (Dupuy et al., 1980). Thus, upon in vivo infection, it appeared that the degree of virus replication correlated with the sensitivity of mouse strains, indicating that some factor(s) plays an important in vivo role in controlling the yield of virus in resistant animals. This factor(s) was shown to operate immediately after virus infection, suggesting that the mechanism involved was related to natural resistance. Restriction of MHV3 replication was also observed in macrophages collected from resistant A/J mice, 4 to 15 days after MHV3 immunization. In such a case, low virus replication was detected in macrophages after in vivo infection or in vitro challenge (Krzystyniak et al., 1980).

Since virus infection of cells requires attachment of virus particles to cell surface and internalization of the virus genome into the cytosol (Dales, 1973; Lonberg-Holm & Philipson, 1974), the present work was undertaken to determine whether the initial steps of MHV3-cell interaction were involved in the differences in virus production seen in mouse strains displaying various genetically related viral sensitivities.

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METHODS

Animals. Inbred mouse strains: A/J (resistant to MHV 3 infection), C57Bl/6 and DBA/2 (susceptible) and (C57Bl/6 × A/J)F 1 hybrids (semi-susceptible) were purchased from Jackson Laboratory, Bar Harbor, Me., U.S.A. C57Bl/6 and (C57Bl/6 × A/J)F 1 will be referred to as B6 and (B6 × A/J)F 1 . Animal care and housing have already been reported (Le Prevost et al., 1975a).

Virus. MHV 3 was passaged in susceptible DBA/2 mice as previously described (Le Prevost et al., 1975a, b). The titre of the virus used for in vivo infection was expressed in LD 50 /0.1 g of tissue. In vitro studies were carried out with the same virus strain passaged on L cells (MHV 3-L). Virus titre was expressed in p.f.u./10 6 cells (Dulbecco & Vogt, 1954). 3H-radiolabelled virus was prepared as described by Lai & Stohlman (1978) with slight modifications. MHV 3-L strain was adsorbed on L monolayers in 150 cm 2 tissue flasks (Corning Glass Works, Montréal, Canada) for 15 min at 37 °C (10 7 to 10 8 p.f.u./bottle). Eagle's minimum essential medium (MEM, Grand Island Biological Co., N.Y., U.S.A.) containing 10% foetal calf serum, antibiotics and 100 μCi of each of the following: 6-[3H]uridine, 2-[3H]adenosine and [3H]leucine (sp. act. respectively 23, 21 and 55 Ci/mmol, Amersham/Searle, Arlington Heights, Ill., U.S.A.) was added to each bottle immediately following adsorption of the virus. Cultures were harvested 18 h post-infection. Purification of radiolabelled virus was carried out by successive centrifugations. The supernatants were clarified by centrifugation at 1200 g for 10 min and 15 000 g for 30 min at 4 °C. The cellular fraction was frozen and thawed three times, homogenized and clarified by centrifugation. The virus obtained from supernatant and cellular fractions was concentrated by pelleting at 90000 g for 2 h at 4 °C, resuspended and gently homogenized. Virus suspensions were then layered on to a discontinuous gradient consisting of 2.5 ml 30% sucrose and 2.5 ml 50% sucrose, and centrifuged at 100000 g for 3 h at 4 °C in a SW65 Beckman rotor. The visible band at the 30 to 50% sucrose gradient interface was removed and concentrated by centrifugation at 90000 g for 2 h at 4 °C. Pellets were then gently homogenized again and virus fractions were stored at −70 °C. In some experiments, virus fractions were further centrifuged on a linear 25 to 50% sucrose gradient at 100000 g for 3 h at 4 °C. Virus titre, 3H radioactivity and absorbance (220 to 320 nm) were determined in all fractions.

Cells. Peritoneal exudate macrophages were obtained and cultured as described by Dupuy et al. (1980). L cells were grown as monolayers in MEM medium supplemented with 10% foetal calf serum and antibiotics. Plastic cell culture bottles (75 cm 2 , Falcon) and in some cases 24-well Linbro plates and Linbro microplates (flat bottom, 0.25 ml vol.) were used. Cells were passaged twice a week and used on the third day after plating.

Spleen and thymus were removed, minced, homogenized and splenic cells and thymocytes were obtained as described by Le Prevost et al. (1975a, b). Splenic T lymphocyte suspensions were enriched using the nylon wool column technique (Julius et al., 1973).

Virus infection and assessment of cytopathic effects. Monolayer cell cultures were infected with MHV 3-L by incubating virus suspensions for 0 to 60 min at 37 °C and in some cases at 0 °C. Cells were then washed three times with MEM, and fresh medium was added. Virus cytopathic effects were measured by using three different techniques. (i) Enumeration of giant multi-nucleated cells (foci) in macrophage cultures at different times post-infection (Dupuy et al., 1980). (ii) 51Cr-release assay from infected cells 24 to 48 h post-infection, as described previously (Dunkley et al., 1974). Cells were labelled with 5 μCi/well of [ 51Cr]sodium chromate (110 mCi/mg Cr, Charles E. Frosst & Co., Radiopharmaceutical Division, Kirkland, Montréal, Canada). The specific 51Cr-release due to virus-induced cytolysis was calculated according to the following formula:

\[
\% \text{ Specific } 51\text{Cr-release} = \frac{B - A}{C - A} \times 100
\]
where $A =$ spontaneous $^{51}\text{Cr}$-release from non-infected control cells, $B =$ $^{51}\text{Cr}$-release from infected cells and $C =$ maximum $^{51}\text{Cr}$-release obtained by lysis of cells in 1% Triton X-100 solution. The maximum releasable counts were usually 80 to 90% of the total counts taken up by the cells. Experiments with 25% or less spontaneous $^{51}\text{Cr}$-release were taken into account. (iii) $[^3\text{H}]$proline assay was used as described previously (Bean et al., 1973), with slight modifications. Cell monolayers were first incubated with 5 $\mu\text{Ci/well} [^3\text{H}]$proline (18 Ci/mmol, Amersham/Searle) for 30 to 48 h and washed four times. The experiment was terminated 48 h post-infection by washing the monolayers four times and adding hyamine hydroxide (J. T. Baker Chemical Co., Phillipsburg, N.J., U.S.A.). Remaining radioactivity in undamaged $[^3\text{H}]$proline-labelled cells was determined.

**Phagocytosis assay.** Phagocytosis of macrophage monolayers was determined by incubation of cells with previously $^{51}\text{Cr}$-labelled sheep red blood cells ($^{51}\text{Cr}-\text{SRBC}$). SRBC were incubated with 200 $\mu\text{Ci}/10^8$ cells of $[^{51}\text{Cr}]$sodium chromate for 45 min at 37 °C and washed four times. $^{51}\text{Cr}-\text{SRBC}$ were used as radioactive markers of phagocytosis by adding $10^7$ $^{51}\text{Cr}-\text{SRBC}/\text{well}$ on to macrophage monolayers followed by a 1 h incubation at 37 °C. Monolayers were then washed four times and 1% Triton X-100 was added. Plates were kept overnight at 37 °C and the chromium release was counted. Cytochalasin B (Sigma) at a concentration of 25 $\mu\text{g/ml}$ (Ito et al., 1979) and prostaglandin E$_1$ (PGE$_1$, Sigma) at a concentration of $10^{-6}$ M (Oropeza-Rondon et al., 1979) were used as inhibitors of phagocytosis.

**Uptake of $^3\text{H}$-labelled MHV$_3$ virus.** One-hundred $\mu\text{l}$ of radioactive $^3\text{H}$-labelled MHV$_3$ virus were added either to cell suspensions ($2.5 \times 10^5$ cells/250 $\mu\text{l}$) or on to cell monolayers and were incubated for 20 min at 37 °C, 5% CO$_2$. After three washings, 0.5 ml hyamine hydroxide was added and cell samples were kept overnight and counted. In some cases, $^3\text{H}$-labelled MHV$_3$ virus was preincubated for 3 h at 4 °C with anti-MHV$_3$ antibody prior to incubation with cells. Controls for non-specific attachment to cells consisted of adding the equivalent amount of radioactive $^3\text{H}$-labelled material (as measured in ct/min) obtained from non-infected L cell cultures and processing them in the same fashion as radiolabelled virus (i.e. 18 h of labelling, 30 to 50% sucrose gradient interface fraction of radiolabelled L cell homogenates). Radioactive $^3\text{H}$-labelled MHV$_3$ did not lose its infectivity during labelling and purification and 48 h after infection with $^3\text{H}$-labelled MHV$_3$, mouse macrophages or L cells showed regular cytopathic effects.

**RESULTS**

**Temperature and time dependency of MHV$_3$ infection of cells**

To determine the early events of MHV$_3$ infection, we measured the effect of different temperatures and times on virus entry into host cells. As shown in Fig. 1 (a, b) incubation of cells with virus particles for 0 to 60 min at 37 °C indicated that the virus uptake was rapid, occurring mostly during the first 15 min of incubation. Both focus enumeration in macrophage cultures 16 h post-infection (Fig. 1 a) and the remaining $[^3\text{H}]$proline radioactivity in L cells 48 h post-infection (Fig. 1 b) showed a relation between virus cytopathic effect and time of incubation. In addition, the specific $^{51}\text{Cr}$-release due to virus-induced cell killing was lower in cultures infected at 0 °C than at 37 °C (Table 1), indicating a temperature dependency of MHV$_3$ uptake by both macrophages and L cells.

**Uptake of $^3\text{H}$-labelled MHV$_3$ by cells**

In order to study virus–host cell interactions, we used $^3\text{H}$-radiolabelled MHV$_3$ virus harvested from L cell cultures 18 h post-infection. Radiolabelled $^3\text{H}$-labelled MHV$_3$ virus was purified from culture medium (supernatant fraction) and from cells (cellular fraction). The absorption spectra of $^3\text{H}$-labelled MHV$_3$ obtained from both cellular and supernatant
Fig. 1. (a) Infection of A/J macrophages with $5 \times 10^5$ p.f.u./10^6 cells of MHV$_3$ for different times of incubation. Cytopathic effects are expressed as the number of foci/10^6 cells ± S.D., 16 h post-infection. (b) Infection of L cells with $5 \times 10^4$ p.f.u./10^5 cells/well of MHV$_3$ at different times of incubation. Cytopathic effects were measured by the remaining [3H]proline radioactivity in cells, 48 h post-infection, and expressed as ct/min ± S.D.

Table 1. Temperature dependency of in vitro infection of cells by MHV$_3$

<table>
<thead>
<tr>
<th>Cell</th>
<th>Virus titre (p.f.u./10^6 cells)</th>
<th>Time post-infection (h)</th>
<th>Specific 51Cr-release (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 °C*</td>
<td>37 °C</td>
<td></td>
</tr>
<tr>
<td>L cells</td>
<td>$5 \times 10^4$</td>
<td>0–18</td>
<td>1.0 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>$5 \times 10^5$</td>
<td>0–18</td>
<td>2.2 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>$5 \times 10^6$</td>
<td>0–18</td>
<td>56.0 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>$5 \times 10^7$</td>
<td>0–18</td>
<td>67.9 ± 2.3</td>
</tr>
<tr>
<td>Macrophages</td>
<td>$5 \times 10^6$</td>
<td>0–16</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$5 \times 10^4$</td>
<td>24–42</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$5 \times 10^5$</td>
<td>24–42</td>
<td>9.0 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>$5 \times 10^6$</td>
<td>42–48</td>
<td>25.5 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>$5 \times 10^7$</td>
<td>42–48</td>
<td>11.0 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>$10^6$</td>
<td>42–48</td>
<td>26.0 ± 2.1</td>
</tr>
</tbody>
</table>

* Incubation of cells at 0 °C was performed without virus for 1 h and with virus for another 1 h. After three washings with ice-cold medium, cells were incubated for 16 to 48 h at 37 °C, 5% CO$_2$. Results represent the mean of four or five samples ± S.D.
† P < 0.001 by Student’s t-test.
Fig. 2. Phagocytosis of $^{51}$Cr-labelled SRBC by A/J macrophages. Cells were preincubated for 60 min at 37 °C with: 1, no virus; 2, 10$^7$ p.f.u./10$^6$ cells MHV$\_3$; 3, 10$^{-6}$ M-PGE$_1$; and 4, 25 μg/ml cytochalasin B. Bars represent the mean of five samples ± s.D.

Fig. 3. Uptake of $^3$H-labelled MHV$\_3$ by A/J macrophages. Cells were preincubated for 60 min at 37 °C with: 1, no virus; 2, 10$^7$ p.f.u./10$^6$ cells of non-labelled MHV$\_3$; 3, 10$^{-6}$ M-PGE$_1$; and 4, 25 μg/ml cytochalasin B. The cells were then incubated with 2.5 × 10$^4$ cts/min/0.25 × 10$^6$ cells $^3$H-labelled MHV$\_3$ for 20 min at 37 °C (1 to 4) or with the equivalent of $^3$H radioactive material (5) obtained in 30 to 50% sucrose centrifugation of the supernatant fraction of radiolabelled non-infected L cells (see Methods). Bars represent the mean of four or five samples ± s.D.

Table 2. Independence of MHV$\_3$ infection of A/J macrophages and inhibition of phagocytosis

<table>
<thead>
<tr>
<th>Cell treatment*</th>
<th>State of donor</th>
<th>No virus</th>
<th>MHV$_3$ infection</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ct/min/10$^6$ cells</td>
<td>Ct/min/10$^6$ cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>49214 ± 3780</td>
<td>48103.0 ± 3108</td>
<td>97.7</td>
</tr>
<tr>
<td></td>
<td>Immunized</td>
<td>48824.5 ± 3628</td>
<td>21846.5 ± 3084</td>
<td>44.7</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>47963.8 ± 2819</td>
<td>43917.0 ± 3961</td>
<td>91.5</td>
</tr>
<tr>
<td></td>
<td>0 °C Normal</td>
<td>48148.5 ± 2863</td>
<td>22876.0 ± 2862</td>
<td>47.5</td>
</tr>
<tr>
<td></td>
<td>PGE$_1$ Normal</td>
<td>46314.5 ± 2706</td>
<td>20263.9 ± 3161</td>
<td>43.7</td>
</tr>
<tr>
<td></td>
<td>Cytochalasin B</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Cells were obtained from MHV$\_3$-immunized or normal, non-immunized mice. Prior to virus infection, cells were preincubated with or without PGE$_1$ (10$^{-5}$ M) or cytochalasin B (25 μg/ml) at 37 °C. After treatment, cells were washed twice.

† Cells were infected with 5 × 10$^4$ p.f.u./10$^6$ cells of MHV$\_3$ at 0 °C for 30 min and washed three times with ice-cold medium (control, 0 °C) or infected at 37 °C for 30 min with the same quantity of virus and washed three times. After 48 h, the non-infected and infected cells were washed three times and the radioactivity of remaining cells was counted as described in Methods. Results represent the mean of three to five samples ±S.E.

‡ P < 0.001 by Student's t-test.

of radiolabelled $^3$H-labelled MHV$\_3$ (Fig. 3) or MHV$\_3$-induced cytopathic effects, as measured by the remaining $[3^\text{H}]$proline radioactivity 48 h after infection of cells with 5 × 10$^4$ p.f.u./10$^6$ cells (Table 2). This indicates that virus uptake and cytolysis are not related to the phagocytic capacities of cells.

The uptake of $^3$H-labelled MHV$\_3$ by normal and immunized macrophages was also measured. Immunization of resistant A/J strain mice with MHV$\_3$, 7 to 14 days prior to in vitro culture of macrophages has been previously shown to protect the cells against in vitro MHV$\_3$ challenge (Krzystyniak et al., 1980; see also Table 2). Since the early stages of virus–host interactions may be critical in antiviral resistance, we measured the uptake of $^3$H-labelled MHV$\_3$ by normal macrophages originating from resistant A/J semi-susceptible
Fig. 4. Uptake of $^{3}$H-labelled MHV$_{3}$ by macrophages originating from non-immunized A/J (1), (C57Bl/6 x A/J)F$_{1}$ (2) and DBA/2 (3), and from immunized A/J mice (4). Cells were incubated for 20 min at 37 °C with $2.5 \times 10^{4}$ ct/min/2.5 x 10$^{5}$ cells of $^{3}$H-labelled MHV$_{3}$, heat-inactivated $^{3}$H-labelled virus (7), anti-MHV$_{3}$ Ab-pretreated $^{3}$H-virus or with the equivalent of $^{3}$H-labelled radioactive material obtained in 30 to 50% sucrose centrifugation technique of the supernatant fraction of radiolabelled non-infected L cells ($^{3}$H control, 9). Bars represent the mean of four or five samples ± S.D. Normal (C57Bl/6 x A/J)F$_{1}$ plus anti-MHV$_{3}$ Ab; 6, immunized A/J plus anti-MHV$_{3}$ Ab; 8, heat-inactivated cells.

Table 3. Uptake of radiolabelled $^{3}$H-labelled MHV$_{3}$ by immunocompetent mouse cells

<table>
<thead>
<tr>
<th>Origin</th>
<th>Cell*</th>
<th>Strain</th>
<th>$^{3}$H radioactivity of infected cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ct/min/2.5 x 10$^{4}$ cells</td>
<td>%</td>
</tr>
<tr>
<td>Peritoneal exudates</td>
<td>A/J</td>
<td>8265.0 ± 788</td>
<td>100</td>
</tr>
<tr>
<td>Spleen</td>
<td>(B6 x A/J)F$_{1}$</td>
<td>6718.5 ± 888</td>
<td>80.5</td>
</tr>
<tr>
<td>Nylon column-purified T cells</td>
<td>(B6 x A/J)F$_{1}$</td>
<td>6696.4 ± 545</td>
<td>80.3</td>
</tr>
<tr>
<td>Thymocytes</td>
<td>(B6 x A/J)F$_{1}$</td>
<td>6712.0 ± 741</td>
<td>80.5</td>
</tr>
<tr>
<td>Peritoneal exudates†</td>
<td>A/J</td>
<td>2451.3 ± 486</td>
<td>29.4</td>
</tr>
</tbody>
</table>

* Cells (2.5 x 10$^{5}$) were incubated with 25 000 ct/min $^{3}$H-labelled MHV$_{3}$ (10 p.f.u.) for 20 min at 37 °C and washed three times. Results represent the mean values from four samples ± S.D.
† The equivalent of $^{3}$H radioactive material obtained in 30 to 50% sucrose gradient centrifugation of the supernatant fraction of radiolabelled, non-infected L cells was used as a control for $^{3}$H-labelled MHV$_{3}$ and added to 2.5 x 10$^{5}$ A/J peritoneal exudates, for 20 min at 37 °C.

(B6 x A/J)F$_{1}$ and susceptible (DBA/2) mouse strains as well as from resistant A/J mice immunized with 100 LD$_{50}$, 7 days prior to testing. Neither strain sensitivity nor prior immunization of animals caused any differences in virus uptake (Fig. 4). In addition, the results were not significantly different when heat-inactivated $^{3}$H-labelled virus was used. Control groups included preincubation of $^{3}$H-labelled MHV$_{3}$ with anti-MHV$_{3}$ antibody at 4 °C for 3 h prior to incubation with cells, incubation of $^{3}$H-labelled MHV$_{3}$ with heat-destructed cells (90 °C for 10 min) and incubation of $^{3}$H-labelled control material (see Methods). Control groups showed a low uptake of $^{3}$H radioactivity, significantly different (P < 0.001) from that of experimental groups.

The uptake of $^{3}$H-labelled MHV$_{3}$ was also studied using unfractionated spleen cells, splenic T lymphocytes and thymocytes from A/J strain mice. The virus uptake obtained using these populations was not significantly different from that seen with A/J macrophages (Table 3).
In vitro MHV$_3$ infection of cells allowed us to study some of the early stages of MHV$_3$–cell interaction. Kinetic data of MHV$_3$ uptake by both macrophages and L cells indicated that the majority of virus particles were taken up by cells within the first 15 min of incubation at 37 °C. The rate of virus uptake was similar in macrophages and L cells. It was significantly reduced, however, when the infection process was performed at 0 °C instead of 37 °C. Maximal cytopathic effects of MHV$_3$ occurred at various times depending on host cells and multiplicity of infection (Table 1). L cells appeared to be more sensitive to relatively low virus doses and cytopathic effects took place faster in L cells than in macrophages. Time dependency of the virus uptake did not seem, however, to be related to host cell types.

Radiolabelled virus allowed us to study the early events of virus–cell interactions. For such experiments, the $^3$H-labelled MHV$_3$ used was obtained from the fraction sedimented at 1.18 g/ml following ultracentrifugation of the supernatant fraction in a sucrose gradient. This band had a good yield of infectious virus ($10^5$ p.f.u./ml) and was considered to represent the most purified fraction of virus. Inhibition of phagocytosis of $^{51}$Cr-SRBC in macrophages did not affect the infection process (Fig. 3, Table 2). Blocking of phagocytic capacities of macrophages affected neither the uptake of radiolabelled $^3$H-labelled MHV$_3$ nor MHV$_3$-induced cytopathic effects of cells. Since it has been shown for several viruses that adsorptive endocytosis is not limited to phagocytic cells and can take place in all animal cells (Palade, 1956), our data suggest that such a mechanism can occur for MHV$_3$ uptake. Morphological studies of the MHV$_3$ entry into host cells will help to clarify this point. Morphological and biochemical data of animal cell infection by viruses indicate that after attachment to the cell surface, most viruses are rapidly trapped into coated pits and internalized by endocytosis (Helenius et al., 1980). This process of receptor-mediated endocytosis has recently become recognized as an important and general mechanism by which animal cells take up nutritional and regulatory proteins from extracellular fluids (Goldstein et al., 1979). Semliki Forest virus enters into cells by adsorptive endocytosis and not by fusion with the plasma membrane (Helenius et al., 1980). Similarly, penetration of Sindbis virus does not introduce virus antigens into the host cell plasma membrane (Fan & Sefton, 1978). Endocytosis is rapid and efficient and the virus is delivered into intracellular vacuoles after a very short time in the coated vesicles (Helenius et al., 1980).

An identical uptake of $^3$H-labelled MHV$_3$ was found in macrophages originating either from immunized or non-immunized A/J mice (Fig. 4), showing that this step is not involved in the restriction of replication observed in macrophages from immunized mice. Similarly, no difference in virus uptake was observed between macrophages originating from resistant A/J mice, semi-susceptible (B6 × A/J)$F_1$ hybrids or susceptible DBA/2 animals. This indicates a lack of relation between in vivo genetically controlled MHV$_3$ sensitivity and virus uptake by macrophages. Furthermore, we observed the same rate of $^3$H-labelled MHV$_3$ uptake by unfractionated spleen cells, purified T cells and thymocytes which was 80% of that by macrophages (Table 3). Although plasma membranes of immunized and non-immunized macrophages appeared to allow MHV$_3$ penetration, virus replication and cell killing were blocked in macrophages from immunized, genetically resistant A/J mice (Table 2). This suggests that virus multiplication, in immunized macrophages, is blocked at a later step in the infectious process. Macrophages play a major role in non-specific as well as in specific defence mechanisms against virus infection. Both ‘intrinsic’ and ‘extrinsic’ activity has been shown to operate in many virus infections (Morahan & Morse, 1979). Macrophages can be intrinsically resistant or permissive to replication. Furthermore, lactate dehydrogenase, lymphocytic choriomeningitis, equine infectious or aleutian mink disease viruses can persist in macrophages without cytopathic effects (Morahan & Morse, 1979).
Although macrophages from adult animals restrict replication of vaccinia (Schultz et al., 1974) or herpes simplex virus (Lopez & Duras, 1979), it was observed that virus adsorbed equally well to normal and even immune macrophages (Avila et al., 1972; Mogensen & Anderson, 1978). Similarly, genetically resistant and susceptible macrophages were found to be equally able to adsorb and to apparently ingest mouse hepatitis virus 2 (Shif & Bang, 1970; Taguchi et al., 1980). In such cases, as for MHV₃, it seems that resistance to the virus is related to the failure to incorporate the virus into the metabolic pool of the cell. Vaccinia virus, for example, enters the cell normally and replication occurs through the initial stage of synthesis of virus thymidine kinase, but formation of the uncoating enzyme is inhibited (Silverstein, 1974). In addition, vaccinia virus uptake and inactivation within macrophages can be enhanced by antiviral antibody.

Although antigen–antibody complexes are efficiently removed by macrophages, it was of interest to observe that preincubation of ³H-labelled MHV₃ with anti-MHV₃ antibody, prior to incubation with cells, resulted in a low uptake of cell-bound radioactivity (Fig. 4). This may be due to the large excess of antibody used for neutralization. It may also be related to a property of MHV₃–antibody complexes similar to the phenomenon observed with flavivirus. It has been reported that, although large amounts of neutralizing antibody prevent in vitro replication of dengue virus in monocytes, subneutralizing concentrations of antisera enhance the infection (Halstead & O'Rourke, 1977). Such results suggest the possibility of a modulation of monocyte infection through antibodies.

Macrophage–virus interactions are still poorly understood and the mechanisms of ‘intrinsic’ and ‘extrinsic’ resistance have to be fully defined. The present work, however, shows that the early step of attachment of MHV₃ to cells is not involved in the resistance to infection.

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MHV₃ macrophage interaction


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