Lack of Virus-Specific Bacterial Adherence to Bovine Embryonic Lung Cells Infected with Bovine Parainfluenza Virus Type 3†

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Infection of bovine embryonic lung cells with bovine parainfluenza virus type 3 did not induce in vitro, virus-specific, hemadsorption-related adherence of Corynebacterium pyogenes, Haemophilus somnus, Staphylococcus aureus, Streptococcus zooepidemicus, Pasteurella haemolytica, Listeria monocytogenes, Escherichia coli, Pasteurella multocida, Brucella sp., or Salmonella typhimurium.

In vitro bacterial adherence to virus-infected cultured cells as a model for the in vivo phenomenon has been investigated with influenza virus (2, 10, 11), measles virus, adenovirus, and rhinovirus (11). Sanford et al. (10) found a positive correlation between the degree and kinetics of the adherence to influenza virus-infected MDCK cells by human erythrocytes and by a group B Streptococcus sp. and by Streptococcus sanguis.

Bovine parainfluenza virus type 3 (BPIV-3) plays a predisposing role in bovine pneumonic pasteurellosis (1, 5, 12). Lopez et al. (6) found that BPIV-3 reduced the pulmonary clearance in cattle of Pasteurella haemolytica on days 7 and 11 after exposure to virus. The mechanism of clearance impairment is not known. Since BPIV-3 is also a hemadsorbing virus, we have investigated the kinetics of hemadsorption (HAd) and in vitro bacterial adherence (BAD) to bovine embryonic lung (BEL) cells inoculated with BPIV-3. The results demonstrate that the virus does not cause in vitro, virus-specific, HAd-related bacterial adherence.

The BEL cell culture was established and propagated by standard procedures. In the present study the fibroblastic BEL cells were used in passages 9 through 11. Eagle minimal essential medium supplemented with antibiotics and fetal bovine serum was used. The BPIV-3 SF-4 strain was propagated in the fifth passage of the BEL cells. Bacteria isolated from live cattle or from cattle specimens submitted to our diagnostic laboratory were used. Original isolations were made on blood agar. Bacteria were identified by colony morphology on original isolation and by biochemical tests (7) of strains grown on blood agar. The following bacteria (number of isolates in parentheses) were isolated, identified, and tested: Corynebacterium pyogenes (3), Escherichia coli (2), Haemophilus somnus (4), P. haemolytica (3), Pasteurella multocida (4), Staphylococcus aureus (14), Staphylococcus zooepidemicus (2), Brucella sp. (1), Listeria monocytogenes (2), and Salmonella typhimurium (1). Except for H. somnus, bacteria were grown in 5 ml volumes of brain heart infusion broth overnight at 37°C. H. somnus was grown on blood agar overnight at 37°C in air containing 10% CO2 and then was washed into 5 ml of brain heart infusion broth. These cultures yielded approximately 10⁹ bacterial cells per ml as determined by McFarland standards (7).

HAd induced by BPIV-3 was tested with guinea pig erythrocytes (GPRBC). The effects of the following parameters were investigated: dose of virus inoculum, virus replication time, and incubation time of GPRBC on virus-inoculated cells. Confluent BEL cell monolayers in four-chamber Lab-Tek cell culture slides were inoculated with 80, 800, or 8,000 50% culture infective doses of virus per chamber. The fourth chamber on each slide was sham inoculated without virus. The slides were incubated for 60 min at 37°C in a humidified atmosphere containing 5% CO2. The inocula were aspirated, the chambers were rinsed twice with Eagle minimal essential medium, the same medium containing 2% serum was added, and the slides were reincubated. At predetermined times post-inoculation, one slide was observed for cytopathic effect, the medium was aspirated, 0.5 ml per chamber of 0.5% GPRBC suspension was added, and the slides were incubated for 60 min.

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at room temperature. The GPRBC suspension was next aspirated, and the slides were rinsed three times and immediately observed for HAd.

To determine the effect of the GPRBC incubation time, the BPIV-3 was allowed to replicate in BEL cells for a period of time (40 to 44 h) which resulted in dose-dependent HAd without cytopathic effect. The GPRBC were incubated on BPIV-3-inoculated BEL cells for different periods ranging from a few seconds to 60 min. Once the kinetics of the HAd were defined, the BAd was tested in four experiments. In the first three trials, the BEL cells were inoculated with the three graded virus doses; in the fourth trial, only a dose of 8,000 tissue culture infective doses per chamber was used. The virus-inoculated BEL cells were incubated at 37°C for 41 to 44 h. The 5 ml of brain heart infusion broth containing the bacteria was centrifuged at 2,000 × g for 10 min at 4°C, and the pellets were suspended in 5 ml of antibiotic-free Eagle minimal essential medium. A 1-ml sample of the suspension containing 10⁷ bacteria was added to each chamber. In each experiment, one slide with virus-inoculated and one with sham-inoculated BEL cells served as HAd-positive and HAd-negative controls. The slides were incubated for 60 min at room temperature, the bacterial suspensions and GPRBC were aspirated, and the chambers were rinsed three times and observed for HAd at ×150 and for BAd at ×150 and ×600 magnification with bright light. Immediately after this observation, the slides were wet mounted and observed again for BAd at ×1,000 magnification by dark-field microscopy.

Cytopathic effect, observed earliest after 72 h of virus replication, was preceded by HAd by at least 48 h at each of the three dose levels. Between 32 and 48 h after viral inoculation, the GPRBC were adsorbed in a viral dose-dependent mode by <5 to >90% of the BEL cells inoculated with BPIV-3. The sham-inoculated control BEL cells were negative at all incubation periods. In tests for the effect of GPRBC incubation time, the virus-inoculated BEL cells were incubated for 44 h. Incubation for 5 to 10 min resulted in adsorption of GPRBC to a maximum proportion of the cells. The proportion of HAd-positive cells was dependent on the virus dose and did not increase upon longer incubation times for up to 60 min. When shorter incubation times were used, no HAd was seen until 20 s. Cells became HAd positive at 40 s in chambers inoculated with the two higher doses and at 180 s in chambers inoculated with the lowest dose. The early HAd appeared on individual cells or in numerous very small foci. Then the foci increased in size and consolidated into medium or large patches which were no longer focal in character.

The results of BAd experiments are summarized in Table 1. The virus-inoculated BEL cells were incubated for 41 to 44 h. BAd was observed for C. pyogenes, H. somnus, S. aureus, and Streptococcus zooepidemicus by both bright-light and dark-field microscopy. One H. somnus isolate did not adhere, but the other three did. The character of the BAd and the proportions of BAd-positive BEL cells in the sham-inoculated and virus-inoculated groups

![](http://jcm.asm.org/)

**TABLE 1.** BAd in sham-inoculated and BPIV-3-inoculated BEL cells

<table>
<thead>
<tr>
<th>Cells tested</th>
<th>No. isolates</th>
<th>BAd at virus dose:&lt;br&gt;80&lt;br&gt;800&lt;br&gt;8,000&lt;br&gt;Control (sham inoculated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corynebacterium pyogenes</td>
<td>3</td>
<td>+, +, +</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Haemophilus somnis</td>
<td>4</td>
<td>-, +&lt;sup&gt;b&lt;/sup&gt;, -, +, - +, +, +</td>
</tr>
<tr>
<td>Pasteurella haemolytica</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Pasteurella multocida</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>3</td>
<td>+, +, +</td>
</tr>
<tr>
<td>Streptococcus zooepidemicus</td>
<td>2</td>
<td>+, +, +</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Brucella sp</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>11</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;, ND, 0–80%, 0–80%</td>
</tr>
<tr>
<td><em>(fourth expt)</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GPRBC<sup>d</sup> NA<sup>e</sup> 10–20% 30–60% 80–100% 0%

<sup>a</sup> Infective dose given in 50% tissue culture infective doses per chamber of four-chamber Lab-Tek slides.

<sup>b</sup> Negative for one isolate, positive for three, for all tests.

<sup>c</sup> ND, Not done.

<sup>d</sup> Data are reported as the proportions of cells adsorbing erythrocytes.

<sup>e</sup> NA, Not applicable.
were similar. For *P. haemolytica* and *L. monocytogenes*, minimal BAd was observed, and that was observed only in the sham-inoculated cells and only by dark-field microscopy. The 11 isolates of *S. aureus* tested in the fourth experiment adhered to widely ranging but equal proportions of virus-inoculated and sham-inoculated cells. Four bacteria (*E. coli*, *P. multocida*, *Brucella* sp., and *S. typhimurium*) did not adhere to BEL cells at all.

The reason for the detailed study of the HAd induced in BEL cells by BPIV-3 was the observation by Sanford et al. (10) of a positive correlation between the kinetics of the influenza virus-induced HAd and the adherence of streptococci. Conditions in our study allowed full development of BPIV-3-specific HAd.

In contrast to the observations of Glorioso et al. (4), who reported that *P. multocida* of rabbit origin adhered to HeLa cells, none of the four *P. multocida* isolates adhered to cultured BEL cells. The BAd was easily detectable by bright-light microscopy at ×600 and was confirmed by dark-field microscopy at ×1,000 for *C. pyogenes*, *H. somnus*, *S. aureus*, and *Streptococcus zooepidemicus*. The observations indicated that none of these bacteria adhered specifically to virus-inoculated cells. The ability of the 11 *S. aureus* isolates tested in the fourth trial to adhere was markedly different, ranging from 0 to 80% of the cells. However, the proportions of BAd-positive cells in the sham-inoculated and virus-inoculated groups were similar for any one isolate, indicating that adherence was not virus specific. Infection with influenza virus has been reported to cause increased BAd in vivo (3, 9) and in vitro (4, 10, 11). Our findings with the BPIV-3 contrast with those in vitro observations. Why one hemadsorbing virus does and another does not enhance BAd is not known. The reason may be differences in the viral membrane receptors expressed on the cell surface by different viruses, differences among strains of the same virus, or differences in the type of cells (fibroblastic versus epithelial) used in the experiments. Our data and the observations of Selinger et al. (11) that infection with measles virus, also a hemadsorbing agent (8), decreased adherence of staphylococci, streptococci, and pneumococci indicate the need to investigate each virus independently for its effect on BAd.

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**LITERATURE CITED**


