Susceptibility of Bovine Macrophages to Infectious Bovine Rhinotracheitis Virus Infection†

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Infectious bovine rhinotracheitis virus replicated in cultured bovine alveolar macrophages (AM). However, yields of infectious virus were low, with maximum titers approximately 100 times that of the residual inoculum. Immunofluorescence and electron microscopic studies indicated that the majority of macrophages produced viral antigen, but after infection at a multiplicity of 0.1, only 4.1% of AM produced infectious centers. Virus-infected AM culture supernatants possessed interfering activity, probably due to interferon. Incubation of fresh AM with these fluids rendered them refractory to infection. Although AM from infectious bovine rhinotracheitis virus-immune and -susceptible donors were equally permissive and their susceptibility was unaltered by incubation with bacterial lipopolysaccharide, bovine mammary macrophages which were elicited with lipopolysaccharide became nonpermissive when further incubated for 48 h with 1 µg of lipopolysaccharide per ml. Under these conditions, infected mammary macrophages failed to synthesize viral DNA, and there was reduced synthesis of “late” viral polypeptides.

Macrophages are an important component of host defense against viral infection due to the roles they play in phagocytosis and killing of viruses, production of interferon, and destruction of virus-infected cells, as well as in the development of immune responses. Although many viruses cannot replicate in macrophages (25), others will infect macrophages and initiate virus replicative activity. This may be manifested by the synthesis of viral proteins and nucleic acid (36), elaboration of viral antigen (5, 26), production of intracellular viral capsids (29, 35), or release of infectious virus to the extracellular environment (6, 20, 28, 37). Viral infection may result in death of the macrophage or, alternatively, may lead to a persistent infection (20, 21, 38, 39) and pathology resulting from various degrees of immunosuppression.

Macrophage permissiveness can be dependent on the age (15, 22) or the immune status (2) of the host and on the anatomical site from which the macrophages were derived (30). Unfortunately, no generalization can be made for a specific family of viruses. For example, both human and mouse alveolar macrophages become restrictive with age of the host for herpes simplex virus infection while remaining permissive for replication of cytomegalovirus (9, 37).

In the case of infectious bovine rhinotracheitis (IBR) virus infection in athymic nude mice, the virus establishes a persistent infection with subsequent transformation of the macrophages (10). In cattle, IBR virus causes disease most commonly in the respiratory tract but also in other organs (16). Extensive virus replication occurs, and, as with most herpesvirus infections, cell-mediated immunity is of major importance in recovery (8, 31, 32). The exact role of the macrophage in IBR virus infection and recovery is unknown. However, the virus has been implicated as a predisposing factor in the development of bacterial pneumonia in cattle (7, 14), and it is likely that this is mediated through an inhibition of alveolar macrophage (AM) function.

In view of this, we have initiated studies to determine whether IBR virus can infect bovine AM and, if so, what effect this has on AM function. This work was designed to determine the susceptibility of bovine AM to infection with IBR virus and factors which may influence the outcome.

MATERIALS AND METHODS

Calves. Holstein calves were obtained at birth and artificially reared in isolation. They were used between the ages of 4 and 12 months. IBR-immune

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calves had been challenged by intranasal instillation of IBR virus (Colorado-1 strain; ATCC VR-864) at least 3 months before use as a source of macrophages. They developed typical clinical signs of disease and showed a serological response. IBR-susceptible calves were periodically monitored serologically to ensure that their status remained IBR negative.

Collection of macrophages and maintenance in culture. AM were collected by the procedure of Wilkie and Markham (42). Calves were sedated with xylazine (Rompun; Cutter Laboratories, Mississauga, Ont., Canada; 100 to 140 mg intravenously) and placed in left lateral recumbency. A fiber optic endoscope was passed via the trachea into the right diaphragmatic lobe. Lung lavage fluid contained 0.15 M saline, 5 mM glucose, 2 mM EDTA, 25 mM HEPES (N-2-hydroxyethyl piperazine-N'-2-ethanesulfonic acid) buffer, 50 µg of gentamicin and per ml, 5 µg of amphotericin B per ml, pH 7.2 (41). An initial wash was made by passing 50 ml of fluid into the lung, of which about half was retrieved under mild vacuum and discarded. The second wash involved injecting a further 250 ml into the lung which was retrieved as above, and the bronchoalveolar cells present in the fluid were used for preparation of AM cultures.

The lavaged cells were filtered through four layers of sterile gauze to remove particulate matter and mucus, centrifuged at 1,000 × g for 10 min, washed once with Eagle minimal essential medium (MEM), and suspended in MEM with 5% fetal bovine serum (FBS). The cells were then inoculated into tissue culture plates. They were incubated for 3 h at 37°C in a 5% CO2-humidified atmosphere, washed twice with MEM to remove nonadherent cells, and reincubated for 18 to 24 h in MEM with 20% FBS.

Macrophage populations (MM) were collected 96 h after the inoculation of a nonlaetactating mammary gland with 5 ml of Escherichia coli lipopolysaccharide (LPS; O128, B12; Difco Laboratories, Detroit, Mich.) at a concentration of 1 µg/ml. A 10-ml amount of MEM was then injected into the gland, and the fluid, containing cells from the lactiferous sinus, was expressed from the teat (40).

The cells were layered onto 3 ml of Ficol-Hypaque (density at 25°C, 1.077 g/cm3) (Ficol was obtained from Pharmacia Fine Chemicals, Inc., Montreal, P.Q., Canada; Hypaque was obtained from Winthrop, New York, N.Y.) in a round-bottom tube and centrifuged at 400 × g for 20 min. The cells at the interface above the Ficol-Hypaque were aspirated, washed three times in MEM, suspended in MEM with 5% FBS, and cultured in the same manner as described for AM.

In experiments carried out to determine the effect of LPS on macrophage permissiveness for viral replication, AM and MM were washed 3 h after plating and reincubated for 48 h in MEM with either 20% endotoxin-free FBS or 20% FBS and LPS at a concentration of 1 µg/ml. Macrophages cultured by these methods were regarded as nonstimulated or stimulated populations, respectively.

Viral replication in macrophages. Macrophage monolayers in 24-well cluster plates (Costar no. 3524; 106 cells per ml) were washed with MEM and inoculated with IBR virus at a high-input multiplicity of infection (approximately 10), based on PFU in Madin-Darby bovine kidney (MDBK) cells and low-input multiplicity (approximately 0.1) and incubated for 1 h at 37°C. The monolayers were then washed twice with MEM to remove unadsorbed virus, reincubated in MEM with 5% FBS, and observed microscopically for cytopathic effect. Monolayers of MDBK cells, known as permissive cells for IBR virus, were similarly infected for comparison.

Infected monolayers were harvested at various times after infection by removing cells from duplicate wells with a rubber policeman and freezing the harvested cells and fluid at −80°C. These were later titrated for infectivity by plaque assay on MDBK cell monolayers in microtiter plates with an antibody overlay (32).

Fluorescent-antibody staining. Cultures were grown in two-well chamber slides (no. 4802; Lab-Tek Products, Westmont, Ill.) and infected at various multiplicities in the usual manner. After infection, cells were assessed for the presence of viral antigens by indirect immunofluorescence. Cells were fixed with acetone for 15 min at room temperature and overlaid with bovine anti-IBR serum. After incubation at room temperature for 30 min, the antibody was removed by washing three times with phosphate-buffered saline (pH 7.2), followed by a further 30 min of incubation with fluorescein-conjugated rabbit anti-bovine immunoglobulin G (Cappel Laboratories, Cochranville, Pa.). Slides were washed, observed, and photographed with the aid of a Leitz Orthomar microscope, using transmitted UV light.

Interferon assays. Interferon levels were determined by assaying the ability of culture fluids from virus-infected macrophages to inhibit vesicular stomatitis virus replication in MDBK cells as described previously (3). Briefly, culture fluids from virus-infected macrophages were incubated for 1 h with anti-IBR serum to neutralize any IBR virus present. Twofold dilutions of the culture fluids were made in MEM plus 5% FBS and incubated with confluent MDBK cells for 24 h. The culture fluids were removed, and cultures were infected with 100 PFU of vesicular stomatitis virus and overlaid with 1% methylcellulose. After 24 h the methylcellulose overlay was removed, cells were fixed and stained, and the interferon titer was determined as the dilution that resulted in a 50% reduction in the number of plaques compared with the control. Reproducibility was checked by including a laboratory standard of bovine interferon prepared in our laboratory.

Electron microscopy. AM and MDBK cells were examined by electron microscopy 24 h after infection with IBR virus at an input multiplicity of 10. Cells which had become nonadherent were centrifuged at 400 × g for 5 min, the supernatant was removed, and the cells were suspended in 250 µl of 0.5 M glutaraldehyde in 0.06 M sodium cacodylate buffer, pH 7.4, delivered rapidly through a 26-gauge needle. They were immediately pelleted again in a soft plastic microcentrifuge tube, the blind end of the tube was cut off, and the pellet was gently washed into glutaraldehyde-cacodylate fixative. After 12 h the pellet was transferred to 0.1 M sodium cacodylate buffer, pH 7.4. The pellet was subsequently embedded in Epon-Araldite, and ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Zeiss EM9S electron microscope.

Infectious center assays. AM and MDBK cells were cultured in 35-mm-diameter wells of cluster plates (Costar no. 3506) and infected with IBR virus at an
Monolayers were fixed in acetone, incubated with bovine anti-IBR serum followed by fluorescein-conjugated anti-bovine serum, and observed under UV light. Almost all MDBK cells, but only approximately 10% of AM, showed fluorescence (x1,640).

Input multiplicity of 0.1 or 0.01. After a 1-h adsorption period, monolayers were washed twice with MEM and overlaid with MEM plus 10% FBS. At various times postinfection (p.i.), the cells were removed from the monolayers. MDBK cells were removed by mild trypsinization, whereas AM were removed by incubation with 9 mM lidocaine hydrochloride (Astra Chemicals Ltd., Mississauga, Ont., Canada) in MEM plus 20% FBS. Both populations were washed, suspended, and diluted in MEM containing anti-IBR serum, and total cells were enumerated. Volumes of 1 ml of various dilutions were added to confluent MDBK cell monolayers in 24-well cluster plates. The cells were allowed to settle undisturbed for 48 h, after which the monolayers were fixed and stained and viral plaques were enumerated microscopically.
Viral polypeptide analysis by polyacrylamide gel electrophoresis. AM, MM, and MDBK cell monolayers were cultured in 35-mm dishes in the usual manner, washed, and infected at a multiplicity of 10 with IBR virus. After the virus was permitted to adsorb for 1 h at 37°C, the monolayers were washed and overlaid with methionine-free MEM (no. 79-0115; GIBCO Laboratories, Grand Island, N.Y.) containing 1% FBS. After incubation for a further 6 h to permit cessation of host protein synthesis, 25 μCi of [35S]methionine (SJ204; Amersham Corp., Arlington Heights, Ill.) per ml was added to each culture. The cells were harvested at 20 h p.i. They were suspended in 100 μl of dissociation buffer (0.06 M Tris [pH 6.8], 0.00125 M bromophenol blue, 1.25% sodium dodecyl sulfate, 12.5% glycerol, 0.15 M 2-mercaptoethanol), sonicated for 10 s at a power setting of 7 in a Sonifier cell disruptor (Biosonic, Plainsview, N.Y.), and boiled for 2 min.

Samples were electrophoresed in the presence of sodium dodecyl sulfate through 7.5 or 10% polyacrylamide gels as described by Laemmli (17), using a 15-cm vertical electrophoresis apparatus (Richter Scientific, Vancouver, B.C., Canada). Gels were dried and autoradiographed on Kodak X-OMAT-R film. Molecular weights of radioactive bands were determined by comparing their Rf values with those of markers with known molecular weights (high- and low-molecular-weight calibration kits; Pharmacia) electrophoresed on the same gel structure.

Detection of viral nucleic acid synthesis. Bovine kidney cells or bovine macrophages that had been incubated in vitro for 48 h in the presence or absence of LPS were infected at an input multiplicity of 10 with IBR virus or mock infected. At 6 h p.i. 10 μCi of [methyl-3H]thymidine (24 Ci/mmoll; Amersham Corp.) per ml was added to infected cultures or 2 μCi of [14C]thymidine (55.7 mCi/mmoll; Amersham Corp.) per ml was added to the mock-infected cultures. At 20 h p.i., cells were removed with the aid of a rubber policeman, and infected and uninfected cultures were mixed, washed with TE buffer (0.01 M Tris, 0.001 M EDTA, pH 8.0), and suspended in 1 ml of TNE buffer (0.01 M Tris, 0.15 M NaCl, 0.001 M EDTA, pH 8.0). Proteinase K and sodium lauryl sarkosine (Sigma Chemical Co., St. Louis, Mo.) were added to 100 μg and 1 mg/ml, respectively, to lyse the cells. After 24 h at room temperature, the lysate was made up to a density of 1.72 g/ml with CsCl and centrifuged for 72 h at 35,000 rpm in a Beckman type 50 Ti rotor. The gradients were fractionated, and amounts of 14C and 3H in each 0.25-ml fraction were determined with the aid of a Beckman LS8000 scintillation counter.

RESULTS

IBR replication in bovine AM. (i) Immunofluorescence and virus yields. Initially, we attempted to determine whether IBR virus could infect bovine AM. Infection at an input multiplicity of 10 resulted in the development of cytopathic changes in some cells as early as 8 h p.i. Also, cells began to show cytoplasmic fluorescence at this time (Fig. 1a). As time progressed, the degree of fluorescence and cytopathology increased such that the entire monolayer began to detach by 16 to 20 h p.i. A comparison of the rate of development of fluorescence demonstrated that AM required approximately 20 h before about 90% were positive, whereas MDBK cells were all positive by 8 h p.i. (Fig. 1b).

Analysis of the supernatant fluids indicated that infection of AM was not abortive, since by 24 h p.i. there was a 100-fold increase in the amount of virus present in AM culture fluids (Fig. 2). As was the case with the immunofluorescence studies, maximum virus yields in AM were achieved at a slower rate than in MDBK cells (Fig. 2). Even though macrophages could replicate IBR virus, the actual yield on a per-cell basis was much lower than in MDBK cells. Thus, MDBK cells released in excess of 100 PFU per cell, whereas AM release was <1 PFU per cell.

(ii) Electron microscopy. Since AM did express IBR antigens as determined by immunofluorescence but only produced very small quantities of infectious virus, attempts were made to determine whether the infection was abortive in a high percentage of cells, possibly by virtue of the inability of virus to assemble into complete virions within macrophages. Examination of infected AM by electron microscopy clearly indicated that virus assembly was occurring and what appeared to be complete virions were budding from the nuclear membrane (Fig. 3a), as well as being released into the extracellular spaces (Fig. 3b and c). Furthermore, much in

![FIG. 2. Replication of IBR virus in AM (○) and MDBK cells (●) after infecting at a multiplicity of infection of 10.](http://iai.asm.org/)
FIG. 3. Replication of IBR virus in AM. At 24 h p.i., naked virions are present in the nucleus and budding through the nuclear membrane (a) and becoming enveloped (a, inset). Virus particles were present extracellularly (b) and showed typical herpesvirus morphology (c). Bars, 100 nm (a, inset, and c) and 1 μm (b).
excess of one virion per cell was evident in the majority of AM, although extracellular virus was associated with only a small percentage of cells. A comparison of virus present in MDBK cells demonstrated that more virus was present within a cell (Fig. 4); however, this difference was not great enough to account for a 3-log difference in titer between MDBK cells and macrophages.

(iii) Infectious centers. Even though we had evidence that most AM did replicate the virus, we could not explain the extremely low yields of virus. Furthermore, if cultures were infected at low multiplicities of infection, the virus did not spread to destroy the entire monolayer (Fig. 5). Thus, the virus produced foci within 48 h p.i., but these foci did not progress to kill the entire monolayer nor were new foci initiated even though no antibody was added. This suggested that most of the spread was initially from cell to cell but that even this mode of spread was halted within 48 h in culture.

In an attempt to see whether the infected macrophages within the foci could produce infectious centers, we plated them on susceptible MDBK cells. Table 1 illustrates that they could initiate infectious centers. However, in contrast to MDBK cells, in which almost all of the cells produced infectious centers by 24 h, <5% of the AM did so as late as 48 h p.i. and there was no

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**Table 1. Production of infectious centers by AM and MDBK cells after infection with IBR virus**

<table>
<thead>
<tr>
<th>Cell type</th>
<th>MOI*</th>
<th>% Producing infectious centers on confluent MDBK cell monolayers at time p.i.:</th>
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<tr>
<td></td>
<td></td>
<td>4 h</td>
</tr>
<tr>
<td>AM</td>
<td>0.1</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>MDBK</td>
<td>0.1</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>1.1</td>
</tr>
</tbody>
</table>

* Multiplicity of infection (MOI) of cells with IBR virus based on PFU in MDBK cells.
TABLE 2. Interferon production in AM cultures infected with IBR virus

<table>
<thead>
<tr>
<th>MOI</th>
<th>Interferon production(^b) at p.i.:</th>
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<tbody>
<tr>
<td></td>
<td>4 h</td>
</tr>
<tr>
<td>0.1</td>
<td>&lt;3(^b)</td>
</tr>
<tr>
<td>0.01</td>
<td>&lt;3</td>
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</table>

\(^a\) Multiplicity of infection (MOI) of AM with IBR virus based on PFU in MDBK cells.
\(^b\) Reciprocal of dilution of AM culture supernatant causing a 50\% reduction in plaques of vesicular stomatitis virus on MDBK cell monolayers.

increase even at 96 h p.i. These results supported previous studies which indicated that the number of permissively infected cells was limited since foci did not increase in size.

Interference with spread of infection in AM cultures. The reason for limited spread of infection through the macrophage monolayer was not due to a lack of IBR virus in the culture medium. Monolayers were infected at an input multiplicity of 0.1, and supernatant fluid and cells were sampled separately at 24, 48, and 96 h p.i. The virus infectivity was distributed almost equally between cells and supernatant fluids at the three sampling times. At present, the only explanation we have for the refractory nature of the cultured AM is that viral infection stimulates the production of high levels of interferon (Table 2). Support for the implication of interferon in limiting IBR virus infection in AM was obtained from experiments in which uninfected AM cultures were incubated for 18 h with culture fluids harvested from macrophages 96 h p.i. and centrifuged at 100,000 \(\times\) g for 3 h to remove IBR virus. Inoculation of treated monolayers with IBR virus at an input multiplicity of 1.0 resulted in very little detectable viral immunofluorescence at 24 h compared with untreated AM, which showed fluorescence in approximately 70\% of cells. After 48 h there was almost no infectious virus production (an approximately twofold increase above the level of the residual inoculum) in treated AM, compared with a >100-fold increase in untreated, control monolayers.

Although the nature of the interference was not positively identified as interferon activity, the activity was only reduced by 50\% on incubation at 56°C for 1 h and was not removed by centrifugation at 100,000 \(\times\) g for 3 h.

Virus replication in immune and nonimmune AM. AM from immune and nonimmune donors infected with IBR virus at an input multiplicity of 0.1 supported virus replication to similar extents (Fig. 6). Comparison of viral protein synthesis in AM and MDBK cells demonstrated that all previously reported IBR structural and nonstructural polypeptides (23) were detected in AM from both immune and nonimmune animals, although in reduced amounts when compared with MDBK cells (Fig. 7). The polypeptides of VP8 and VP13 have been defined as "late" polypeptides and are only synthesized in appreciable amounts in cells that are synthesizing viral DNA. These polypeptides were synthesized in AM. In addition to all of the polypeptides synthesized in MDBK cells, AM-infected cultures contained an additional 45,000-dalton protein.

Viral DNA synthesis was examined by labeling infected cells with \(^3\)H\(^\text{thymidine followed by analysis of the DNA on CsCl gradients. Each gradient contained 14C-labeled cellular DNA as a marker. The results supported the above experiments which showed that virus was synthesized. However, the quantity of DNA produced in AM, as determined by \(^3\)H\(^\text{thymidine incorporation, was approximately 1/10 that produced in MDBK cells."

Effect of LPS stimulation of macrophages on IBR virus replication. Previous studies have demonstrated that MM from immune animals were sometimes resistant to infection with IBR virus (unpublished data). Since the immune status of the animal did not alter the AM's ability to be infected by virus, we assumed that the LPS used in eliciting MM may activate the macrophages, making them refractory to infection. To examine whether macrophages could become
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This was confirmed by analysis of the viral DNA from these cells. Thus, no [3H]thymidine label was present at the density of viral DNA in LPS-stimulated MM cultures (Fig. 8).

DISCUSSION

IBR virus replication in bovine AM was demonstrated by a variety of methods. This evidence activated to alter their ability to support IBR virus replication, both AM and MM were cultured in vitro for 48 h in the presence or absence of LPS before infection. Bacterial LPS treatment did not significantly alter viral DNA (Fig. 8) or protein synthesis (Fig. 7) in AM. However, it did reduce the quantity of late polypeptides VP8 and VP13 (Fig. 7, line 10) in LPS-treated MM. This implied that viral DNA synthesis was blocked in these stimulated macrophages. This was confirmed by analysis of the viral DNA from these cells. Thus, no [3H]thymidine label was present at the density of viral DNA in LPS-stimulated MM cultures (Fig. 8).

FIG. 7. [35S]methionine-labeled polypeptides synthesized in uninfected and IBR virus-infected MDBK cells and AM and MM. MDBK cells (lanes 1 and 2), AM from immune animals (lanes 3 and 4), AM from nonimmune animals (lanes 5 and 6), and MM from immune animals (lanes 7, 8, 9, and 10) were mock infected (lanes 1, 3, 5, 7, and 9) or infected with IBR virus (lanes 2, 4, 6, 8, and 10). After being labeled with [35S]methionine from 6 to 24 h p.i., the cells were lysed and analyzed by polyacrylamide gel electrophoresis. MM were either incubated with LPS (lanes 9 and 10) or maintained without LPS (lanes 7 and 8) for 48 h before infection. White arrows indicate positions of molecular-weight markers (white numbers = g x 103). Black numbers denote IBR virus structural polypeptide, and black letters denote nonstructural polypeptides. Numbers 8 and 13 have been identified as late polypeptides (23). Black arrow indicates a polypeptide only observed in infected macrophages.

FIG. 8. DNA synthesized in LPS-treated and untreated, IBR virus-infected AM and MM from IBR-immune animals. Infected macrophages were labeled with [3H]thymidine from 6 h after infection. At 24 h p.i., DNA from cells was analyzed by banding in CsCl gradients. Each gradient contained 14C-labeled DNA from uninfected cells as an internal marker. (A) Analysis of infected AM (○) and MM (□) without LPS treatment; (B) AM (■) and MM (□) treated with LPS. Closed arrow indicates position of viral DNA, and open arrow indicates position of cell DNA.
suggests a mechanism for the implication of IBR virus in bovine respiratory disease. Cattle succumbing to pneumatic pasteurellosis often have evidence of concurrent viral infection, including IBR virus (19), and IBR virus infection renders cattle susceptible to subsequent experimental challenge with Pasteurella haemolytica (14). Since it is believed that the AM is the major mechanism for pulmonary bacterial clearance (13), and if IBR virus infection of AM inhibits macrophage function either directly by infection or indirectly due to immunosuppressive effects, then this could explain its predisposing effect in bovine respiratory disease. It is possible that other sequelae of viral infection, such as reduced mucociliary clearance or altered secretion of bactericidal factors by the bronchotracheal mucosa (27), may also contribute to increased host susceptibility to P. haemolytica. These possibilities are presently being investigated.

Although AM were susceptible to IBR virus infection, we could not fully explain the relatively low yield of infectious virus in macrophages when compared with MDBK cells. The number of particles assembled in infected macrophages, as seen by electron microscopy, was much in excess of one virus particle per cell. However, the actual yield of infectious virus from AM was <1 PFU per cell (Fig. 2 and 6). Thus, most particles were not capable of initiating replication. Since the particle/infectivity ratio in most herpesvirus infections can approach 100:1 to 1,000:1, it is possible that the ratio in AM was no different than that in MDBK cells. Thus, it is possible that AM, although they replicate the virus, produce a smaller quantity of viral product on a per-cell basis. This is partially supported by the observation that [3H]thymidine incorporation into viral DNA was 90% lower in AM than in MDBK cells. Similarly, incorporation of [35S]methionine into cells as viral protein was also lower in AM. Alternatively, we could not exclude the possibility that a large proportion of the particles produced in AM were defective.

If defective interfering particles were released from AM, this could partially explain why infected foci did not increase in size with continued in vitro incubation. Otherwise, we must assume that the lack of spread of virus in AM cultures was due to the release of high levels of interferon (Table 2) which rendered contiguous cells refractory to infection. Although we found that interference to virus replication was inhibited by cell-free supernatant fluids from IBR-infected AM cultures and that activity in our vesicular stomatitis virus plaque reduction assay was not removed by centrifugation at 100,000 x g or destroyed by heating at 56°C, we did not fully characterize the activity as being due to interferon. Since there is controversy regarding the sensitivity of herpesviruses to interferon (1, 3, 29, 34) and macrophages are possibly refractory to their own interferon (12), the identity of the interfering factor in our AM cultures requires further confirmation.

The role of AM in producing interferon in vivo also requires investigation. It may play an important role in defense, not only by preventing virus replication but also by activating other macrophages (4), which can then influence antigen presentation to lymphocytes or directly affect lymphocytes (11, 18).

AM from immune and nonimmune donors were equally susceptible to IBR virus infection. This finding is similar to that of Brautigam et al. (6), who found that peritoneal macrophages from susceptible and resistant mice were equally permissive for murine cytomegalovirus. However, contrasting results have been obtained by Bryans (personal communication), who found that equine rhinopneumonitis virus could replicate in peripheral blood monocytes from nonimmune horses only. Hence, even within the herpesvirus family, there appears to be a variation in this respect.

Variation in macrophage susceptibility to viral infection, depending on anatomical site or activation, has also been recorded by a number of workers (6, 24, 30). Although incubation of AM with bacterial LPS did not alter their susceptibility to IBR virus infection, it rendered MM resistant to infection. Our MM differed from AM in their site of derivation and also by virtue of being elicited by inoculation of LPS into the mammary gland. Whatever the reason, it is of interest that they were resistant, and we are currently investigating the circumstances under which bovine macrophage populations can be rendered refractory to virus infection and the mechanisms involved in activation of various macrophage populations, as well as what effect such activation may have on virus-cell interactions.

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LITERATURE CITED


