THE DEMONSTRATION OF THE K-ANTIGEN OF CAMPYLOBACTER FETUS VENEREALIS USING A MICROTITRE AGGLUTINATION TEST

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ABSTRACT


Using specific antisera prepared against heat-treated and untreated cells in a microtitre system, 7 strains of *Campylobacter fetus venerealis* were examined for the presence of K-antigen. The technique was effective for distinguishing between strains and it was found that 4 out of 7 isolates contained microcapsules, therefore being potentially suitable for vaccine production.

INTRODUCTION

The K-antigen (microcapsule) of *C. fetus* was recognized by Berg, Jutila & Firehammer (1971) as an important factor in the immunity to bovine genital campylobacteriosis. This antigen is an extracellular microcapsule around the organisms (Winter, McCoy, Fullmer, Burda & Bier, 1978). It has antiphagocytic properties (McCoy, Doyle, Burda, Corbeil & Winter, 1975) and renders the organisms resistant to phagocytosis in the genital tract. Only after opsonizing antibodies specific for this antigen have bonded with the antigen will the organisms be phagocytized. A vaccine against bovine genital campylobacteriosis should elicit an opsonin response to be effective against microcapsule-carrying campylobacters. Vaccine strains should thus have microcapsules. A number of *C. fetus venerealis* strains, whose microcapsule status was unknown, were examined serologically to select a possible vaccine strain.

The microcapsule covers the somatic (O-) antigens of *C. fetus* organisms (Berg et al., 1971) and organisms with microcapsules are therefore not agglutinated by O-specific antibodies (Border, Myers & Firehammer; 1974; Ristic, White, Doty, Herzberg & Sanders, 1957). The microcapsule is a glycoprotein (Winter et al., 1978) and is destroyed by heat at 121 °C for 120 min (Berg et al., 1971). Antiserum produced from such heat-treated cells will have only O-antibodies and will not cause agglutination of cells with microcapsules, as these cover the O-antigen.

Several workers have done serum agglutination tests with *C. fetus* organisms (Marsh & Firehammer, 1953; Berg & Firehammer, 1978; Ristic et al., 1957; Schurig, Hall, Burda, Corbeil, Duncan & Winter, 1973; Walsh & White, 1968; Winter & Dunne, 1962), all of whom performed the tests in tubes. The disadvantages of their systems were: (i) long incubation periods (20 h), (ii) manual performance of the test which could result in inaccuracy, and (iii) large volumes of sera and antigen were needed. To overcome these disadvantages a serum agglutination test that could be performed in microtitre plates (Herr, Te Brugge & Guiney, 1982) was therefore investigated.

MATERIALS AND METHODS

**Bacterial strains**

The origin of the 7 *C. fetus venerealis* strains used in the study are listed in Table 1. Catalase positive isolates with typical *Campylobacter* morphology were identified as *C. fetus venerealis* as a result of absence of H₂S-production and inability to grow in the presence of 1 % glycine and 3.5 % NaCl (Garcia, Eaglesome & Rigby, 1983).

**Antigen production**

The organisms were grown on blood tryptose agar plates for 48 h at 37 °C in anaerobic jars, containing a microaerophilic atmosphere (5 % O₂, 10 % CO₂ and 85 % N₂).

**Untreated antigens**: Growth from the agar plates was suspended in 1 % formol saline and washed 3 times by centrifugation for 10 min at 1 000 g. The cells were finally resuspended in formol saline and stored at 4 °C until used for antiserum production or in the agglutination test.

**Heat-treated antigens**: Growth from the agar plates was suspended in 0,1 M phosphate buffered saline (PBS), pH 7,2 and autoclaved for 2 h at 121 °C. Cells were then washed in PBS, as described for untreated antigens, resuspended in formol saline and stored at 4 °C, until used for antiserum production.

**Antigen concentration for the agglutination test**

Untreated antigens were used in the agglutination test. Antigen solutions were adjusted to an optical density of 0.6 absorbance at 420 nm with a Bausch & Lomb Spectronic 21 spectrophotometer.

**Antiserum production**

Rabbits were hyperimmunized, following the method of J. H. Bryner (personal communication, 1978) as set out in Table 2. Rabbits were exsanguinated on Day 16, serum was separated and stored in 2 ml aliquots at −18°C.

**TABLE 1 Origin of *C. fetus venerealis* strains**

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>796/1</td>
<td>Sheath washing, VRI, OP</td>
</tr>
<tr>
<td>6829</td>
<td>Institute Pasteur, Paris</td>
</tr>
<tr>
<td>1336</td>
<td>Florent, Paris</td>
</tr>
<tr>
<td>157/5</td>
<td>Sheath washing, VRI, OP</td>
</tr>
<tr>
<td>725/2</td>
<td>Sheath washing, VRI, OP</td>
</tr>
<tr>
<td>311/2</td>
<td>Sheath washing, VRI, OP</td>
</tr>
<tr>
<td>413/17</td>
<td>Sheath washing, VRI, OP</td>
</tr>
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</table>

**TABLE 2 Injection schedule for the production of antisera raised to heat-treated and untreated cells**

<table>
<thead>
<tr>
<th>Day</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>7</th>
<th>9</th>
<th>11</th>
<th>14</th>
</tr>
</thead>
<tbody>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Antigen concentration in % light transmission at 420 nm**

<table>
<thead>
<tr>
<th>Day</th>
<th>40</th>
<th>40</th>
<th>40</th>
<th>40</th>
<th>20</th>
<th>20</th>
<th>20</th>
</tr>
</thead>
</table>

**Amount injected intravenously**

1 ml 2 ml 2 ml 2 ml 2 ml 2 ml 2 ml

(1) J. H. Bryner, personal communication, 1978

(2) Bausch & Lomb Spectronic 21 spectrophotometer light transmission (L.T.) readings at wave length 420 nm

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1 B.B.L. anaerobic jars, Laboratory and Scientific Equipment Co. (Pty) Ltd., P.O. Box 4512, Mayfair, Johannesburg 2108

2 Protea Laboratories, P.O. Box 39127, Bramley, Johannesburg 2018
The demonstration of the K-antigen of *Campylobacter fetus venerealis*

**RESULTS**

The results of the homologous and heterologous agglutination reactions of antisera raised to heat-treated and untreated cells with untreated antigen preparations of the various strains are given in Table 3.

It was fairly easy to differentiate between a positive and a negative reaction. In a positive reaction agglutination was seen as a clearly visible clumping of cells, with either a granular mat or a smooth shield of agglutinated cells. In the case of positive reactions, the supernate was clear. The negative reaction showed no agglutination, formed a button of cells in the wells and had a cloudy supernatant.

High titres, ranging from 2,560 to 10,240, were encountered in the homologous reactions of untreated antigens with untreated antisera (Table 3). This was the case for all strains except 413/17, whose titre was lower (Table 3).

In the heterologous reactions, titres varied from low to high. The heat-treated antisera gave more negative reactions than the untreated antisera. The degree of cross-agglutination was high. The heat-treated antisera generally reacted with lower titres to both homologous and heterologous antigens than did untreated antisera (Table 3).

The untreated antigen preparation of strain 413/17 showed the lowest titre of all the untreated antigens in the homologous agglutination reactions. This antigen showed high titres by heterologous antisera, although antisera of this strain agglutinated the antigens of the other strains poorly, except for antigen 725/2 (Table 3).

The untreated antigen preparations of strains 796/1, 1336 and 413/17 in their homologous reactions were agglutinated to almost the same titres by both their untreated and heat-treated antisera.

The untreated antigenic forms of strains 6829, 157/15, 311/2 and 725/2 were either not agglutinated or showed lower titres by their homologous heat-treated antisera, and they reacted equally poorly with heat-treated antisera of these 3 strains in the heterologous reactions (Table 3).

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Table 3: Serological reactions of strains to homologous and heterologous antisera

<table>
<thead>
<tr>
<th>Antigen</th>
<th>1336</th>
<th>157/15</th>
<th>6829</th>
<th>725/2</th>
<th>311/2</th>
<th>413/17</th>
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<td>5120</td>
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<td>2560</td>
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<td>5120</td>
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<td>5120</td>
<td>5120</td>
<td>2560</td>
</tr>
<tr>
<td>311/2</td>
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<td>5120</td>
<td>5120</td>
<td>5120</td>
<td>5120</td>
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<td>5120</td>
<td>5120</td>
<td>5120</td>
<td>5120</td>
<td>5120</td>
<td>2560</td>
</tr>
</tbody>
</table>

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3. Linbro U-bottom microtitre plates, Seravac, P.O. Box 5933, Johannesberg 2000
4. General Diagnostics, Warner Pharmaceuticals (Pty) Ltd, P.O. Box 1718, Cape Town
DISCUSSION

The serum agglutination test performed in microtitre plates has many advantages over a manually performed test:

(i) the results are available earlier because of a shorter incubation period (3 h as opposed to 20 h).
(ii) the accuracy and reproducibility are superior because of the very accurate microdiluter apparatus.
(iii) only small quantities of antisera and antigen are required. This is of great benefit when the slow-growing C. fetus is used for antigen.

The test was developed with the aim of determining the presence of the K-antigen (microcapsule) among various C. fetus venerealis strains. The microcapsule is heat-labile (Berg et al., 1971) and covers the somatic antigens of an organism, rendering it inagglutinable in somatic-antigen-specific antisera.

Antisera produced from untreated organisms which possess microcapsules would contain antibodies against the microcapsule, flagellar antigens and, if some somatic antigens are exposed, antibodies against these as well.

Antiserum produced from untreated strains which do not have this microcapsule in their natural state would contain antibodies against the exposed somatic antigens, as well as against flagellar antigens. Thus, antisera produced from such an untreated strain, as well as antisera from any heat-treated strain, would both contain antibodies against somatic antigens and would give similar agglutination reactions. However, it should be kept in mind that antisera from untreated strains contain antibodies against flagellar antigens, while flagellar antigens are destroyed by the heating process (Berg et al., 1971). Also, some incompletely covered somatic antigens may elicit an antibody response, even in untreated strains with a microcapsule.

On the basis of the inagglutinability of strains 6829, 157/5, 725/2 and 311/2 by their heat-treated antisera (Table 3), it could be assumed that these strains do possess microcapsules which cover somatic antigens. Strains 793/1, 1336 and 413/17 are agglutinated to more or less the same degree by homologous heat-treated and untreated antisera (Table 3). It seems as if these latter 3 strains do not possess microcapsules.

The high degree of cross-agglutination can be expected, as an antigenic relationship exists between all strains of C. fetus (Winter & Dunne, 1962). All C. fetus venerealis strains possess the same somatic antigen (Berg et al., 1971), thus heat-treated antisera of all C. fetus venerealis strains would have the same specificity. The titres as well as the degree of cross-agglutination were comparable with the results of other workers (Berg et al., 1971; Walsh & White, 1968; Winter & Dunne, 1962; Ristic et al., 1957).

All strains of C. fetus venerealis, except strain 413/17, seem to be highly immunogenic. Apparently all C. fetus venerealis strains have microcapsules while in their natural host. A strain which seemed not to have a microcapsule was mentioned only once in the available literature (Winter & Dunne, 1962; Winter, 1963). C. fetus venerealis strain 793/1 could have been laboratory passaged and adapted, but have lost, through mutation, its ability to form a microcapsule. Strain 1336, a strain obtained in 1972, could have lost its microcapsule through the same mechanisms as strain 793/1. All the other strains used in the study were more recent field isolates of imports (6829) which had not been passaged to the same extent as strain 796/1.

C. fetus has 7 antigenically different heat-labile (microcapsular) antigens (Berg et al., 1971; Corbeil, Schurig, Bier & Winter, 1975). Since an organism can have between 2 and 5 of the different antigenic forms (Berg et al., 1971), untreated antisera from strains 6829, 311/2, 157/5 and 725/2 would not have exactly the same microcapsule specificity. This explains why these 4 strains did not show agglutination reactions identical to those of different heterologous untreated antisera.

The microtitre serum agglutination test was developed to examine C. fetus venerealis strains antigenically to determine which strains possess microcapsules. Such strains would be suitable to be used as vaccine strains. This study shows that strains 6829, 311/2, 157/5 and 725/2 all possess microcapsules. However, to use a strain like 157/5, whose antisera did not give non-specific cross-reactions, would be an advantage. Further studies should be carried out on strains 157/5, 725/2, 6829 and 311/2, before the final selection of a vaccine strain could be made.

REFERENCES


