Area: Microbial Pathogenesis

Microorganism: *Vibrio cholerae*

Reference: Sengupta, T. K., Sengupta, D. K., and Ghose, A. C. 1993. A 20-kDa pilus protein with haemagglutination and intestinal adherence properties expressed by a clinical isolate of non-01 *Vibrio cholerae*. FEMS Microbiology Letters 112: 237-242.

 The gastrointestinal disease cholera is caused by the Gram-negative bacterium *Vibrio cholerae*. This bacterium colonizes the mucosa of the small intestine and produces a toxin that alters membrane permeability. Sengupta et al. (FEMS Microbiol. Lett. 112: 237-241, 1993) recently described an unusual clinical isolate of *V. cholerae* and studied its ability to attach to host tissues.

1. Which of the following structures in the Gram-negative envelope is least likely to be involved in cell adhesion.

 a. cell membrane

 b. outer membrane

 c. S-layer

 d. pili

 Sengupta et al. first compared the ability of the bacteria to cause hemagglutination (the aggregation of red blood cells) and to bind to isolated rabbit intestinal slices after growth in two different media: tryptic soy broth (TSB) and tris-buffered salts medium (T medium). The results are shown in the following table.

 growth medium hemagglutinating activity adhesion index

 TSB 128 12.9

 T medium 4 2.0

2. These results indicate that:

 a. the bacteria cannot cause hemagglutination.

 b. the bacteria cannot adhere to intestinal cells.

 c. hemagglutination and adhesion are greater after growth in TSB.

 d. hemagglutination and adhesion are greater after growth in T medium.

 When Sengupta et al. analyzed the lipopolysaccharide profiles of the bacteria after growth in TSB or T medium, they found no significant differences. However, when they analyzed the protein profiles of the cell envelopes after growth in each medium, they obtained the following results (a - indicates a protein of a certain mass was absent, a + indicates a protein of a certain mass was present).

 protein molecular mass TSB T medium

 1 66,000 - +

 2 55,000 - +

 3 47,000 + +

 4 39,000 + +

 5 28,000 + +

 6 20,000 + -

3. These results indicate that:

 a. the protein composition of the cell envelope is the same under all growth conditions.

 b. the protein composition of the cell envelope is completely different in the two media.

 c. there are more different proteins in the cell envelope after growth in TSB.

 d. there are more different proteins in the cell envelope after growth in T medium.

 Sengupta et al. then tried to determine which of these proteins was important in adhesion of *V. cholerae* to intestinal cells. They isolated protein 1 (66,000 daltons), protein 4 (39,000 daltons), and protein 6 (20,000 daltons) and used each preparation to make antibodies that would bind to that particular protein. They then tested the effects of these antibodies on the ability of *V. cholerae* to adhere to rabbit intestinal slices. The results are shown below.

 additions adhesion index

 *V. cholerae* + buffer 12.9

 *V. cholerae* + antibodies to protein 1 12.8

 *V. cholerae* + antibodies to protein 4 12.9

 *V. cholerae* + antibodies to protein 6 1.2

4. From this, one can conclude that:

 a. protein 1 (66,000) is important in adhesion.

 b. protein 4 (39,000) is important in adhesion.

 c. protein 6 (20,000) is important in adhesion.

 d. none of the proteins is important in adhesion.

 To identify the portion of the cell envelope with which protein 6 (20,000) was associated, they linked the antibodies specific to protein 6 to gold particles and used these particles to "stain" the bacteria for electron microscopy. The results are diagrammed below, where the dots represent the gold particles:



 From this, one can concluded that protein 6 is:

 a. a pilin

 b. a flagellin

 c. an integral outer membrane protein

 d. a peripheral

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